



# CHILDREN'S EXERCISE PHYSIOLOGY

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# CHILDREN'S EXERCISE PHYSIOLOGY

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# Editorial: Children's Exercise Physiology

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## Editorial on the Research Topic

### Children's Exercise Physiology

Stimulated by the need to understand the specific effects of exercise on children, the current Frontiers Research Topic was carried out to collect a set of studies that highlight important findings related to the impact of exercise in this population. Childhood is a very specific and sensitive period for a great number of characteristics that are a part of human development. Among them, motor and functional changes, supported by growth (nature) and experience (nurture) play a key role in the performance trajectories of current and future development of children's physical fitness, motor competence, and physical activity behavior (Rodrigues et al., 2016) with relevance to future health profiles in adulthood (WHO, 2010; ODPHP, 2018). Exercise physiology research in this specific population has not always been a major concern, probably because maximal performance and competitive sports are not the intended targets in childhood; nonetheless, it is crucial to better understand children's aptitudes and to define exercise guidelines and optimization. That is why we expect that this Frontiers Research Topic on children's exercise physiology will help to boost the science and practice in childhood exercise and training.

With 20 articles published in this Research Topic, six main areas of research were defined: (a) performance, (b) physical fitness, (c) motor skill and fundamental motor competence, (d) measurement methodologies, (e) overweight subjects, and (f) pathological subjects. Most of the articles examined consider these areas of research.

Based on the diversity of study designs and objectives, we now have the opportunity to better understand the mechanisms that explain the effects of exercise on children and how performance and health can be mediated by different covariates.

It is not easy or straightforward to attribute an area to each article published in our Research Topic, though we have tried to do so. We have also summarized the most noteworthy evidence of each study.

## PERFORMANCE

Using a cross-sectional design and focusing on analyzing weightlifting performance from 2013 to 2017 in the World Championships and Olympic Games, a study conducted by Huebner and Perperoglou aimed to estimate the age of peak performance and quantify performance development during the transition from adolescence to adulthood.

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By following the characterization of anthropometric variables and adding the maturity information of handball players, Hammami et al. aimed to determine the importance of such variables on the fitness status of players from under-14 to under-18 age groups. The findings reveal that age was the strongest predictor of performance (e.g., jumping, sprinting, change of direction, and strength), and that only body mass contributed to the prediction of jumping ability (Hammami et al.).

Also within the topic of performance—and considering the hypothesis that physical activity and fitness status can be associated with school achievements—Padulo et al. suggests that agility is moderately associated with academic achievement in English ( $r = -0.400$ ), Italian ( $r = -0.337$ ), maths ( $r = -0.423$ ), music ( $r = -0.315$ ), sports ( $r = -0.622$ ), and technology ( $r = -0.381$ ). In the same study (Padulo et al.), it was found that lifestyle and socio-demographics significantly impacted school achievements. Specifically, lifestyle fully moderated the impact of family context on academic achievement.

Finally, a study conducted on under-14 soccer players tested the effects of multidirectional plyometric training on vertical jump height, change of direction, and dynamic postural control (Jlilid et al.). After an 8-week training period (2 days per week), meaningful improvements were found for squat jump (+11.14%), countermovement jump (9.91%), and *t*-test (−3.07%) in the experimental group, while no significant changes were observed in the control group.

## PHYSICAL FITNESS

A study involving a sample of 18,295 schoolchildren was conducted to test the possibility of aerobic capacity related to other aspects of health-related physical fitness (Venckunas et al.). According to the results, aerobic capacity was most strongly related to agility shuttle run and standing broad jump, which explained more than 10% of the variance in players' performance in these tests (Venckunas et al.). Aerobic capacity also explained 6 to 7% of performance in terms of abdominal curls and bent arm hang time. The positive contribution of aerobic capacity was revealed for both sexes and all age groups (Venckunas et al.).

Sánchez-Sánchez et al. investigated the influence of age group on athletic performance and muscle response after a repeated sprint ability test. The differences between the first and the last sprint, as well as the percentage of decrease, between age groups were insignificant (Sánchez-Sánchez et al.). The musculo-mechanical properties of participants in the under-16 and under-18 age-groups changed after repeated sprints.

Kokstejn et al. tested whether fundamental motor skills contribute to the acquisition of soccer-specific motor skills while considering the physical fitness and biological maturation of young soccer players. The linear regression results reveal that fundamental motor skills and physical fitness were significant predictors of speed dribbling performance, although only the effect of fundamental motor skills was statistically significant (Kokstejn et al.).

Branco et al. tested the effects of two-concurrent training programs conducted over 12 weeks in obese children. They

found significant increases in musculoskeletal mass and resting metabolic rate, significant reductions in fat mass and body fat percentage, significant improvements in maximum isometric handgrip strength, maximum isometric lumbar-traction strength, maximum isometric lower-body strength, and maximal oxygen consumption, and significant reductions in insulin, homeostatic model assessments, triglycerides, total cholesterol, and low-density lipoprotein.

A mini-review written by Armstrong and Welsman highlights the importance of properly understanding the results of peak oxygen uptake and emphasizes some flaws and fallacious interpretations of the peak oxygen uptake ratio with regard to body mass. It has been demonstrated that peak oxygen uptake increases in accordance with sex-specific, concurrent changes in age- and maturity-status-driven morphological covariates (Armstrong and Welsman). Interestingly, fat-free mass has been suggested as the most powerful morphological variable in terms of its impact on the development of youth aerobic fitness considering cycle ergometry and treadmill running tests (Armstrong and Welsman). Finally, the mini-review also recommends that future cross-sectional studies should consider sex-specific and maturation-driven changes in fat-free mass as covariate factors.

## MEASUREMENT METHODOLOGIES

Forbregd et al. assessed the effects of four different exercise modalities and body positions—treadmill walking/running (modified Bruce protocol), sitting, tilted (45 degrees), and lying flat ergometer bicycling (viz., with patient less in-motion)—on peak oxygen uptake ( $\text{VO}_2$ ), stroke volume, heart rate, and cardiac output in 31 9 to 15-year-old children under cardiopulmonary exercise testing (CPET). When compared with participants who completed the treadmill test, those who performed bicycling elicited lower peak  $\text{VO}_2$ s. Peak heart rate decreased from both treadmill to upright bicycle and from upright bicycle to lying flat bicycle. Overall, considering the higher  $\text{VO}_2$  with treadmill testing, both sitting and lying flat bicycling tests are judged proper for CPET with concomitant MRI-scanning, PET-scanning, and echocardiography.

Gao et al. investigated differences between sedentary and non-sedentary activities in 35 7 to 11-year-old children (21 girls) in terms of  $\text{VO}_2$ , triaxial accelerometry, and thigh muscle electromyography (EMG) during eight different energy-demanding activities: lying down supine while watching a children's program, sitting quietly and playing a mobile game, standing quietly and playing the mobile game, walking on a treadmill at either 4 or 6 km/h, and walking around an indoor track at freely chosen speed ( $5.0 \pm 0.8$  km/h). Optimal sedentary-to-non-sedentary thresholds, based on a receiver operating characteristic (ROC) curve analysis, revealed values of 1.3 for METs (sensitivity = 82%, specificity = 88%), 0.0033 g for accelerometry mean amplitude deviation (sensitivity = 80%, specificity = 91%), and 11.9% for EMG (sensitivity = 79%, specificity = 92%).



Duncan et al. used  $\text{VO}_2$  and triaxial accelerometry to assess physical activity in 30 8 to 11-year-old children (14 girls) while lying down supine, playing with Lego, walking on a treadmill at either 4.5 or 6.5 km/h, running on a treadmill at 6.5 km/h, overarm throwing and catching, instep passing a football, and cycling (35 W). Participants wore accelerometers on their non-dominant wrist, dominant wrist, waist, and ankle. Based on  $\text{VO}_2$  values, lying down supine and playing with Lego were categorized as sedentary activities ( $<1.5$  METs), walking and throwing and catching were light activities (1.51–2.99 METs), and running, cycling, and instep passing were moderate activities ( $>3$  METs). According to the ROC curve analysis, sedentary-to-non-sedentary and sedentary-to-moderately-non-sedentary activity discrimination were excellent for all accelerometer placements (with the best results being associated with the ankle accelerometer), even when cycling was neglected.

The protocol article by Petraconi et al. describes a child-friendly Go/No-Go paradigm to assess inhibitory control of the foot with a physical activity measurement methodology based on a dance mat, tested in 31 3 to 4-year-old children (17 girls). Go and No-Go stimuli were modeled within the context of a fishing game, and children's behavioral responses were assessed by recording the latency to touch the mat and the accuracy of the touches. The dance mat protocol can be used by researchers who are interested in the development of foot motor response inhibition in young children, including hand and foot specialization, exercise, and/or sport performance, and in neuroimaging (i.e., with fNIRS).

## MOTOR COMPETENCE

Coppens et al. followed the development of the motor competence of over 550 children over 2 years. Gender, BMI, age, and physical fitness measures (speed and explosivity) were cross-sectionally related to motor competence at the baseline, which is in line with findings presented in the literature published in past decades. From a longitudinal perspective, girls made less progress than boys in terms of motor competence during the 2-year study. Surprisingly, though, apart from BMI—which was negatively associated with MC development—none of the physical fitness measures affected the rate of development.

A study conducted by Hill et al. provides new insights into the development of a crucial component of motor competence, namely, dynamic postural control. Specifically, they studied the contribution of the upper extremities to the performance of three dynamic balance tests that were performed with free and restricted arm movements. Arm movements were clearly beneficial to performance on the Y-balance test and on a dynamic walking test. However, when participants were made to regain their stability on one leg after the execution of a jump, this advantage did not emerge. Apparently, the neuromuscular response strategies of the ankle, knee, and hip might be sufficient to effectively guide the transition from dynamic to static balance. This study sheds light on the underexplored role of the arms in maintaining postural balance and, therefore, might have implications for training and therapy.

Physical performance measures are assumed to support the development of general motor competence. González-Víllora et al. compared two methods of small-sided futsal games to improve physical (e.g., distance covered during the game) and physiological (e.g., heart rate) variables in elementary school children. They showed that the Contextualized Sport Alphabetization Model (CSAM; Kirk, 2017) was associated with higher scores regarding the physical and physiological aspects of small-sided futsal games when compared to the well-known Teaching Games for Understanding (TGfU) approach. The relevance of this study lies in underlining the value of the CSAM model for introduction in small-sided games, focusing on the development of cognition, technique, and social skills, while respecting the necessity that PE sessions at school should promote optimal involvement at the physiological level of each pupil.

Along similar lines of thinking, Garcia-Angulo et al. used principles of non-linear pedagogy by modifying games so that the level of play suited the children's developmental characteristics. The impact of changing the task and environment of the physical activity levels during the game was studied by measuring heart rate in young players between 6 and 12 years of age. The results show that these games led to a physical activity rate that meets the international PA recommendations favoring health benefits.

The quest to identify young athletes with the potential to excel in a particular sport is prominent in the scientific literature. Zhao et al. applied a test battery of 24 non-sport-specific tests on a squad of under-15 youth elite athletes in six different sports. The proposed test battery showed a medium-to-high validity for discriminating between basketball, fencing, judo, swimming, table tennis, and volleyball. In addition, the tests could discriminate between athletes from one sport and those from the other five sports with an acceptable level of accuracy.

Carvalho et al. studied the evolution of the physiological performance characteristics of youth female basketball players during the pubertal years. They discuss their findings with respect to calendar age (CA), biological age (BA, based upon age of menarche), and sports age (SA, exposure to training). They showed that the developmental rate of explosivity and speed/agility starts to level off at around the age of 14, although the models keep increasing linearly until 3 years after the age of menarche. Contrarily, a linear trend of improvement is observed when endurance and the overall performance index are aligned for CA and BA. It was observed that when the effects of maturation reach their end, all girls evolve in a similar way.

## OVERWEIGHT AND OBESITY

Given the many health-related correlates of overweight and obesity, the availability of reference values and validated cut-offs in different populations is crucial. In their study, López-Sánchez et al. assessed the prevalence of overweight and obesity in a sample of 1,000 children and adolescents in Italy and Spain. Overweight or obese status was determined by means of three different international references: BMI, according to the World Health Organization (WHO) International Obesity Task Force

(IOTF), and fat mass (FM), according to the Child Growth Foundation (CGF). The three classifications produced different levels of prevalence of overweight and obesity.

Overweight and obesity are generally considered obstacles to participation in physical activities and a healthy lifestyle. Pojskic and Eslami focused on the interrelationship between age, indices of overweight/obesity, physical activity, and cardiorespiratory fitness (CRF) in a sample of >750 children and adolescents in Bosnia and Herzegovina. Overall, CRF was associated with gender, age, indices of overweight/obesity, and PA levels. A worrisome finding was that over 80% of the participants were categorized as having low CRF, which puts them at risk for metabolic diseases. At the fundamental level, overweight status was not associated directly with CRF. Rather, old male participants with high levels of PA exhibited better measures of CRF than other participants.

While overweight and obesity are known to affect movement control and efficiency in tasks that demand the movement of large parts of the body, relatively little is known about how body fat percentage is related to mechanical efficiency during cycling at different intensities and how this might be associated with hormonal responses to exercise. To this end, Jabbour and Majed had male adolescents perform an incremental exercise test to exhaustion while measuring energy consumption, lipid oxidation rate, and concentrations of epinephrine and norepinephrine. As expected, mechanical efficiency decreased as weight increased. Interestingly, the authors show that the mechanisms of lower ME vary with intensity. At a low intensity, overweight and obese boys exhibited increased energy consumption, leading to lower ME. Meanwhile, at a higher intensity, lower ME is primarily explained by low power output and hormonal responses to exercise.

Sögüt et al. also compared central adiposity, cardiovascular fitness, and physical activity in children, revealing that moderate to vigorous levels of physical activity were negatively associated with body mass index and waist circumference and positively correlated with cardiovascular fitness in both sexes.

## PATHOLOGICAL SUBJECTS

A study conducted by Pianosi and Smith dealt with the ventilatory limitation of exercise in children with exertional dyspnea. The traditional method for evaluating ventilatory limitation considers breathing reserve (Pianosi and Smith). However, this approach is problematic in that it ignores maximal

voluntary ventilation (MVV). Therefore, Pianosi and Smith used multiples of the FEV<sub>1</sub> method. Their results reveals that evaluating ventilatory limitations in children using 30FEV<sub>1</sub> is superior to using MVV-based methods.

A study conducted by Rochette et al. measured the fat and carbohydrate oxidation rates of children with inactive JIA and healthy children (control group) using a submaximal incremental exercise test. The results show lower lipid oxidation rates and higher respiratory exchange ratio at 50% of VO<sub>2</sub> peak in the JIA group in comparison to the control group. However, there were no differences in heart rate or percentage of VO<sub>2</sub> peak at the maximal fat oxidation rate (MFO), and healthy subjects reached their MFO at a higher exercise power than JIA subjects did Rochette et al.

Regarding metabolic-related diseases, Dring et al. examined the risk factors for cardio-metabolic disease [e.g., multi-stage fitness test (MSFT), VO<sub>2</sub> peak, and adiposity] in children. Children were separated into quartiles based on the distance run in the MSFT. The worst-performing group had the highest blood IL-6 (3.25 ± 0.25 pg/mL) and IL-1β (4.78 ± 0.54 pg/mL) levels and the lowest concentrations of IL-10 (1.80 ± 0.27 pg/mL).

Cystic fibrosis (CF) is a serious genetic disease that typically affects the lungs and seriously reduces exercise performance (Shei et al.). In a review article authored by Shei et al. it is stated that few studies have provided evidence for physiological differences in CF related to exercise capacity through aging. Therefore, they recommend that exercise studies in CF should consider factors like pulmonary function declination, chronic airway colonization, endocrine comorbidities, and nutrition-related factors because these are age-specific factors that affect the exercise capacity of CF patients.

A study comparing 16 children after coarctation repair and 20 healthy control subjects revealed that children after coarctation repair exhibited meaningfully lower peak power and maximal oxygen uptake than healthy children (Vandekerckhove et al.). The amount of muscle tissue oxygenated was also meaningfully lower in patients (from 10 to 70% peak power output), and muscle deoxygenated hemoglobin was significantly higher in patients (from 20 to 80% peak power output).

## AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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# Relationship Between Obesity, Physical Activity, and Cardiorespiratory Fitness Levels in Children and Adolescents in Bosnia and Herzegovina: An Analysis of Gender Differences

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This study aimed to examine: (i) the level of physical activity (PA), obesity indices and cardiorespiratory fitness (CRF) among boys and girls in primary school, and (ii) to determine the association of obesity indices and PA with CRF for the total number of participants, and then separately for boys and girls. 753 sixth to ninth grade girls and boys aged 10–14 years took part in this cross-sectional study. The PA was assessed by the “Physical Activity Questionnaire – Children” and CRF was assessed by the Maximal multistage a 20 m shuttle run test. Body mass index (BMI), waist circumferences (WC), and waist to height ratio (WHtR) were considered as obesity indices. Multiple linear regression analyses were performed to explore correlates of CRF. The results obtained showed the prevalence of general overweight and obesity was 25.5% in our sample which was lower than that in the regional estimate (e.g., ~28%) for Eastern Europe. Among all participants, CRF was associated with male sex, older age, a lower WC percentile, higher WHtR, and higher level of PA. The model accounted for 24% of the variance. CRF was associated with older age and higher level of PA among girls and boys. Lower WC percentile was a significant determinant of CRF among boys. In conclusion, general overweight/obesity was not independently associated with CRF. Those with better CRF were more likely to be male and older, had a higher level of PA and lower central adiposity. These findings emphasize the importance of supporting school age children to take a part in programmed physical activity regardless of their body composition.

**Keywords:** BMI, overweight, shuttle run test, VO2max, waist circumference

## INTRODUCTION

Child and adolescent obesity is a significant health problem worldwide (Flodmark et al., 2004; Gomes et al., 2014) reaching epidemic proportions both in developed (Ogden et al., 2002; Gomes et al., 2014) and less developed countries (Ebbeling et al., 2002). The prevalence of general overweight and obesity has been reported to be between 20 and 45% in European children

(Lobstein and Frelut, 2003; Gomes et al., 2014, 2015) with a regional estimate of 28% for the eastern region of the European Union (Jackson-Leach and Lobstein, 2006). In the most recent report of the World Health Organization (2017), was shown that the prevalence of overweight and obesity continued to increase across most of European countries and regions in school children and adolescents (e.g., 11–15 years old) from 2002 to 2014. Furthermore, the report revealed differences in the prevalence between sexes (i.e., the average prevalence of 24% for boys and 14% for girls), between age groups (i.e., higher in younger age), and between the countries (i.e., the highest prevalence in southern European and Mediterranean countries) in 2014. However, the WHO's data was based on self-reported height and weight which could underestimate the truth data (Elgar and Stewart, 2008). Comparing to the regional estimate the situation in Bosnia and Herzegovina is somewhat better with the reported prevalence of ~21%, but it should be noted that the study was conducted with schoolchildren from only one canton in the country (Hasanbegovic et al., 2010). Unfortunately, the major concern with children obesity is that 80% of obese children become obese adults (Whitaker et al., 1997) and that overweight children and adolescents show an increased rate of mortality due to cardiovascular and digestive diseases in adulthood (Mossberg, 1989). Additionally, obese children are at increased risk to be diagnosed asthma at school age (Loid et al., 2015), they are more likely to have extremity fractures, to die of traumatic injuries than nonobese children (Kim et al., 2016) and to have a lower cardiorespiratory fitness (CRF) in childhood (Tuan et al., 2018) and more than 10 years later (Pahkala et al., 2013).

In that regard, there are several indices which are frequently used to assess obesity in children, including body mass index (BMI), waist circumference (WC), and waist to height ratio (WHtR). Although BMI has been commonly used as a sensible indicator of overall adiposity in children (Ogden et al., 2002), it seems that BMI has limitations in assessing the prevalence of age- and sex-specified obesity as well as body composition and fat distribution (Savva et al., 2000). On the contrary, WC and WHtR have been reported to be stronger indicators of central obesity and better predictors of cardiovascular disease risk factors (e.g., high levels of plasma lipids, lipoprotein levels) in children (Savva et al., 2000). In this regard, all these indicators of obesity are recommended to be used in epidemiological studies as they are low-cost and simple to measure.

Furthermore, it has been suggested that a low level of physical activity (PA) is one of the main cause of children's obesity (Page et al., 2005) and worldwide the forth risk factor related with mortality (World Health Organization, 2009). However, obesity may cause physical inactivity and not to be a consequence (Metcalf et al., 2011). Physically active children compared to inactive children are less susceptible to the risk factors of chronic diseases and obesity, and they are also more likely to stay physically active with a higher level of CRF during adolescence and adulthood (Janz et al., 2000). Even though it was assumed that PA and CRF are, similarly, related to obesity indicators, some previous studies have reported inconsistent findings suggesting that CRF is more related to abdominal adiposity (measured by WC) compared to the level of PA

(Ortega et al., 2007). Additionally, it is shown that the level of CRF in childhood and adolescence is a better predictor of cardiovascular diseases (CVD) in adulthood compared to the level of PA *per se* (Janz et al., 2002), so it is recommended that the both correlates should be permanently and independently monitored in children (Esmailzadeh et al., 2013). However, comparing to anthropometric indicators of obesity (BMI, WC, WHtR), some studies reported CRF to be weaker predictor of CVD in children (Goncalves et al., 2015), which emphasize the importance of concurrent assessment of all three predictors (i.e., anthropometrics, PA and CRF) of CVD whenever is possible.

Moreover, studies frequently report inconsistent results on the prevalence of obesity in boys and girls, as well as a gender specific association between obesity indicators, PA and CRF levels in children (Al-Nakeeb et al., 2007; Ostojic et al., 2011). In that regard continuous monitoring of these items is very important for researchers and health care providers in order to develop adequate and gender specific obesity prevention and intervention strategies (Esmailzadeh et al., 2013). To our knowledge, there is only one study that systematically investigated the prevalence of overweight and obesity, and PA level in school-age children (i.e., 6–15 years) from Bosnia and Herzegovina (Hasanbegovic et al., 2010). However, even though the study provides valuable data, it involved only children from one part of the country (i.e., Sarajevo canton) and did not investigate the predictors of CRF. Moreover, the aforementioned WHO's report (World Health Organization, 2017) did not include data from Bosnia and Herzegovina. To fill the gap, current study aims were (i) to separately examine the level of PA, obesity indices and CRF among boys and girls in primary school, and (ii) to determine the association of obesity indices and PA with CRF for the total number of participants, and then separately for boys and girls. We hypothesized that children with a normal weight and higher level PA would have a higher level of CRF.

## MATERIALS AND METHODS

### Study Design, Participants, and Procedure

This cross-sectional study recruited 753 sixth to ninth grade school children aged 10–14 years between February and May 2015. With an assumed prevalence of obesity and overweight of 25% and precision of 0.05, the calculation indicated that the required sample size was around 288 per group (Pourhoseingholi et al., 2013). Geographically stratified random sampling was used. Ninety-seven primary schools were identified from four main and biggest urban areas in the Federation of Bosnia and Herzegovina (i.e., Sarajevo, Tuzla, Zenica, and Mostar). Rural schools that belong to the named municipalities were not included in the selection process to avoid the potential socio-economic differences between the areas. Eight schools were randomly selected from each of the four areas for the study. From each selected school, one randomly selected class (sixth to ninth grade) was included in the study with the final number of schools (classes) being 32 (4 areas × 8 schools/classes). Eight classes per grade were included in the study to maintain the proportion of

grades, age and gender. A total number of 1025 students from 32 classes were invited to participate in the current study. The randomization process of the identified schools and classes was generated by the Excel 2003. Socio-economic data of the selected urban areas and schools were not collected and considered in the current study.

Principals and physical education teachers of the schools were informed about the study aim and methodology and once they agreed for their schools to take part in the study, an informative letter and medical history questionnaire were distributed to parents or guardians and a short briefing session was organized for interested parents at the schools. Written informed consent was received from all children and their parents or guardians after a detailed verbal and written explanation of the purpose of the study, experimental design, testing protocols, research benefits and potential risks of the study were provided to them. Children were informed that they were free to withdraw from the study at any time without consequences. Only children who provided their own and parents' or guardians' written informed consent were included in the study. The study was approved by the Tuzla University Ethics Committee (02/11-2842/14-3) and conformed to the principles of the Declaration of Helsinki on human experimentation (World Medical Association, 2013).

Only healthy children aged 10–14 years old were included in the study. Those who reported that they used any medication or had a history of neuromuscular or heart disease or injuries, and or had any limitations in PA for the previous 6 months, were excluded from the study. Children who were regularly involved in some kind of sports activity were asked to refrain from this training and to avoid sleep deprivation for at least 2 days prior to the testing sessions. The participants were asked to consume a light meal at least 3 h prior to the beginning of testing (i.e., VO<sub>2</sub>max testing) and to make sure that they were properly hydrated before and during testing.

## Data Collection

All data collection for one child was conducted in 1 day in three separate stages between 8 and 12 am. First, the level of PA was evaluated by a self-reported questionnaire and took place in the schools' classrooms. The questionnaire sheet was given to each child with clear instructions on how to complete it by an experienced research staff member. The children were instructed to independently fill in the questionnaire within 30 min time framework. The same researcher supervised the data collection in the classroom. Afterward, all anthropometric measurements were taken in the changing rooms of the gym. In the final phase, the maximal oxygen uptake was estimated for each child in the schools' gyms. The measurements were conducted in the gender-specific groups of 12–15 children.

## The Level of Physical Activity

To evaluate the level of PA during the children's leisure time, the Physical Activity Questionnaire–Children (PAQ-C) was used (Crocker et al., 1997; Kowalski et al., 1997). The self-reported questionnaire was developed to assess levels of moderate to vigorous PA in children from grade 4 upward. It showed to be reliable and valid tool in assessing level of activity in children

(Crocker et al., 1997; Janz et al., 2008). The questionnaire consists of nine questions (items) specifically evaluated on a 5-point Likert type scale, with higher scores indicating a higher level of PA. The mean of the nine items was used in the calculation of the summary of total activity which is the composite score that can range from 1 to 5 (Kowalski et al., 1997). To overcome the language barrier, we used the Croatian translated version of PAQ-C that was understandable for the selected participants in Bosnia and Herzegovina speaking area too. The version showed satisfactory internal consistency value (0.80) in assessing the level of PA in children (Samarzija and Misigoj-Durakovic, 2013).

## Anthropometric Data

To estimate the children's anthropometric characteristics and obesity indices, the subjects were asked to remove their shoes and socks and to be with underwear only. All measurements were taken by the same trained operator following standard procedures. The following anthropometric variables were measured: body height (BH), body weight (BW), waist circumference (WC). Based on these measures, we calculated the BMI for each child [body weight (kg)/body height squared (m<sup>2</sup>)], BMI percentile, and WHtR.

Body height was measured to the nearest 0.01 m with a portable stadiometer (Astra scale 27310, Gima, Italy). Body mass was measured using a bioelectric body composition analyzer (Tanita TBF-300 increments 0.1%; Tanita, Tokyo, Japan). To define underweight, overweight and obese according to BMI, the 5th, 85th and 95th BMI for age, the Centers for Disease Control and Prevention (CDC) reference percentiles were used (Ogden et al., 2002; Goncalves et al., 2015). Waist-circumference (WC) was measured using a flexible measuring tape at the midpoint between the superior edge of the iliac crest and the inferior border of the ribcage, with the average of three measurements used in analysis (Goncalves et al., 2015). WC and WHtR were used as indirect measures of the amount of abdominal fat. For the WC, values above 85th percentile (Taylor et al., 2000) and a cut off of 0.5 for WHtR (McCarthy and Ashwell, 2006) have been used to identify central obesity in children.

## Cardiorespiratory Fitness

Maximal aerobic power (VO<sub>2</sub>max) as a CRF level indicator was estimated by the Maximal Multistage 20 Meter Shuttle Run Test (Leger and Lambert, 1982). The test consisted of a shuttle running at a pre-set pace by the shuttle run test protocol and played on a CD recorder. In the test, the participant ran 20 m long shuttles after a signal was sounded. At the start of the test, the participant had to run at a speed of 8 km/h to reach the opposite line before another signal was given. The running speed increased every minute by 0.5 km/h. When the participants were unable to maintain the pace, that is when they failed to reach the lines with the audio signals on 2 consecutive occasions, or when they stopped because of fatigue, the last shuttle covered was used to estimate the maximal oxygen uptake (VO<sub>2</sub>max). The VO<sub>2</sub>max was estimated from the following equation: VO<sub>2</sub>max = 3.46 \* (L + NS/(L \* 0.4325 + 7.0048)) + 12.2, where L is the reached level and NS is a number of shuttles covered at the respective level. The test has shown to be a valid and reliable tool for the evaluation

of maximal aerobic power in children (Van Mechelen et al., 1986). The participants were asked to perform 7 min running based warm-up at low intensity. They started with the test 2 min after the warm-up. The test was performed in groups of 12–15 children. Verbal encouragement was provided for all participants during the test.

## Statistical Analyses

Categorical variables (e.g., gender) were presented as absolute frequencies and percentages, while continuous variables by mean and standard deviation. In bivariate analyses, the independent *t*-test and the Mann–Whitney *U*-test were used to compare girls and boys in continuous and ordinal variables, respectively. Differences across the age groups and body weight categories (based on BMI percentiles) were assessed using one-way ANOVA for continuous variables (e.g., VO<sub>2</sub>max) and the Kruskal–Wallis test for ordinal variables (e.g., PA level). When statistically significant difference between the groups was detected, pairwise comparisons were performed using a Bonferroni *post hoc* test to investigate where the difference lies. We performed multiple linear regression analyses to determine whether there was any association between independent variables (i.e., obesity indices and PA) and CRF (dependent variable) once for the total sample, and then separately for girls and boys controlling for other potential confounders (e.g., age). Altogether three multiple regression models were calculated. The data was presented in the form of coefficient ( $\beta$ ), 95% Confidence Intervals, and *p*-values.

The significance level for all statistical tests was set at  $p \leq 0.05$ . All statistical analyses were completed with Microsoft Excel (2003) and PASW statistic package 24.0 (IBM/SPSS Inc., Chicago, IL, United States).

## RESULTS

After the selection process, a total of 1025 students from 32 classes aged 10–14 years were invited to participate in this study, of whom 753 (361 girls and 392 boys) consented and were enrolled which resulted in a 73.5% participation rate. About 48% of participants were girls.

### Girls vs. Boys: Obesity Indices, Physical Activity, and Cardiorespiratory Fitness

The prevalence of general overweight and obesity (based on BMI) was 25.5% while the prevalence of central obesity (based on WC) was 16% among the total sample. As shown in **Table 1**, about 16% of girls and 18% of boys were overweight. Whereas, obesity was observed among 6.4% of girls and 10.9% of boys ( $p = 0.038$ ). The prevalence of central obesity (waist circumference percentile  $\geq 85\%$ ) was 19% and 13% among boys and girls, respectively ( $p = 0.029$ ). Compared to boys, girls were shorter ( $p < 0.001$ ) with a smaller waist circumference ( $p = 0.016$ ), and had a lower level of PA ( $p < 0.001$ ). Moreover, girls scored lower than boys in CRF ( $p < 0.001$ ). Otherwise, there were no differences in weight, BMI percentile, and weight to height ratio between girls and boys.

**TABLE 1** | Students' *t*-test and chi-square tests comparing age, anthropometric data, obesity indices, physical activity, and fitness among primary school age girls and boys (a total sample).

Variables	Girls		Boys		<i>p</i>
	Mean (SD)	n (%)	Mean (SD)	n (%)	
Total		361 (47.9)		392 (52.1)	
Age (years)	12.47 (1.06)		12.38 (1.09)		0.236
Height (meters)	1.60 (0.08)		1.63 (0.11)		<0.001
Weight (Kg)	53.27 (11.68)		54.96 (13.90)		0.074
BMI-percentile	59.75 (27.45)		58.22 (30.88)		0.473
BMI categories <sup>a</sup>					0.075
Under-weight		12 (3.3)		14 (3.6)	
Normal		268 (74.2)		265 (67.6)	
Over weight		58 (16.1)		70 (17.8)	
Obese		23 (6.4)		43 (10.9)	
Waist circumference (cm)	73.35 (8.90)		75.06 (10.33)		0.016
Central obesity <sup>b</sup>		47 (13.0)		74 (18.9)	0.029
Waist to height ratio	0.46 (0.05)		0.47 (0.28)		0.278
Level of PA	2.91 (0.63)		3.16 (0.65)		<0.001
Normal <sup>c</sup> – level of PA	3.33 (0.43)	207 (57.3)	3.48 (0.44)	280 (71.4)	
Low <sup>d</sup> – level of PA	2.34 (0.34)	154 (42.7)	2.37 (0.34)	112 (28.6)	
Cardiorespiratory fitness <sup>e</sup>	32.08 (4.85)		35.86 (6.46)		<0.001
Normal <sup>f</sup> – cardiorespiratory fitness	39.75 (3.12)	62 (17.2)	46.3 (2.91)	67 (17.1)	
Low <sup>g</sup> – cardiorespiratory fitness	30.49 (3.41)	299 (82.8)	33.71 (4.63)	325 (82.9)	

SD, standard deviation; n, number; BMI, body mass index. <sup>a</sup>Underweight, overweight and obese according to the 5th, 85th and 95th BMI percentiles. <sup>b</sup>Waist circumference percentile  $\geq 85\%$ ; PA, physical activity (1–5 point scale). <sup>c</sup>PA  $\geq 2.73$ . <sup>d</sup>PA  $\leq 2.73$ . <sup>e</sup>based on VO<sub>2</sub> max (ml · kg<sup>-1</sup> · min<sup>-1</sup>). <sup>f</sup>VO<sub>2</sub>max  $\geq 37.0$  (for girls) and VO<sub>2</sub>max  $\geq 42.1$  (for boys). <sup>g</sup>VO<sub>2</sub>max  $\leq 37.0$  (for girls) and VO<sub>2</sub>max  $\leq 42.1$  (for boys).

There was a significant increase in VO<sub>2</sub>max across age groups for boys  $F(4, 387) = 9.1, p < 0.005, \eta^2 = 0.086$ . VO<sub>2</sub>max increased from age 10 ( $30.27 \pm 3.83$ ), to age 11 ( $34.66 \pm 6.48$ ), to age 12 ( $34.67 \pm 4.79$ ), to age 13 ( $36.45 \pm 7.15$ ), and to age 14 ( $39.15 \pm 6.20$ ). The same pattern was observed among girls, but without significant differences observed between the age groups ( $p = 0.063$ ). Boys showed to have significantly higher CRF comparing to girls in age groups 11–14. The mean differences between them increased with the age. At age 11 it was  $3.08 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  (95% CI, 1.19–4.96),  $t(144) = 3.23, p = 0.002$ , at age 12 it was  $2.91 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  (95% CI, 1.60–4.21),  $t(209) = 4.04, p = 0.00$ , at age 13 it was  $4.41 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  (95% CI, 2.83–6.00),  $t(238) = 5.49, p = 0.00$ , and at age 14 it was  $5.85 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  (95% CI, 3.77–7.63),  $t(132) = 5.85, p = 0.00$  (Figure 1).

Boys who were classified in normal and overweight body weight group showed to have a higher VO<sub>2</sub>max ( $36.81 \pm 6.56$  and  $35.28 \pm 5.31$ , respectively) comparing to those who were in underweight and obese group ( $34.88 \pm 7.13$  and  $31.27 \pm 5.01$ , respectively),  $F(3, 388) = 9.9, p < 0.001, \eta^2 = 0.071$ . There were not significant differences in VO<sub>2</sub>max between the body weight categories in girls ( $p > 0.05$ ). Boys from normal and overweight category had higher VO<sub>2</sub>max ( $36.81 \pm 6.56$  and  $35.28 \pm 5.31$ , respectively) comparing to girls ( $32.13 \pm 4.86$  and  $31.74 \pm 4.09$ , respectively), while there were not significant differences between sexes in obese and underweight group (Figure 2).

PA level significantly decreased across age groups in girls ( $p = 0.010$ ), but not in boys ( $p = 0.086$ ) (Figure 3). *Post hoc* test did not reveal any significant pairwise differences among age groups in girls (Figure 3). Boys showed to be significantly more physically active than girls in age 12 and 13 ( $p = 0.00$  and  $p = 0.01$ , respectively). Boys who were classified in normal body weight

group showed to have higher level of PA comparing to those who were in underweight, overweight and obese group,  $\chi^2(3) = 9.29, p = 0.026$  (Figure 4). Girls did not showed significant differences in PA level across the body weight categories,  $\chi^2(3) = 1.97, p = 0.579$ . Boys from normal and overweight category were physically more active comparing to girls ( $p < 0.01$ ), while there were no significant differences between them in obese and underweight group ( $p > 0.05$ ) (Figure 4).

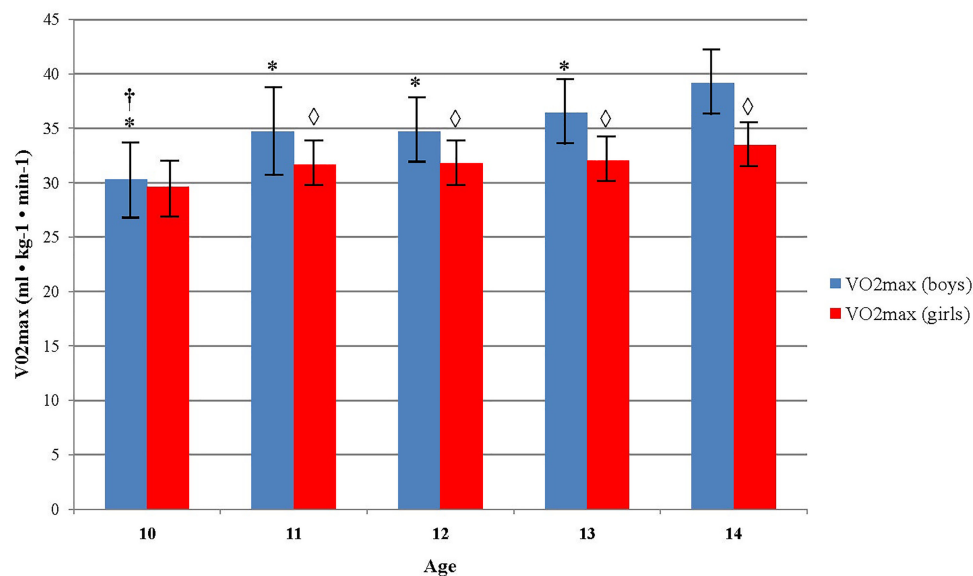
## Factors Associated With Cardiorespiratory Fitness

Visual inspection of a normal probability plot showed that residuals were normally distributed. Analyses among all participants ( $n = 753$ ) revealed that better CRF was associated with male sex ( $p < 0.001$ ), older age ( $p < 0.001$ ), lower waist circumference percentile ( $p = 0.034$ ), higher weight to height ratio ( $p = 0.045$ ) and higher level of PA ( $p < 0.001$ ). The model accounted for 24% of the variance of CRF.

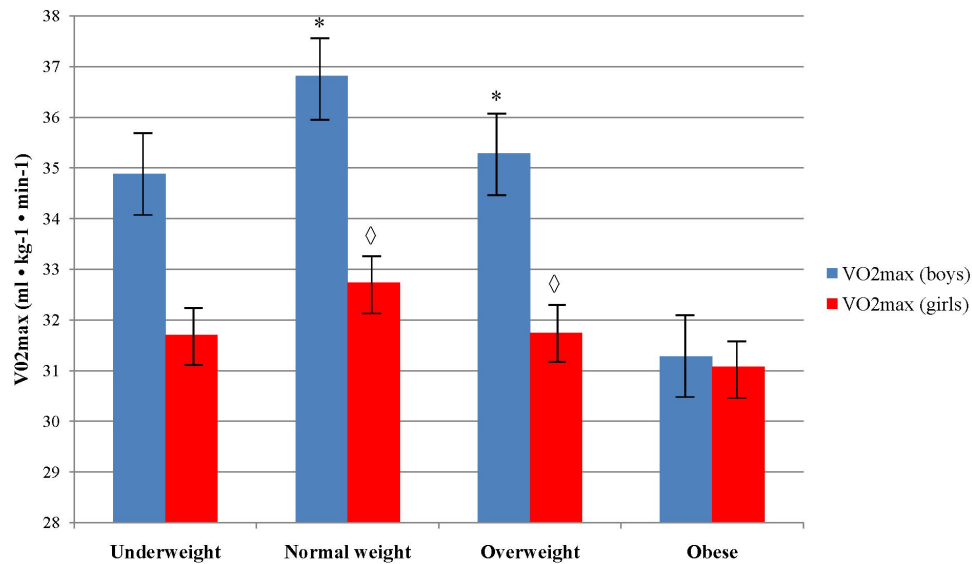
As shown in Table 2, CRF was associated with older age and a higher level of PA among primary school age girls and boys. For boys, having lower waist circumference percentile was also a significant determinant ( $p = 0.048$ ). The model could explain 22% of the variance of CRF among boys and 10% among girls, respectively.

## DISCUSSION

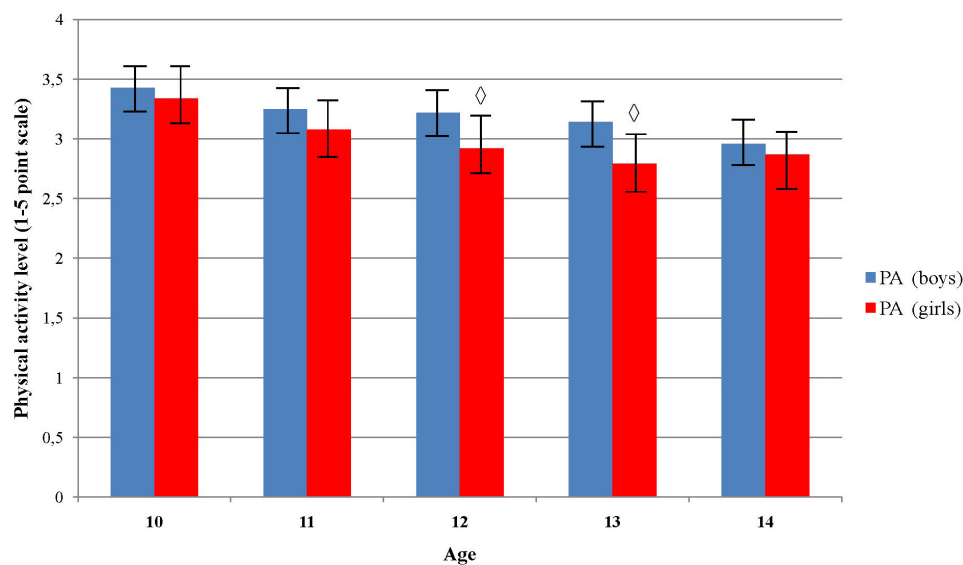
The aim of the present study was twofold. Firstly, it attempted to separately examine the level of PA, obesity indices and CRF among boys and girls in primary school, and secondly to determine the association of obesity indices and PA with



**FIGURE 1** | Differences in aerobic fitness level across age and sex. \*Indicates values significantly different from those obtained in 14 years old boys at  $p < 0.05$ ; †Indicates values significantly different from those obtained in 13 years old boys at  $p < 0.05$ ; °Indicates significant differences between boys and girls in accompanied age group at  $p < 0.05$ .



**FIGURE 2** | Differences in aerobic fitness level across body weight categories and sex. \*Indicates values significantly different from those obtained in obese weight category among boys at  $p < 0.05$ ; ◊Indicates significant differences between boys and girls in accompanied body weight category at  $p < 0.05$ .

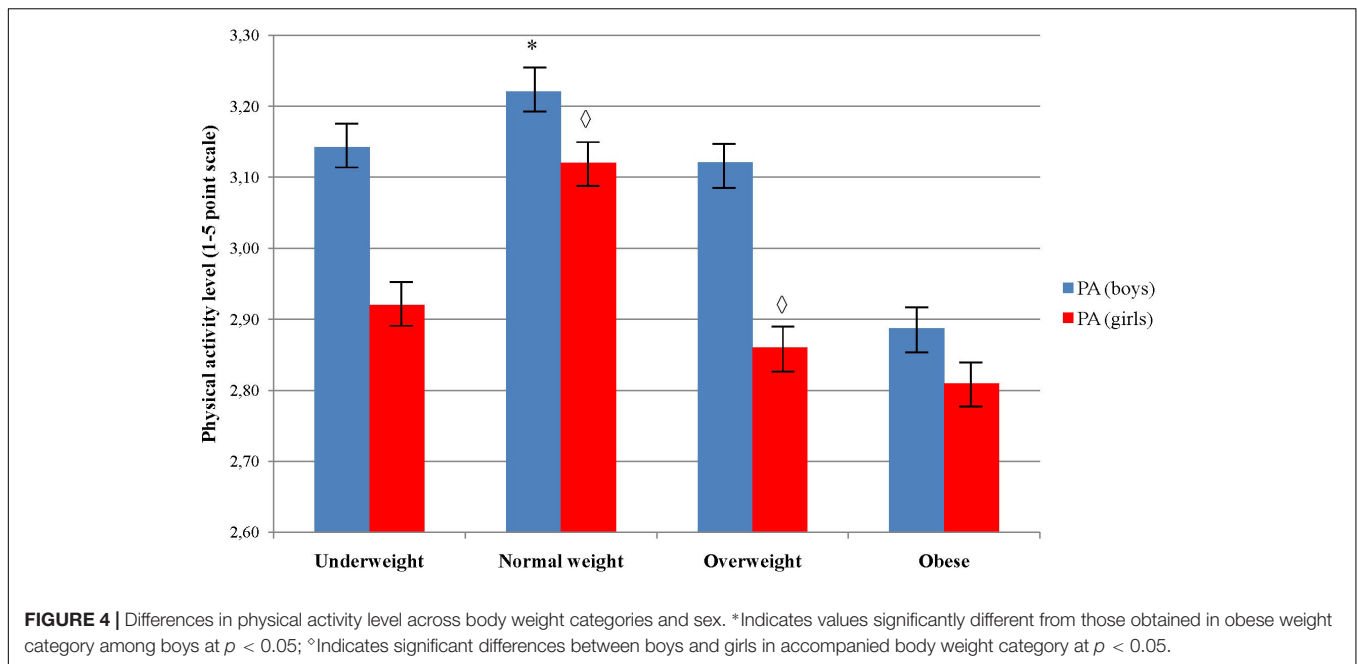


**FIGURE 3** | Differences in physical activity level across age and sex. ◊Indicates significant differences between boys and girls in accompanied age group at  $p < 0.05$ .

CRF for all participants and then separately for boys and girls. The results revealed several important findings that should be emphasized here. First, even though the results showed relatively low prevalence of overweight/obesity and relative a high number of children who met recommended PA time, it is a worrying fact that more than 80% of them had a low CRF which put them at high metabolic risk. Secondly, although boys were more frequently obese than girls, girls had a lower level of PA and CRF. Thirdly, sex, age, PA level and central adiposity showed to be independent determinants of CRF among school age children.

Before discussing these findings, we will provide a brief overview of the established prevalence. The prevalence of general overweight and obesity was 25.5% in our sample which was lower than that in the regional estimate (e.g., ~28%) for Eastern (Jackson-Leach and Lobstein, 2006) and 30.3–45% Southern Europe (Gomes et al., 2014), but higher than it was reported for schoolchildren from Sarajevo Canton in Bosnia and Herzegovina (21%) (Hasanbegovic et al., 2010). However, Ostojic et al. have reported an even higher prevalence of obesity (e.g., ~39%) for Serbian school age children from 6 to 14 years (Ostojic et al., 2011). The central obesity, which was shown to be correlated with





**TABLE 2 |** Factors associated with cardiorespiratory fitness among all participants ( $n = 753$ ) and separately for girls ( $n = 361$ ) and boys ( $n = 392$ ) in multiple linear regression analyses.

Independent variables	Total sample		Girls		Boys	
	$\beta$	95% CI	$\beta$	95% CI	B	95% CI
Sex						
Boys						
Girls	-0.28	-4.12 to -2.54***				
Age	0.30	1.23-2.08***	0.22	0.49-1.54***	0.39	1.64-2.94***
BMI percentile	-0.07	-0.03 to 0.01	0.01	-0.02 to 0.03	-0.10	-0.05 to 0.01
Waist circumference percentile	-0.16	-0.06 to -0.002*	-0.15	-0.07 to 0.01	-0.20	-0.09 to 0.00*
Waist to height ratio	0.14	0.001-0.06*	0.16	-0.01 to 0.06	0.16	-0.00 to 0.08
Physical activity level	0.30	2.21-3.42***	0.29	1.44-2.98***	0.32	2.32-4.13***
Total $R^2$		0.243		0.105		0.220

CI, confidence interval; BMI, body mass index;  $\beta$ , a standardized beta coefficient;  $R^2$ , the coefficient of determination; \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .

CVD risk factors and unfavorable metabolic profiles in children and adults (Savva et al., 2000), was observed among 16% of the current sample. Our results seem to suggest that even though overweight and obesity among Bosnian children, is somewhat lower than in other developing/developed countries (Jackson-Leach and Lobstein, 2006; Ostojic et al., 2011; Gomes et al., 2014, 2015), it is still high and should be considered as a potential public health concern. This should be taken into account by policy makers in Bosnia and Herzegovina investing in health education of children, to help these children maintain an appropriate weight to decrease adverse health effects in adulthood.

In the current study, girls had lower values in obesity indices than boys, which was in accordance with some previous studies (Ortega et al., 2007; Gomes et al., 2014, 2015; World Health Organization, 2017; Tuan et al., 2018) and at odds with others (Al-Nakeeb et al., 2007; Ostojic et al., 2011; Dencker et al., 2012). Lower obesity indices among girls in this age range may

not necessarily pertain to their healthier lifestyle as our results showed that girls had a lower level of PA and CRF compared to boys across all age groups with a more prominent difference in age of 12 and 13. Likewise, Gomes et al. (2017) have reported that boys participate in more PA than girls with a trend of decreasing the level of PA being more rapid in girls compared to boys in the 11-13 year age group (Armstrong et al., 2000). Furthermore, one could argue that our findings could be related to girls' nutritional status, as the girls at this age might be more concerned about their body image (Mond et al., 2011). This is an issue which also needs to be considered in studies assessing health behavior among school age children. However, the differences in age range, ethnicity and socio-cultural factors may likely explain the inconsistent results in the above mentioned studies.

Our findings were in agreement with research revealing that school age boys performed better in the maximal multistage fitness test across all age groups and therefore they had a higher

estimated VO<sub>2</sub>max (Ruiz et al., 2007; Ostojic et al., 2011; Dencker et al., 2012; Tuan et al., 2018). PA level, body composition (greater muscle mass), cardiac size and function as well as mechanical efficiency (e.g., larger levers) may explain the gender differences in cardiovascular fitness among children (Rowland et al., 2000; Manna, 2014). Moreover, in the current sample, based on suggested cut-off values (2.73) for PAQ-C (Benítez-Porres et al., 2016), a higher percentage of girls 43.2% ( $N = 156$ ) did not meet recommended MVPA (moderate-vigorous PA) time (>60 min) (Strong et al., 2005) comparing to the boys, 28.8% ( $N = 113$ ), which is in line with some recently published studies (Gomes et al., 2015, 2017; World Health Organization, 2017). In this context, the discrepancy in MVPA between the sexes could additionally explain their differences in CRF too.

Furthermore, it is reported (e.g., Ostojic et al., 2011; Esmaeilzadeh et al., 2013; Tuan et al., 2018) that there is a negative correlation between central obesity (WC and WHtR) and cardiovascular fitness which is in line with our findings. Those children with higher central obesity have shown to have lower CRF estimated by using the maximal multistage 20 m shuttle run test. The negative relationship can be explained by the test's "stop and go" nature that continuously required subjects to accelerate and decelerate while overcoming their body inertia which is more demanding in obese subjects who have more fat as a nonfunctional, ballast mass (Katic, 1996). On the other side, there was not a difference in VO<sub>2</sub>max between boys who belong to the normal and overweight group (estimated by BMI) which possibly indicates a higher level of fat free mass in the overweight group and point out the limitation of BMI to differentiate fat and muscle mass in children (Vanderwall et al., 2017). This was a pattern which was observed only among boys in our study. The multivariate analyses of our sample showed that none of the obesity indices were independently associated with CRF among girls. On the contrary, the higher level of PA was positively associated with better CRF in the both groups which is consisted with other contemporary data on school age children (Esmaeilzadeh et al., 2013; Chen et al., 2018). Namely, Chen et al. (2018) showed that fifth-grade students, both girls and boys, who had bigger total PA time and were more engaged in organized PA had better developed CRF and muscular endurance. In particular, our findings emphasize the importance of encouraging school age children to do PA independent of their level of obesity or weight, as PA directly improves their physical fitness that is associated to many health-related benefits at all ages (Ortega et al., 2008; Chen et al., 2018). Moreover, comparing to WC, BMI has not been correlated to CRF which emphasize the importance of regular measurements of central obesity. This finding confirms the importance of WC measurement that has been proved to be a stronger predictor of cardiovascular disease risk factors (Savva et al., 2000) than BMI and emphasize beneficial effects of CRF on children health.

Furthermore, the recent studies showed a negative relationship between PA, MVPA and obesity indices (e.g., BMI, WC, body fat percentage) (Gomes et al., 2015, 2017). Unfortunately, it was reported that only few children achieve the sustained period of moderate to vigorous PA (Al-Nakeeb et al., 2007; World Health Organization, 2017), recommended by

guidelines to maintain good health (World Health Organization, 2006; Tremblay et al., 2011), and the situation is even worse among overweight and obese children (Dorsey et al., 2011). In the current sample, even though 64.3% of children met the recommended PA time, only 17.1% ( $N = 67$ ) boys and 17.2% ( $N = 62$ ) girls were at a hypothetically low metabolic risk according to established cut-off values for VO<sub>2</sub>max level of 37.0 and 42.1 ml/kg/min in girls and boys, respectively (Ortega et al., 2008). In this regard, only advising or educating the children to do PA is not equivalent to doing an effective activity leading to better physical fitness. Thus, obesity preventive interventions should focus on designing physical activities that would increase MVPA time (e.g., high-intensity interval based activities) which has been proved to improve children's physical fitness effectively (Racil et al., 2016) and reduce obesity indices such as BMI scores (Bingham et al., 2013; Trinh et al., 2013; Burke et al., 2014). Another key point to remember is that obesity may cause physical inactivity and not to be a consequence (Metcalf et al., 2011). This can lead to a vicious circle. That is to say, physical inactivity may negatively affect skills acquisition and execution required for many sport and physical activities which, as a result, has less self-confident children who are discouraged from being a part of such activities and consequently are at higher risk to gain a weight and lower fitness level (Pietiläinen et al., 2008; Esmaeilzadeh et al., 2013; Pahkala et al., 2013).

We have found that the older age was associated with better cardiovascular fitness, which is in line with older (Houlsby, 1986) and recent studies (Tuan et al., 2018) that showed that maturation positively affected CRF in children. The results could pertain to the growth, increase in muscle mass, strength and mechanical efficiency, and maturation of the cardiovascular system (Rogol et al., 2000; Manna, 2014). Or simply, we can speculate that those who were older could perform multistage fitness test more precisely. Contrary, one may suggest that by aging, children may have a better self perception and perception of health and health behavior benefits (Strong et al., 2005) which in turn can lead to the higher level of PA and CRF, respectively. However, we found the level of PA decreased progressively with age for both boys and girls, but more rapidly in girls at age 12 and 13 years, which was in line with a study by Telama and Young showing a remarkable decline in frequency of PA after the age of 12 (Telama and Yang, 2000).

## Limitations

This study has provided new insights into the physical health of school age children in Bosnia and Herzegovina. The strengths of the study were a relatively large sample size which was selected randomly from four urban areas in the Federation of Bosnia and Herzegovina, implying that this sample may represent the entire urban population of students at age 11–14 years. However, the study has several limitations which must be acknowledged. The cross-sectional characteristic of the data does not allow the establishment of firm causal links. The optimal study design to predict the CRF from PA and obesity indices would be a longitudinal follow-up research design. Moreover, the level of PA was assessed by a subjective measure. Even though the questionnaire PAQ-C is widely used and has a good reliability

and validity (Kowalski et al., 1997; Janz et al., 2008; Samarzija and Misigoj-Durakovic, 2013), no objective measures were taken to verify students' responses. Furthermore, PAQ-C was originally developed to assess general levels of PA and does not provide information regarding the caloric expenditure, frequency, time, and intensity of the activity. Although, the age of the selected sample was likely to differ in the maturation, we did not assess the pubertal stage which somewhat hindered us in clarifying and interpreting our data regarding sex differences in obesity, PA and CRF that we have observed. Moreover, we did not directly measure body fat percentage, but we used BMI to categorize the children in different body weight groups (e.g., overweight and obese) which could lead to unreliable data in terms of the accurate estimation of children's body composition and the group affiliation. Additionally, these results may not be generalizable to school age children living in rural areas too. Moreover, the amount of explained variance in the models was small and yet more not so important because a lot of variance in the model might be explained by the confounding variables that were not controlled. It indicates that further investigations should include other possible confounding variables (e.g., socio-cultural and ethno-religious characteristics, nutritional behavior, screen time, sedentary time, maturation etc.), which in return could explain the roots of the obesity indices and PA level in the sample and thus reveal their cause-and-effect relationship with the CRF.

## CONCLUSION

In conclusion, even though the results showed relatively low prevalence of overweight/obesity (comparing to regional estimate) and relative a high number of children who met recommended PA time in the current study, it is a worrying fact that more than 80% had a low CRF that put them at high metabolic risk. Moreover, this study demonstrated that general overweight/obesity was not independently associated with CRF. We observed that those with better CRF were more likely to be male, to be older and to have a higher level of PA. Therefore, it is

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important to support school age children, especially girls, to take a part in programmed PA regardless of their body composition.

Furthermore, elementary schools appear to be an ideal environment to implement a holistic obesity preventive strategies with physical education (PE) being the best tool in keeping children physically active during a day (Burke et al., 2014). In light of our study, this is even more important, when we know that children who participated in the present study had PE only two times a week for 45 min due to the school curriculum in Bosnia and Herzegovina, which is definitely not sufficient for inducing a health related benefits and combat against obesity. This should be taken into account by policy makers investing in physical and health education of children, to help these children maintain an appropriate weight to decrease adverse health effects in adulthood. Preventive strategies should target two main causes of children obesity: poor diet (increased energy intake) and a lower level of PA (decreased energy expenditure) (Anderson and Butcher, 2006).

## DATA AVAILABILITY

The datasets for this study can be found at: <https://www.dropbox.com/s/2grbsuoawn005hd/Data-primary-school%20-%20DROPBOX.xls?dl=0>.

## AUTHOR CONTRIBUTIONS

HP conceptualized and designed the study and organized and supervised data collection. BE undertook the data analysis and interpretation. HP and BE led together the writing of the paper.

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# Aerobic Capacity Is Related to Multiple Other Aspects of Physical Fitness: A Study in a Large Sample of Lithuanian Schoolchildren

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This study evaluated how aerobic capacity is related to performance in other aspects of health-related physical fitness among schoolchildren. The study involved >15,200 schoolchildren of both genders aged 11–18 years, who were tested with a reliable tests from Eurofit battery for most important aspects of exercise capacity and anthropometrics from 1992 to 2012. The analysis showed that aerobic capacity was weakly but significantly positively related to all other aspects of exercise abilities tested in all age groups for both genders. Variance of performance in agility shuttle run and standing broad jump were each explained by aerobic capacity the strongest (>10%), followed by weaker but still significant positive relation of aerobic capacity with the abilities in bent arm hang and abdominal curl tests (aerobic capacity explaining ~6.5% of the variance of the performance in these tests), as well as in balance and flexibility tasks (aerobic capacity significantly explaining ~3% of the variance). Thus, while aerobic capacity in schoolchildren of all ages and both genders can explain the performance in other aspects of physical fitness and especially leg muscle power, the percent of explained variance in the results of any these tests was not high and therefore aerobic capacity should be tested as a separate important fitness parameter which cannot be substituted by other tests from the Eurofit battery.

**Keywords:** children, adolescents, testing, musculoskeletal fitness, motor fitness, cardiorespiratory capacity

## INTRODUCTION

Health-related physical fitness is a multifactorial construct that encompasses cardio-respiratory (aerobic) capacity, muscular strength, speed/agility, balance and flexibility components (Huang and Malina, 2002). The level of physical fitness harbors important information about current and future cardio-vascular, skeletal and mental health (Catley and Tomkinson, 2013). In particular, aerobic capacity, which is the highest amount of oxygen consumed during maximal exercise in activities that uses the large muscle groups, is associated with a risk of developing metabolic syndrome or diabetes (Carnethon et al., 2003; Ruiz et al., 2009) as well as with a risk of cardiac events (Laukkanen et al., 2004). More than that, it has been shown that low aerobic capacity is not only a substantial risk factor for a vast range of modern diseases such as cancer, cardiovascular diseases (Antero-Jacquemin et al., 2018) and diabetes (Laine et al., 2017), but it is the most powerful predictor of overall mortality equally among healthy people and patients (Blair et al., 1989, 1995; Myers et al., 2002).

It has been recognized that risk factors for modern chronic diseases have their origins in childhood and adolescence (Berenson et al., 1998; Katzmarzyk et al., 2001; Ebbeling et al., 2002; Ortega et al., 2008; Dwyer et al., 2009; Ruiz et al., 2009, 2016). It is metabolic health that is particularly strongly associated with aerobic capacity, and it has been reported that about 4% of schoolchildren in the United States already have metabolic syndrome (Welk et al., 2011). The sharp decrease in aerobic capacity among schoolchildren during the last decades (Tomkinson et al., 2003; Tomkinson and Olds, 2007; Venckunas et al., 2017) raise serious concerns about the health of the upcoming generations. Recent declines in children's aerobic capacity have been attributed to increases in obesity (Albon et al., 2010) and to reduced physical activity (Huotari et al., 2010; Pahkala et al., 2013), which are interrelated (Lamboglia et al., 2013).

While aerobic capacity in schoolchildren has been found to be related to the abilities in explosive- and motor skill-demanding movements involving either the legs or arms (Okely et al., 2001), changes in various components of physical fitness during childhood and adolescence do not always change the same as aerobic capacity and in addition may depend on other factors such as gender (Catley and Tomkinson, 2013). In addition, it has been shown that various aspects of motor fitness in children are not following the same trend of change across decades (Runhaar et al., 2010). It is thus still unclear as to what extent cardio-respiratory fitness is related to muscle strength/power, balance and flexibility parameters among children and adolescents.

The aim of this study was to test the relationship of aerobic capacity with performance in other aspects of physical fitness among children and adolescents. We hypothesized that there would be a positive relationship of aerobic capacity with other aspects of fitness, indicative of the general dependence of fitness on activity levels. Declines in multiple aspects of fitness would then exacerbate the disease risk in lower fitness percentiles of schoolchildren. Moreover, if a significant relationship between cardio-respiratory (aerobic) capacity and other aspects of physical fitness is found, this would imply a substantial interplay between motor abilities and confirm the idea of the importance of comprehensive physical development as by sports practices to ensure general well-being through regular participation in active leisure time (Runhaar et al., 2010).

## MATERIALS AND METHODS

### Participants

The study included data from participants from the three nationally representative cohort studies performed in Lithuania in the years 1992, 2002, and 2012 among 11- to 18-year-old schoolchildren. In total, 18,294 schoolchildren were recruited for testing. Only those who had their body weight and height measured, and completed a shuttle endurance test and at least one other test were included in the analysis. The final number included 15,213 participants (7,608 boys and 7,605 girls). The distribution of numbers of participants for both genders across age groups and the three decades are presented in **Table 1**.

### Procedures

The study was carried out in accordance with the recommendations listed in Declaration of Helsinki. The protocol was approved by the Lithuanian Bioethics Committee (permission no. BE-2-45). Informed consent was obtained from all participants, and written informed consent was also provided by their parents/guardians. Data were collected during the spring in all three time points. Physical fitness evaluations using the Eurofit test battery and anthropometrical measurements were taken by a team of qualified testers—graduates of our sports university—who had all been trained by the same chief investigator across the three decades. Schools were selected from a national registry including major cities and districts across the country ( $n = 14$ ,  $n = 14$  and  $n = 19$  in 1992, 2002, and 2012, respectively). Schools were classified into groups by type of location (urban or rural). Selection was made by randomly selecting school code from the boxes of urban and rural school codes, with each school having an equal probability of selection. Schoolchildren were selected based on grade (from 5th to 12th) and were included if healthy and attended physical education classes at the time of the study. Physical fitness tests were performed wearing gym attire. Body weight and height were measured before the tests. To avoid fatigue, tests were split into two non-consecutive days within 1 week. Tests were administered and performed by the children in the following order: day 1—Flamingo balance, sit-and-reach, standing broad jump, sit-ups, bent arm hang and  $10 \times 5$  m agility shuttle run; day 2—endurance 20 m shuttle run (Venckunas et al., 2017).

Testing procedures were standardized among testers before testing in each decade. Reliability of Eurofit tests has been investigated in a population of young subjects (Tsigilis et al., 2002) in whom intraclass correlation revealed satisfactory coefficients  $>0.70$  for the tests used in the current study ( $r$  was 0.57 for the plate-tapping test, the one which had not been included into the current study). All testing procedures were meticulously explained to children on the day of testing. Testing equipment calibration was performed periodically (before each testing session in each of the schools) in the university settings. Measurements were taken in accordance with standard methodology for anthropometrics and Eurofit physical fitness tests.

### Physical Fitness Tests

The different components of physical fitness—balance, flexibility, muscular strength, power and endurance, agility and cardio-respiratory fitness—were assessed by the standardized Eurofit test battery as described previously (Venckunas et al., 2017).

In brief, **Flamingo balance** test measures static balancing ability by the number of attempts required to complete the total of balancing standing on the rod for 1 min on one foot. **Sit-and-reach** test measures lower body flexibility while attempting to reach forward as far as possible keeping knees straight in a sitting position. **Standing broad jump** measures jumping distance from a standing start ('frog leap'). **Sit-up test** measures abdominal muscles function as number of sit-ups completed from lying position (knees bent at a  $90^\circ$ ) in 30 s. **Bent arm hang test**

**TABLE 1** | The distribution of number of participants (*n*) across age groups and decades.

	Boys					Girls				
	11–12 years	13–14 years	15–16 years	17–18 years	Total	11–12 years	13–14 years	15–16 years	17–18 years	Total
<b>1992</b>										
<i>n</i>	880	922	551	232	2585	870	916	649	369	2804
% within decade	34.0	35.7	21.3	9.0	100.0	31.0	32.7	23.1	13.2	100.0
<b>2002</b>										
<i>n</i>	805	696	635	418	2554	758	727	620	438	2543
% within decade	31.5	27.3	24.9	16.4	100.0	29.8	28.6	24.4	17.2	100.0
<b>2012</b>										
<i>n</i>	411	691	761	606	2469	412	600	716	530	2258
% within decade	16.6	28.0	30.8	24.5	100.0	18.2	26.6	31.7	23.5	100.0

measures function of upper muscles as time which participants are able to sustain the both hand grasp hang with the chin above the bar. **Agility shuttle run** measures the time required to complete 50 m shuttle run test from a standing start during which the participants run forth and back five times to complete five 10 m laps. **Endurance shuttle run** measures cardiorespiratory fitness (aerobic capacity) as the number of stages completed during every-minute increasing pace of 20 m shuttle run test which begins with walking and proceeds to running. The result of the test provides a valid estimate of treadmill maximal oxygen uptake in young adults (Paradisis et al., 2014).

A standard warm-up (mostly running and dynamic stretching exercises) for 7–8 min was carried out before testing. Before each of the tests, the participants were given a try, and the importance of concentration and maximal efforts was reminded. The better result of two attempts was recorded in sit-and-reach and standing broad jump tests, while in other tests only one successful attempt was allowed. All tests have been conducted indoors (school's gym) in the comfortable sporting attire; jumping and running tests were carried out on wooden non-slippery floor.

## Anthropometric Measurements

Barefoot stature was measured to the nearest 0.1 cm, and body weight was measured to the nearest 0.1 kg when participants were wearing minimal clothing. The body mass index (BMI) was calculated as body weight per height squared ( $\text{kg}/\text{m}^2$ ).

## Statistical Analysis

IBM SPSS Statistics v. 24.0 for Windows (IBM Corp., Armonk, NY, United States) was used for data processing. Normality tests for each gender and age group were applied to identify outliers, which were subsequently excluded. Means, standard deviations and frequencies were calculated using descriptive statistics. Participants were allocated into quintiles according to the result of their endurance shuttle run test. Classification of schoolchildren to cardio-vascular fitness quintiles of “very low” to “very high” fitness was based on previous studies (Blair et al., 1989, 1995; Myers et al., 2002; Catley and Tomkinson, 2013). For the analysis, data from the three different decades were pooled. A generalized linear model univariate analysis using BMI

and decade (the year in which the measurements were taken) as covariates was performed to test for the differences in the performance in other physical fitness tests between the quintiles of cardio-respiratory fitness level. Analyses for boys and girls were performed separately. A Bonferroni *post hoc* test was used for multiple comparisons, and two-sided *p*-values of  $<0.05$  were considered statistically significant.

## RESULTS

Quintiles of the number of completed endurance shuttle run stages revealed that aerobic capacity increased with age in boys but remained similar across age groups in girls (Table 2). Fewer subjects were found at the two highest quintiles in all age groups and in both genders. Aerobic capacity was positively related to all other aspects of physical fitness tested in all age groups and both genders of schoolchildren. Aerobic capacity was most strongly related to performance in agility shuttle run and standing broad jump, where it explained  $>10\%$  of the variance in performance in these tests; then followed bent arm hang time and number of abdominal curls, the results of each of which were explained by  $\sim 6\text{--}7\%$  of the variance in aerobic capacity; the performance in Flamingo balance and sit-and-reach flexibility tests were each explained by  $\sim 3\%$  of the variance in aerobic capacity.

Agility was significantly related to aerobic capacity level in both genders across all age groups. BMI had a marginal effect for this relationship, while the decade of study was a significant covariate in all age groups for both genders (Table 3). Bent arm hang time differed between quintiles of aerobic capacity in both genders and across all age groups. BMI was a significant co-factor in this measure for all age groups and both genders (Table 4). Numbers of abdominal curls completed in 30 s differed between quintiles of aerobic capacity in both genders and across all age groups. BMI had a small but significant effect on this relationship (except for the oldest group of boys), and decade was a significant covariate for this relationship in all age groups in both genders (Table 5). Standing broad jump results differed between quintiles of aerobic capacity in both genders and across all age groups, with a minor effect of BMI and decade (Table 6). Balancing ability was moderately but significantly affected by aerobic capacity



**TABLE 2 |** Quintiles of number of completed endurance shuttle run stages (proxy for aerobic capacity) in schoolchildren.

Gender	Age, years	Quintiles of aerobic capacity				
		1st <20% (very low)	2nd 20–40% (low)	3rd 40–60% (average)	4th 60–80% (high)	5th >80% (very high)
♂	11–12 (n = 2204)	≤4 (n = 626; 28.4%)	5 (n = 531; 24.1%)	6 (n = 397; 18%)	7–8 (n = 320; 14.5%)	≥9 (n = 330; 15%)
	13–14 (n = 2412)	≤4 (n = 644; 26.7%)	5–6 (n = 498; 20.6%)	7 (n = 550; 22.8%)	8–9 (n = 328; 13.6%)	≥10 (n = 392; 16.3%)
	15–16 (n = 1995)	≤5 (n = 477; 23.9%)	6–7 (n = 421; 21.1%)	8 (n = 440; 22.1%)	9 (n = 400; 20.1%)	≥10 (n = 257; 12.9%)
	17–18 (n = 1315)	≤5 (n = 314; 23.9%)	6–7 (n = 288; 21.9%)	8 (n = 299; 22.7%)	9 (n = 275; 20.9%)	≥10 (n = 139; 10.6%)
♀	11–12 (n = 2130)	≤3 (n = 661; 31%)	4 (n = 440; 20.7%)	5 (n = 395; 18.5%)	6 (n = 281; 13.2%)	≥7 (n = 353; 16.6%)
	13–14 (n = 2328)	≤3 (n = 619; 26.6%)	4 (n = 461; 19.8%)	5 (n = 479; 20.6%)	6 (n = 370; 15.9%)	≥7 (n = 399; 17.1%)
	15–16 (n = 2027)	≤3 (n = 550; 27.1%)	4 (n = 483; 23.8%)	5 (n = 436; 21.5%)	6 (n = 286; 11.8%)	≥7 (n = 272; 13.4%)
	17–18 (n = 1367)	≤3 (n = 428; 31.3%)	4 (n = 315; 23%)	5 (n = 279; 20.4%)	6 (n = 186; 13.6%)	≥7 (n = 159; 11.6%)

**TABLE 3 |** Agility shuttle run(s) in schoolchildren of different aerobic capacity levels.

Gender	Age, years	Quintiles of aerobic capacity					Quintile's effect ( $\eta_p^2$ )	Covariates' effect ( $\eta_p^2$ )	
		1st <20% (very low)	2nd 20–40% (low)	3rd 40–60% (average)	4th 60–80% (high)	5th >80% (very high)		Body mass index	Decade
♂	11–12 (n = 1989)	22.1 (2.1) <sup>b–e</sup>	21.5 (1.8) <sup>d,e</sup>	21.5 (1.7) <sup>d,e</sup>	21.2 (1.6) <sup>e</sup>	20.8 (1.4)	0.136 ***	0.004 **	0.121 ***
	13–14 (n = 2217)	21.2 (2.3) <sup>b–e</sup>	20.8 (1.8) <sup>c–e</sup>	20.5 (1.8) <sup>d,e</sup>	20.4 (1.5) <sup>e</sup>	20.0 (1.2)	0.097 ***	0.004 **	0.070 ***
	15–16 (n = 1872)	20.3 (2.1) <sup>b–e</sup>	19.9 (1.8) <sup>c–e</sup>	19.8 (1.7) <sup>e</sup>	19.3 (1.9)	19.4 (1.5)	0.105 ***	<0.001	0.102 ***
	17–18 (n = 1199)	20.3 (2.3) <sup>b–e</sup>	19.4 (1.6) <sup>d,e</sup>	19.2 (1.6) <sup>e</sup>	18.7 (1.9)	18.8 (1.6)	0.109 ***	<0.001	0.025 ***
♀	11–12 (n = 885)	23.3 (2.2) <sup>b–e</sup>	23.1 (1.8) <sup>c–e</sup>	22.5 (1.7) <sup>d,e</sup>	22.3 (1.5) <sup>e</sup>	22.0 (1.4)	0.139 ***	0.001	0.083 ***
	13–14 (n = 2140)	22.5 (2.1) <sup>b–e</sup>	22.2 (1.8) <sup>c–e</sup>	21.8 (1.7) <sup>d,e</sup>	21.7 (1.6) <sup>e</sup>	21.2 (1.5)	0.101 ***	0.005 **	0.065 ***
	15–16 (n = 1889)	22.6 (2.0) <sup>b–e</sup>	21.8 (1.9) <sup>d,e</sup>	21.6 (1.6) <sup>d,e</sup>	21.3 (1.5) <sup>e</sup>	20.9 (1.3)	0.112 ***	0.004 **	0.020 ***
	17–18 (n = 1290)	22.4 (1.9) <sup>b–e</sup>	21.7 (1.8) <sup>d,e</sup>	21.4 (1.6) <sup>d,e</sup>	20.9 (1.7)	20.7 (1.4)	0.114 ***	0.018 **	0.016 ***

Data presented as mean (SD). Faster times mean better agility. <sup>b,c,d,e</sup> differ at  $p < 0.05$  from 2nd, 3rd, 4th, and 5th quintiles, respectively. \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , respectively.

level in both genders and across all age groups, with a minor effect of BMI and some more substantial effect of decade on the relationship (Table 7). Lower body flexibility was moderately but significantly interrelated to aerobic capacity level in both genders and across all age groups except for the oldest (17- to 18-year-old) boys group in which were no significant differences between aerobic capacity quintiles in sit-and-reach flexibility (Table 8); small but significant effects of BMI and decade were detected for this relationship (Table 8).

## DISCUSSION

Our analysis of the data collected over three decades revealed that aerobic capacity was weakly positively related with all aspects of exercise abilities tested in all age groups for both genders of children. The agility shuttle run and standing broad jump results were most strongly related to aerobic capacity. Part of this relatively strong relationship could be because of the similarity of movement pattern between shuttle endurance and agility

shuttle run, and involvement of the same muscle groups (leg extensors) that are critical for locomotion in these tests. The endurance shuttle run, agility shuttle run and jumping are all weight-bearing exercises, as well as execution of all three requires acceleration (propulsion) and deceleration (landing) whole body. Therefore, both intrinsic muscular characteristics and proficiency in movement patterns such as synchronization of leg and arm swings in single leg (running) or both leg jumps to gain efficient momentum might have affected the results in the performance in these three tests (though to probably a different extent) compared with the influence on the performance in other tests. It is indeed that measures of anaerobic power such as a jumping ability explain considerable variation in endurance performance which clearly suggests some common shared physiologic mechanism (Houmard et al., 1991; Sinnett et al., 2001). Somewhat weaker but still significantly positively related to aerobic capacity were the results of bent arm hang, abdominal curls, balance, and flexibility tasks. Bent arm hang time and number of abdominal curls, representing upper body muscle fitness, were each explained by ~6–7% of the variation in aerobic capacity. Differences in aerobic

**TABLE 4 |** Bent arm hang time(s) in schoolchildren of different aerobic capacity levels.

Gender	Age, years	Quintiles of aerobic capacity					Quintile's effect ( $\eta_p^2$ )	Covariates' effect ( $\eta_p^2$ )	
		1st <20% (very low)	2nd 20–40% (low)	3rd 40–60% (average)	4th 60–80% (high)	5th >80% (very high)		Body mass index	Decade
♂	11–12 (n = 1983)	13.0 (10.7) <sup>b–e</sup>	17.8 (12.0) <sup>d,e</sup>	20.9 (13.3) <sup>d,e</sup>	25.0 (14.3) <sup>e</sup>	28.8 (15.7)	0.112 ***	0.037 ***	0.006 **
	13–14 (n = 2198)	18.0 (14.7) <sup>b–e</sup>	20.2 (12.8) <sup>c–e</sup>	26.4 (14.1) <sup>d,e</sup>	28.4 (13.5) <sup>e</sup>	31.9 (15.5)	0.073 ***	0.018 ***	<0.001
	15–16 (n = 1855)	24.6 (14.3) <sup>b–e</sup>	30.6 (15.0) <sup>d,e</sup>	33.6 (15.8) <sup>e</sup>	37.8 (15.7)	39.0 (16.3)	0.056 ***	0.017 ***	0.008 ***
	17–18 (n = 1198)	25.0 (15.5) <sup>b–e</sup>	32.7 (18.2) <sup>d,e</sup>	34.3 (18.8)	38.7 (17.3)	42.9 (19.8)	0.045 ***	0.037 ***	0.022 ***
♀	11–12 (n = 838)	7.5 (6.3) <sup>b–e</sup>	10.5 (8.4) <sup>c–e</sup>	11.7 (8.1) <sup>e</sup>	12.1 (8.1) <sup>e</sup>	16.6 (9.5)	0.106 ***	0.031 ***	0.009 **
	13–14 (n = 2029)	8.4 (7.2) <sup>b–e</sup>	11.1 (8.9) <sup>e</sup>	12.8 (9.2) <sup>e</sup>	12.4 (9.2) <sup>e</sup>	16.2 (9.6)	0.054 ***	0.037 ***	0.001
	15–16 (n = 1814)	10.0 (8.4) <sup>b–e</sup>	11.9 (9.2) <sup>d,e</sup>	12.7 (9.1) <sup>e</sup>	14.7 (9.9)	16.2 (9.7)	0.039 ***	0.057 ***	0.002
	17–18 (n = 1177)	9.3 (8.4) <sup>c–e</sup>	11.2 (8.1) <sup>d,e</sup>	12.5 (9.2) <sup>e</sup>	15.5 (11.0) <sup>e</sup>	16.3 (12.2)	0.061 ***	0.082 ***	0.009 **

Data presented as mean (SD). <sup>b,c,d,e</sup> differ at  $p < 0.05$  from 2nd, 3rd, 4th, and 5th quintiles, respectively. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , respectively.

**TABLE 5 |** Abdominal curls (no. per 30 s) in schoolchildren of different aerobic capacity levels.

Gender	Age, years	Quintiles of aerobic capacity					Quintile's effect ( $\eta_p^2$ )	Covariates' effect ( $\eta_p^2$ )	
		1st <20% (very low)	2nd 20–40% (low)	3rd 40–60% (average)	4th 60–80% (high)	5th >80% (very high)		Body mass index	Decade
♂	11–12 (n = 2020)	22.8 (4.3) <sup>b–e</sup>	24.1 (4.1) <sup>d,e</sup>	25.0 (3.8) <sup>d,e</sup>	25.0 (3.8) <sup>e</sup>	26.0 (3.4)	0.076 ***	0.007 ***	0.020 ***
	13–14 (n = 2228)	24.6 (4.8) <sup>b–e</sup>	25.7 (4.6) <sup>d,e</sup>	26.6 (4.1) <sup>d,e</sup>	27.1 (3.8) <sup>e</sup>	28.0 (3.8)	0.059 ***	0.007 ***	0.007 ***
	15–16 (n = 1870)	26.5 (4.5) <sup>b–e</sup>	27.3 (4.0) <sup>d,e</sup>	27.9 (4.1) <sup>e</sup>	28.8 (3.8) <sup>e</sup>	29.2 (3.5)	0.062 ***	0.003 *	0.014 ***
	17–18 (n = 1219)	27.3 (5.1) <sup>b–e</sup>	28.6 (4.3) <sup>d,e</sup>	28.7 (3.9) <sup>e</sup>	29.5 (3.9)	30.2 (4.3)	0.060 ***	0.001	0.024 ***
♀	11–12 (n = 899)	21.1 (4.1) <sup>b–e</sup>	21.8 (3.8) <sup>d,e</sup>	22.0 (3.6) <sup>e</sup>	22.6 (4.0)	23.3 (3.5)	0.055 ***	0.010 **	0.026 ***
	13–14 (n = 2138)	21.9 (4.7) <sup>b–e</sup>	23.2 (4.3) <sup>e</sup>	23.8 (4.0) <sup>e</sup>	23.5 (3.9) <sup>e</sup>	24.6 (3.7)	0.056 ***	0.012 ***	0.025 ***
	15–16 (n = 1906)	23.4 (4.3) <sup>b–e</sup>	24.1 (4.2) <sup>d,e</sup>	24.1 (3.8) <sup>d,e</sup>	24.7 (3.8)	24.8 (3.7)	0.027 ***	0.007 ***	0.022 ***
	17–18 (n = 1287)	24.3 (4.3) <sup>b–e</sup>	24.9 (4.0) <sup>d,e</sup>	25.4 (3.6) <sup>e</sup>	26.1 (3.9)	26.4 (3.7)	0.069 ***	0.011 ***	0.058 ***

Data presented as mean (SD). <sup>b,c,d,e</sup> differ at  $p < 0.05$  from 2nd, 3rd, 4th, and 5th quintiles, respectively. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , respectively.

capacity explained only ~3% of the variance in balance and lower body flexibility, even though lower body musculature is largely responsible for performance in both of these tests and the shuttle run test.

The positive interrelation between aerobic capacity and other physical capacities suggests that each of the applied tests from the Eurofit (or probably any other) battery has its own value in estimating 'overall fitness' of the individual. Even though at the highest level of multidisciplinary athletes there could be some trade-offs in performance between power and endurance events

(Van Damme et al., 2002), this does not look to be true for the general non-athletic young population. On another end of age spectrum, it seems that not only endurance, but also speed-power training could somewhat 'compensate' for the lack of aerobic training stimulus and thus improve cardiovascular capacity as evidenced in the study of master athletes from different track-and-field disciplines (Kusy and Zieliński, 2014). It could be argued of course that it is not the parallel change in different aspects of physical fitness (i.e., comprehensive development of all aspect of exercise capacity) that is of primary importance

**TABLE 6** | Standing broad jump (cm) in schoolchildren of different aerobic capacity levels.

Gender	Age, years	Quintiles of aerobic capacity					Quintile's effect ( $\eta_p^2$ )	Covariates' effect ( $\eta_p^2$ )	
		1st <20% (very low)	2nd 20–40% (low)	3rd 40–60% (average)	4th 60–80% (high)	5th >80% (very high)		Body mass index	Decade
♂	11–12 (n = 2033)	155.3 (20.7) b–e	169.1 (17.7) d,e	170.1 (17.8) d,e	174.4 (17.0) e	178.8 (16.1)	0.138 ***	0.004 **	0.007 ***
	13–14 (n = 2236)	181.3 (26.1) b–e	185.1 (23.5) c–e	195.5 (21.7) d,e	195.6 (21.2) e	201.9 (18.6)	0.100 ***	0.004 **	0.008 ***
	15–16 (n = 1875)	201.7 (27.2) b–e	211.3 (23.5) c–e	215.7 (22.3) e	222.9 (20.1) e	226.1 (17.5)	0.120 ***	0.002 *	0.007 ***
	17–18 (n = 1217)	210.9 (33.8) b–e	227.8 (24.3) e	229.5 (22.0)	234.1 (20.6)	238.0 (19.0)	0.105 ***	0.002	0.001
	11–12 (n = 900)	141.2 (18.5) b–e	148.5 (17.4) c–e	153.6 (15.3) d,e	159.5 (15.6) e	163.9 (15.6)	0.123 ***	0.010 **	<0.001
♀	13–14 (n = 2157)	151.7 (20.1) b–e	161.3 (18.4) c–e	166.8 (17.9) e	170.3 (15.6) e	176.0 (17.0)	0.125 ***	0.015 ***	<0.001
	15–16 (n = 1914)	158.7 (21.2) b–e	166.1 (20.4) c–e	170.5 (19.5) d,e	176.9 (18.4) e	182.1 (14.6)	0.102 ***	0.013 ***	<0.001
	17–18 (n = 1265)	162.3 (20.8) b–e	169.2 (17.4) d,e	173.4 (16.7) d,e	182.4 (17.3) e	188.5 (19.8)	0.130 ***	0.053 ***	<0.001

Data presented as mean (SD). *b,c,d,e* differ at  $p < 0.05$  from 2nd, 3rd, 4th, and 5th quintiles, respectively. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , respectively.

**TABLE 7** | Flamingo balance (no. of attempts in 1 min) in schoolchildren of different aerobic capacity levels.

Gender	Age, years	Quintiles of aerobic capacity					Quintile's effect ( $\eta_p^2$ )	Covariates' effect ( $\eta_p^2$ )	
		1st <20% (very low)	2nd 20–40% (low)	3rd 40–60% (average)	4th 60–80% (high)	5th >80% (very high)		Body mass index	Decade
♂	11–12 (n = 2052)	13.1 (6.0) b–e	12.5 (5.5) d,e	12.3 (5.4) d,e	12.0 (5.1)	11.4 (4.9)	0.030 ***	0.013 ***	0.047 ***
	13–14 (n = 2255)	10.7 (6.0) d,e	11.4 (6.0) d,e	11.3 (5.5) d,e	10.8 (5.1)	10.9 (5.0)	0.025 ***	0.011 ***	0.072 ***
	15–16 (n = 1905)	10.3 (5.2) c–e	9.9 (5.2) e	10.3 (5.2) e	10.0 (5.0)	10.0 (4.6)	0.022 ***	0.003 **	0.067 ***
	17–18 (n = 1238)	10.3 (4.9) d,e	9.9 (5.0) d,e	9.8 (4.7) e	8.7 (4.5)	8.5 (4.8)	0.032 ***	0.002	0.018 ***
♀	11–12 (n = 892)	12.9 (6.3) c–e	12.7 (5.5) d,e	12.8 (5.6)	12.3 (5.6)	13.6 (5.5)	0.022 ***	0.022 ***	0.089 ***
	13–14 (n = 2190)	11.8 (5.9) b–e	11.1 (5.4) d,e	11.4 (5.5) e	11.6 (5.3)	11.0 (4.9)	0.044 ***	0.021 ***	0.143 ***
	15–16 (n = 1946)	10.3 (5.2) c–e	10.1 (5.4) c–e	9.7 (5.1)	10.2 (5.1)	10.8 (5.0)	0.029 ***	0.003 *	0.130 ***
	17–18 (n = 1301)	10.2 (4.7) b–e	9.4 (4.8) d,e	9.5 (5.2)	9.1 (4.6)	9.6 (4.8)	0.039 ***	0.020 ***	0.099 ***

Data presented as mean (SD). The lower number of attempts means better balance. *b,c,d,e* differ at  $p < 0.05$  from 2nd, 3rd, 4th, and 5th quintiles, respectively. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , respectively.

and should be pursued but rather at least minimal level of any of the exercise capacities that is essential for a good quality of life and long years of independent subsistence. This seems to be especially relevant to the cardiorespiratory fitness as former elite endurance athletes have not increased life expectancy (van Saase et al., 1990; Farahmand et al., 2003; Sanchis-Gomar et al., 2011; Kettunen et al., 2015; Antero-Jacquemin et al., 2018) but also better life quality even long after discontinuation of

athletic career (Bäckmand et al., 2010). However, as it is not only the endurance type of training bring about the beneficial adaptations (Bäckmand et al., 2010; Antero-Jacquemin et al., 2018), it could be recommended that in fact any of the vigorous exercise or any sports activity should be promoted among children.

The significant effect of the decade in which the testing was conducted indirectly supports the importance of recently

**TABLE 8** | Sit-and-reach flexibility (cm) in schoolchildren of different aerobic capacity levels.

Gender	Age, years	Quintiles of aerobic capacity					Quintile's effect ( $\eta_p^2$ )	Covariates' effect ( $\eta_p^2$ )	
		1st <20% (very low)	2nd 20–40% (low)	3rd 40–60% (average)	4th 60–80% (high)	5th >80% (very high)		Body mass index	Decade
♂	11–12 (n = 1993)	15.4 (5.6) <sup>b–e</sup>	17.4 (5.6) <sup>d</sup>	18.1 (5.4) <sup>d</sup>	19.7 (5.7)	19.3 (5.2)	0.034 ***	0.007 ***	0.017 ***
	13–14 (n = 2198)	17.2 (7.0) <sup>b–e</sup>	18.6 (7.0) <sup>d,e</sup>	20.9 (6.8)	21.1 (6.7)	21.8 (6.9)	0.026 ***	0.024 ***	0.013 ***
	15–16 (n = 1843)	18.8 (8.3) <sup>b–e</sup>	21.9 (7.7) <sup>e</sup>	23.8 (7.6)	24.7 (8.1)	25.9 (7.8)	0.033 ***	0.021 ***	0.042 ***
	17–18 (n = 1202)	22.3 (9.4)	24.1 (8.1)	24.5 (8.4)	25.6 (8.3)	27.3 (8.2)	0.008	0.015 ***	0.057 ***
	11–12 (n = 883)	17.9 (5.8) <sup>b–e</sup>	19.2 (5.7) <sup>d,e</sup>	20.4 (6.2)	21.2 (4.9)	20.9 (6.3)	0.036 ***	0.021 ***	0.001
♀	13–14 (n = 2113)	21.5 (6.7) <sup>b–e</sup>	23.7 (6.5) <sup>d,e</sup>	25.0 (6.3)	25.7 (6.0)	25.4 (5.9)	0.041 ***	0.010 ***	0.005 **
	15–16 (n = 1926)	23.5 (8.0) <sup>b–e</sup>	25.8 (6.9)	26.9 (6.3)	28.0 (6.6)	28.8 (6.0)	0.029 ***	0.019 ***	0.043 ***
	17–18 (n = 1298)	23.4 (8.7) <sup>b–e</sup>	26.1 (7.3)	28.3 (6.6)	28.7 (6.0)	29.8 (6.2)	0.038 ***	0.018 ***	0.056 ***

Data presented as mean (SD). <sup>b,c,d,e</sup> differ at  $p < 0.05$  from 2nd, 3rd, 4th, and 5th quintiles, respectively. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , respectively.

changed activity patterns among schoolchildren as world trends show physical activity is on constant decline (Sallis et al., 2016). Lithuanian schoolchildren have also shown a continual decrease in physical activity levels during the period from 1998 to 2010 (Currie et al., 2012), which could well be the major reason of the declining aerobic capacity over decades in our cohort (Venckunas et al., 2017). Indeed, there is strong evidence to suggest that exercise capacity is largely determined by the level of physical activity (Huang and Malina, 2002; Huotari et al., 2010; Pahkala et al., 2013), even at a very early age (Lintu et al., 2016; Leppänen et al., 2017). For instance, it was reported that in 6- to 8-year-old children, physical activity and static balance were both linked to aerobic capacity (Lintu et al., 2016). Importantly, health-related behaviors including physical activity are to a significant extent transferred between school age and adulthood (Kelder et al., 1994; Telama et al., 1996). It is of interest that aerobic capacity in both genders of schoolchildren has also been found to be related to efficacy in explosive- and motor skill-demanding movements involving either the upper or lower musculature (Okely et al., 2001). Therefore, the idea that prevention of the decline in motor fitness in children is important for the overall engagement of kids into different exercise activities (Runhaar et al., 2010) is so relevant, and training various aspects of health related physical fitness via, e.g., participation in sports activities might prove indispensable for the accumulation of health capital.

The importance of aerobic capacity is well recognized at a young age already. During the school ages, low aerobic capacity is associated with an increased risk of cardio-vascular disease (Ruiz et al., 2009). Furthermore, after adjustment for age, ethnicity, gender, smoking, and family history of diabetes, hypertension, or premature myocardial infarction, it was shown that young adults with low cardio-vascular

fitness (1st quintile) were threefold to sixfold more likely to develop diabetes, hypertension and metabolic syndrome than participants with high fitness (4th and 5th quintiles) during a 15-year period (Carnethon et al., 2003). Perhaps even more importantly, cardio-vascular fitness is the most powerful predictor of overall mortality among healthy people and cardiopulmonary patients (Blair et al., 1989, 1995; Myers et al., 2002).

The increase in aerobic capacity with age in our cohort was evident in boys but not girls, which is consistent with findings of other authors where non-athletic girls improved their aerobic capacity during maturation much slower than did boys (Catley and Tomkinson, 2013) while aerobic capacity in preadolescent schoolboys improves very slowly (De Miguel-Etayo et al., 2014). Our results for the distribution of endurance shuttle run lap numbers between quintiles are largely similar to data for Australian schoolchildren collected during a similar period (Okely et al., 2001; Catley and Tomkinson, 2013) and also those pooled from 50 countries (Tomkinson et al., 2017), implying that the data are representative of larger populations than those of particular regions.

## Limitations

We applied the progressive shuttle run (“beep”) test for measuring aerobic capacity, which in the later stages requires substantial muscle power for repeated acceleration and deceleration of the body. It could be that flat running in a circle (i.e., a stadium oval), such as the Cooper test, would produce different results, as partially reflected in the study on Australian children and adolescents where the beep test and flat 1-mile running results showed some differences across ages in that flat running seemingly improved less than the shuttle run for both genders (Catley and Tomkinson, 2013). However, as is

usually the case with not well-trained subjects, the problems of proper pacing would have precluded a self-paced running field test from being really informative.

The relationships detected in the current study may to some extent also reflect the level and pattern of engagement in of part of the participants in physical activities and sports where increased participation would be associated with superior performance in the tests because of the enhanced physiological functions as a consequence of adaptation to regular training and higher competitive/motivational levels via learning effects during activities. This would be expected as those children who are physically active do not usually undertake one particular sport or concentrate on single event in that sport until the age of roughly 16 years but are rather encouraged by the physical education teachers and/or coaches to be involved in different exercise activities and learn many sports, which is (allegedly) a good relic of the Soviet time sports education culture in Lithuania. Consequently, it could be speculated that such comprehensive development ought to improve their overall fitness and most aspects of exercise capacities, while those schoolchildren leading largely sedentary life would not benefit from sports in any of the conditioning aspects. This scenario would create 'scissors' in the population tested, but as long as we alas cannot investigate this hypothesis in the frame of the current cohort because the information on the physical activity levels or sports participation is completely lacking in the database, this remains to be proved in other studies.

Our results do not necessary suggest a direct causal relationship of aerobic capacity with other exercise abilities. It might well be that increased activity levels augment aerobic capacity along with other physical abilities. However, as aerobic capacity was shown to be consistently associated with a vast range of other exercise abilities in both genders and all age groups of schoolchildren, this highlights its importance in overall motor abilities and overall well-being. Testing for aerobic capacity in schoolchildren and upgrading its level to higher quintiles by regular training could allow for improvements in other

physical abilities, in addition to enabling continued interest and involvement in daily exercise activities including sports. Therefore, physical education teachers are encouraged to refer to the presented norms of aerobic capacity to monitor children and especially guide those in the lower quintiles (Myers et al., 2002) toward lifestyle modifications through implementation of individualized training and physical education programs.

## CONCLUSION

Aerobic capacity in all age groups and in both genders of schoolchildren is positively related to all other aspects of physical fitness, with the relationship being strongest with lower body muscular power.

## AUTHOR CONTRIBUTIONS

TV, BM, and AE designed the study, and collected and analyzed the data. All authors contributed to interpretation of the data, drafting, and revising the manuscript, and approved the final version of the manuscript.

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# Dynamic Postural Control in Children: Do the Arms Lend the Legs a Helping Hand?

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There is growing empirical evidence lending support to the existence of an “upper body strategy” to extend the ankle and hip strategies in maintaining upright postural stability among adults. Both postural stability and arm movement functions are still developing in children. Therefore, enquiry concerning arm contribution to postural stability among children is needed. This proof of concept study seeks to determine whether the arms play a functionally relevant role in dynamic postural control among children. Twenty-nine children (girls,  $n = 15$ ; age,  $10.6 \pm 0.5$  years; height,  $1.48 \pm 0.08$  m; mass,  $42.8 \pm 11.4$  kg; BMI,  $19.2 \pm 3.7$  kg/m<sup>2</sup>) completed three dynamic balance tests; (1) Y Balance test<sup>®</sup>, (2) timed balance beam walking test, (3) transition from dynamic to static balance using the dynamic postural stability index (DPSI). Each test was performed with free and restricted arm movement. Restricting arm movements elicited a marked degradation in the Y Balance reach distance (all directions,  $P \leq 0.001$ ,  $d = -0.85$  to  $-1.13$ ) and timed balance beam walking test ( $P \leq 0.001$ ,  $d = 1.01$ ), while the DPSI was the only metric that was not different between free and restricted arm movements ( $P = 0.335$ ,  $d = -0.08$ ). This study provides direct evidence that the arms play a functionally relevant role in dynamic balance performance among children. These findings may provide the impetus to develop training interventions to improve the use of the arms in activities of daily living.

**Keywords:** balance regulation, upper extremities, arm movements, mobility, children

## INTRODUCTION

The ability to maintain postural control plays an important role in child development, representing a fundamental pre-requisite to competently perform skilled movements and complex motor skills (Mickle et al., 2011; Verbecque et al., 2016). Although the etiology of falls is complex, maturational and/or experiential immaturities in static (i.e., increased postural sway) and dynamic (i.e., gait disorders, such as a loss of symmetry or slowing of gait speed) postural control have been identified as important intrinsic factors increasing the risk of falling and sustaining an injury in children (Khambalia et al., 2006; Granacher et al., 2011). Therefore, an improved understanding of postural control is important for the identification of children with an increased risk of falling and the development of fall-prevention interventions.

To maintain an upright position, the central nervous system (CNS) must continually integrate and (re)weigh information from visual, vestibular and proprioceptive systems to elicit coordinated muscular responses (Mickle et al., 2011). Nashner and McCollum (1985) proposed the existence of two distinct modes of operation to maintain upright posture, referred to as the ankle and hip strategies. Mechanically, the ankle strategy is predominantly used during slow and small amplitude perturbations (i.e., quiet bipedal standing) by moving the whole body as a single segment inverted pendulum controlled by ankle joint torque (Runge et al., 1999). In contrast, the hip strategy, which moves the body as a double-segment inverted pendulum with counterphase motion at the ankle and hip, is expected to be employed for fast or large amplitude perturbations, or when the support surface is narrow so that only little ankle torque can be applied (Horak and Nashner, 1986). However, it has been suggested that postural control is multivariate (as opposed to bivariate) in nature (Hsu et al., 2007; Kilby et al., 2015). Although an upright posture can usually be maintained by the ankle and hip during most scenarios, movements of the upper body are not taken into account by these two control strategies. Indeed, most clinical balance and mobility tests do not evaluate arm movements. Although many studies acknowledge that the arms play a major role in maintaining dynamic postural control (Bruijn et al., 2010; Sawers and Ting, 2015), some studies allow free arm movements (Faigenbaum et al., 2014), others restrict arm movement (Muehlbauer et al., 2013), while others do not provide specific details of arm position (Geldhof et al., 2006; Humphriss et al., 2011). Given these observations, it is important to investigate the potentially important role of arm movements on postural stability, which will be valuable in elucidating some of the fundamental aspects of postural control development in children.

There is growing empirical evidence lending support to the existence of an “upper body strategy” to enhance the body of work that includes ankle and hip postural stability strategies (Hsu et al., 2007), particularly during challenging dynamic tasks (e.g., walking across a narrow beam) (Boström et al., 2018). Accordingly, the influence of arm movements on postural control only appears to be evident underchallenging constraints or task demands. For example, restricting arm movements impairs performance in functional mobility tests (i.e., timed-up and-go) (Milosevic et al., 2011), reduces dynamic postural control (i.e., Y Balance test) (Hébert-Losier, 2017) and impairs mechanisms to minimize postural sway during quiet tandem standing (Patel et al., 2014). In addition, arm movements play a functional role in postural recovery during standing (Allum et al., 2002; Maki and McIlroy, 2006) and walking (Marigold et al., 2003; Roos et al., 2008; Pijnappels et al., 2010), further strengthening the hypothesis that the upper extremities play an important role during challenging postural tasks. Therefore, it is reasonable to suggest that the contribution of the arms becomes important after a certain “threshold” of postural stress has been met.

The demands placed on the postural control system during dynamic postural tasks (i.e., walking across a narrow beam or standing on a single limb and reaching with the contralateral limb), are considerably greater than standing or walking

(Boström et al., 2018). Although evidence has demonstrated a degradation in postural control with restricted arm movements in young (Patel et al., 2014; Hébert-Losier, 2017; Boström et al., 2018) and intermediate aged (Milosevic et al., 2011) adults, little is known about the role of arm movements on dynamic postural control in children. Indeed, there is a reasonable theoretical basis for expectation that arm movements will make a substantial and functionally relevant contribution to dynamic postural tasks in children, because their neuromuscular system is not yet fully matured and fundamental motor skills are still emerging (Granacher and Gollhofer, 2012). Determining whether arm movements play a role in performance of dynamic postural control tasks will be influential in guiding future efforts to incorporate/exclude upper body movements into rehabilitation, training and assessment protocols designed to test and improve postural control in children. These findings will also be influential in providing the impetus for future research to clearly define and describe arm placement and movement to avoid misinterpretation of dynamic postural tasks and for replication purposes.

Therefore, the aim of this study was to investigate the effects of arm movements on the performance of dynamic postural tasks in children. Considering the important contribution of arm movements to dynamic postural control in adults (Milosevic et al., 2011; Patel et al., 2014; Hébert-Losier, 2017) we hypothesize that free arm movements lead to better postural performance among children than restricted arm movements. The difference in performance between restricted and non-restricted conditions will provide a specific quantitative assessment of individuals reliance on lower body postural control mechanisms.

## MATERIALS AND METHODS

### Participants

An *a priori* power analysis (statistical power = 0.80, alpha = 0.05, effect size = 0.48) was conducted for composite Y Balance score (Hébert-Losier, 2017) and revealed that 29 participants would be sufficient for finding statistically significant effects of arm restriction on dynamic balance performance. Thus, the present study consisted of a group of twenty-nine (girls,  $n = 15$ ; age,  $10.6 \pm 0.5$  years [range, 10.1–11.2 years]; height,  $1.48 \pm 0.08$  m; mass,  $42.8 \pm 11.4$  kg; BMI,  $19.2 \pm 3.7$  kg/m<sup>2</sup>, waist circumference,  $68.6 \pm 9.1$  cm; right foot dominant,  $n = 27$ ; dominant leg length,  $79.0 \pm 5.3$  cm) children, recruited from their primary schools in the city of Coventry, United Kingdom. Foot dominance was defined as the foot used to kick a ball. Physical Maturity was assessed by predicting the age at peak height velocity (APHV) using the equation (Mirwald et al., 2002), which is a method based on the growth patterns of the upper body and legs of every individual and is compared to the average population with the aim to classify children between early, average and late maturers. This technique of measuring APHV was chosen because it had the advantages of being non-invasive and more economical in relation to labor and monetary cost (Mirwald et al., 2002). All parents completed a health screen questionnaire prior to participation. This requested information relating to any



physical, cognitive or other issues that prevented participation in physical activity. This includes details in relation to chronic disease (e.g., diabetes), special educational needs (e.g., ADHD), injuries, muscular deficits or cardiovascular impairments as well as confirming that children had normal vision and no auditory impairments. Following institutional ethics approval and prior to conducting the experiment, all participants as well as the children's parents gave their written informed consent. The study was carried out in accordance with the guidelines outlined in the Declaration of Helsinki (1964).

## Experimental Procedure

Participants completed dynamic postural tasks of varying difficulty under two different verbally conveyed instructions of arm position; (1) arms placed flat across the chest touching the contralateral shoulder (i.e., restricted arm movement) and (2) arm movement without restriction (i.e., free arm movement). To ensure familiarization and to remove potential learning effects, each participant completed three practice trials and three recorded trials for each test condition (i.e., arms vs. no-arms). The order of balance tasks was randomized, as were the arm position instructions. For the free arm movement, participants were instructed to be able to move their arms freely during the tasks. For the restricted arm position, compliance to the instructions was monitored visually by the investigators. If the arms moved away from the chest the trial was discarded and repeated. Given the age of the participants, minor arm adjustments were permitted. The investigators were always available to assist the participants to complete the tests safely.

## Y Balance Test

The Y Balance Test Kit<sup>TM</sup> was used to determine dynamic postural control. As described by Plisky et al. (2006), the Y Balance Test Kit<sup>TM</sup> consists of a stance platform to which three pieces of plastic pipe are attached in the anterior, posteromedial, and posterolateral reach directions. The posteromedial and posterolateral pipes are positioned 135° from the anterior pipe with 45° between the posterior pipes. Participants stood on the center of a foot plate with the most distal point of the great toe at the starting line. While maintaining a single-leg stance with the dominant limb, participants were asked to push a target (reach indicator) along the pipe with the contralateral limb (i.e., non-dominant limb) in the anterior, posteromedial and posterolateral directions. Maximal reach distance was measured by reading the tape measure at the edge of the reach indicator, reflecting the point where the most distal part of the foot reached. The trial was discarded and repeated if the participant (1) failed to maintain single limb stance (i.e., touch the floor with the reach limb), (2) failed to remain in contact with the reach indicator at the most distal point (i.e., kicked the reach indicator to achieve greater distance), (3) used the reach indicator to support weight (i.e., mechanical support) or (4) failed to return to the reach foot at the center of the foot plate. Although the reach direction was randomized, to improve reproducibility of the testing protocol, participants performed three consecutive reach attempts for each direction. The greatest reach distance for each direction was used for subsequent analysis. Reach distance was

normalized to limb length (reach distance/limb length \* 100) (Plisky et al., 2006). Each participant's dominant limb length was measured in centimeters from the anterior superior iliac spine to the most distal portion of the medial malleolus using an anthropometric measuring tape (Gribble and Hertel, 2003). Additionally, the composite reach score was also calculated as the sum of the three reach distances divided by three times the limb length and multiplied by 100. A composite reach score was calculated as the sum of the three reach directions divided by three times limb length, and then multiplied by 100 (Plisky et al., 2006). A composition score below 94% is related to neuromotor deficit and a greater probability of injuries (Plisky et al., 2006). Therefore, we used this criterion to determine the clinical relevance of changes in Y Balance performance with restricted arm movements."

## Dynamic Postural Stability Index

Dynamic postural stability index (DPSI) was assessed using an anterior jump-landing task on the dominant limb (Sell, 2012). DPSI is a unitless composite score of anteroposterior (*y*), mediolateral (*x*), and vertical (*z*) ground reaction forces (GRF) and is similar to the static postural stability task, in that a higher DPSI indicates worse postural control (Sell, 2012). Participants were instructed to stand on two legs at distance of 40% of their body height from the center of the force platform (AMTI, AccuGait, Watertown, MA, United States). Each participant was instructed to jump forward over a 6-inch hurdle on to the force platform and land on their dominant limb, stabilize as quickly as possible and, balance for 10 s. The hurdle was positioned at a distance of 20% of their body height (i.e., half way between the starting point and the center of the force platform). Each participant completed a minimum of three practice attempts. Trials were discarded and repeated if the participants contralateral limb touched the floor. Data were sampled at 200 Hz (AMTI, Netforce, Watertown, MA, United States) and data were passed through a fourth order low pass Butterworth filter with a 20 Hz cut-off frequency. DPSI was calculated using the first 3 s of the ground reaction forces following initial contact, defined as the instant the vertical ground reaction force exceeded 15 N (equation below). An average DPSI from the three trials in each condition was used for further analysis.

$$DPSI = \left( \frac{\sum (GRFx)^2 + \sum (GRFy)^2 + \sum (body\ weight - GRFz)^2}{number\ of\ data\ points} \right)$$

## Tandem Walk

For the tandem walk test, participants were asked to walk along a 2-m length balance beam (8 cm width), starting with the dominant foot, and complete three measurements for each arm position. The width of the beam was chosen based on previous research (Sawers and Ting, 2015) and prior feasibility testing. All participants wore comfortable shoes. Walking speed was self-selected, but participants were aware they were being timed.

The time taken to step on to the balance beam, walk along the balance beam, step off, turn around, step back onto the balance beam and return back to the original position was recorded in seconds by two raters using a stopwatch. Task failure was defined as stepping off the beam during the trial. For safety, two members of staff walked either side of the participant to prevent falling, but without interfering with the test. The fastest times for free and restricted arm movements were used in subsequent analysis.

## Data Analysis

Data were analyzed using SPSS version 24.0 (IBM Inc., Chicago, IL, United States). Paired *t*-tests were carried out to determine differences in dynamic balance and mobility performance between free arm and restricted arm movements. For all analyses, normality (Shapiro–Wilk test) and homogeneity of variance/sphericity (Levene’s test) were performed and confirmed prior to parametric analyses. Data were also analyzed for practical meaningfulness using magnitude-based inferences. Magnitude of effect size (Cohen’s *d*) was calculated for all metrics and were interpreted using thresholds of  $\leq 0.2$  (trivial), 0.2 (small), 0.6 (moderate), 1.2 (large), and 2.0 (very large) (Hopkins et al., 2009). Statistical significance was accepted at  $P \leq 0.05$ .

## RESULTS

Recognizing that gender and maturation status may influence the performance of balance assessments, as part of our initial exploratory analyses we conducted a 2 (*gender*; male and female)  $\times$  2 (*arm contribution*; free and restricted) way analysis of co-variance (ANCOVA), controlling for APHV to determine the effects of gender as a between-subject factor. There were no significant interactive or main effects of gender for any of the outcome measures. Arm movement had the greatest effect in the balance beam test (**Table 1**). Mean balance beam walking time increased by 1.5 s (19.2%) when participants

arm movements were restricted ( $t_{(28)} = -10.889$ ,  $P < 0.001$ ,  $d = 1.01$ ) (**Figure 1A**). In contrast, the DPSI did not show statistically significant changes with restricted arm movement ( $t_{(28)} = 0.940$ ,  $P = 0.335$ ,  $d = -0.08$ ) (**Figure 1B**). The Y Balance reach distance decreased in the anterior (mean diff; 6.3 cm,  $t_{(28)} = 11.563$ ,  $P < 0.001$ ,  $d = -1.13$ ), posteromedial (mean diff; 9.6 cm,  $t_{(28)} = 6.627$ ,  $P < 0.001$ ,  $d = -0.85$ ) and posterolateral (mean diff; 10.6 cm  $t_{(28)} = 8.653$ ,  $P < 0.001$ ,  $d = -0.92$ ) directions when arm movements were restricted. Accordingly, composite Y Balance score also decreased by 9.6% ( $t_{(28)} = 7.638$ ,  $P < 0.001$ ,  $d = -0.63$ ) (**Figures 2A–C**). Although none of the participants were classified as at-risk with free arm movements, four participants were identified as at-risk when the arms were restricted (**Figure 2D**).

## DISCUSSION

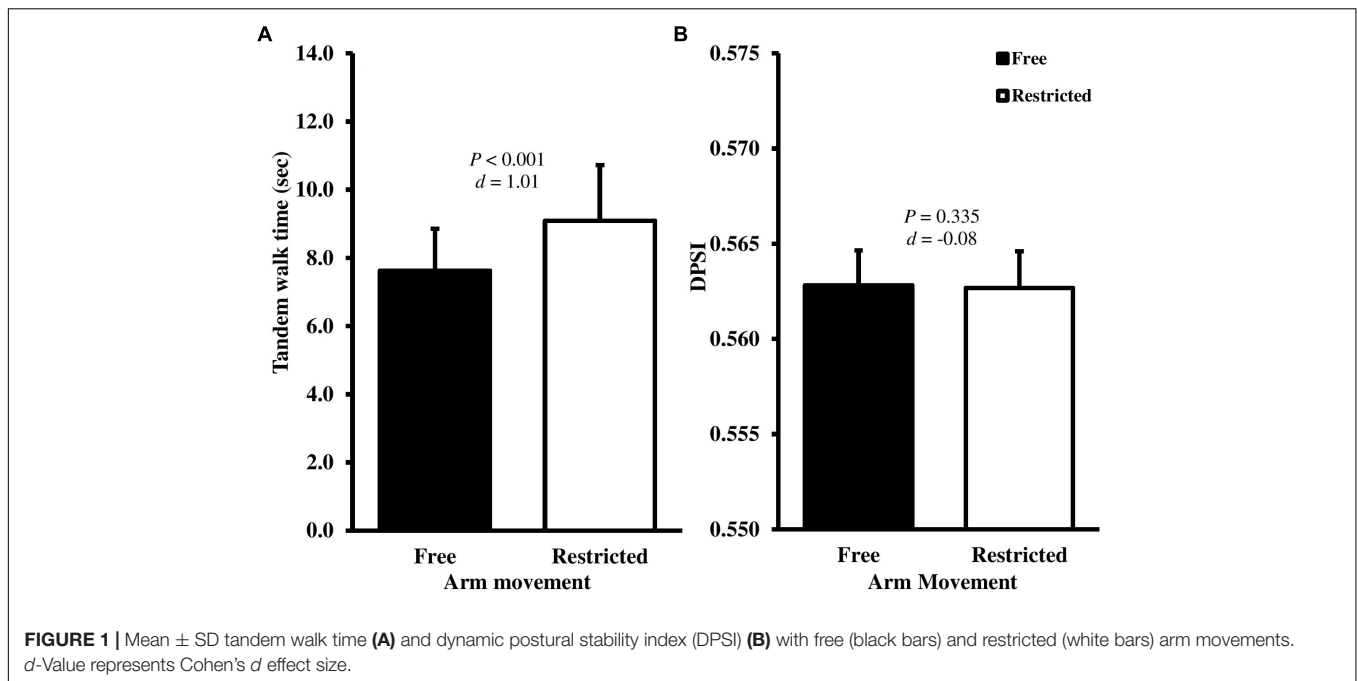
This is the first study to examine the effect of the use of the arms on multiple measures of dynamic postural control in a pediatric population. The results are therefore novel and have practical application for physical therapists, sport and exercise scientists, physical educators and strength and conditioning coaches who work with children to enhance movement performance. We found moderate to large magnitude reductions in the performance of two out of three dynamic postural control tests when arm movements were restricted, partially supporting our hypothesis. The results of the present study suggest that arm movements significantly influence performance in dynamic postural situations in children. Such findings align with prior work conducted in adults which suggests the existence of an “upper body strategy” (Hsu et al., 2007; Boström et al., 2018). These important findings also indicate that upper body movements should be incorporated into assessment protocols designed to test and improve postural control in children. More specifically, arm movements could be standardized (i.e., arms stretched out at an angle of 90° shoulder abduction in the frontal plane) or completely restricted (i.e., hands across chest).

Previous research has indicated that a composite Y Balance reach score of less than or equal to 94% was significantly associated with lower extremity injury (Plisky et al., 2006). As expected, a significant degradation in reach distance was observed when arm movements were restricted. Although only four participants fell below the 94% criterion line with restricted arm movements, all participants demonstrated a reduction in reach distance ( $\Delta -2$  to  $-22\%$ ), indicating a general decline in dynamic postural control. Although the result of the Y Balance test are not intended to infer an increased risk of injury, this study seeks to evaluate the extent to which the arms contributed (or not) to performance in dynamic tasks in children, as no study to date had examined this in children. This is an important first step before additional exploration could be undertaken in relation to injury risks. Importantly, we found that anterior reach distance was the most affected direction with restricted arm movements. These findings are in direct contrast to previous reports in young adults, where anterior reach distance did not

**TABLE 1** | Mean  $\pm$  SD and Cohen’s *d* effects size for the difference in dynamic balance performance between free and restricted arm movement conditions.

Variable	Free-arm movement	Restricted arm-movement	Cohen’s <i>d</i>
Tandem walk (sec)	7.6 $\pm$ 1.2	9.1 $\pm$ 1.6*	1.01
Dynamic postural stability index	0.563 $\pm$ 0.002	0.563 $\pm$ 0.002	-0.08
Y Balance test anterior direction (% leg length)	74.0 $\pm$ 5.3	67.7 $\pm$ 5.9*	-1.13
Y Balance test posteromedial (% leg length)	108.4 $\pm$ 9.3	98.8 $\pm$ 11.6*	-0.92
Y Balance test posterolateral (% leg length)	107.9 $\pm$ 11.3	97.7 $\pm$ 13.3*	-0.83
Composite score (% leg length)	122.5 $\pm$ 14.7	113.1 $\pm$ 15.8*	-0.62

\*Indicates significant difference between free and restricted arm conditions ( $P < 0.001$ ).



differ between free and restricted arm movements (Hébert-Losier, 2017). Anterior reach with the lower limb involves posterior displacement of body mass away from the base of support, which is an uncommon task (i.e., upper body leaning backward). In contrast, posterior reach directions require the body mass to be displaced anteriorly (i.e., upper body reaching forward), which is more functionally relevant and familiar to children. Therefore, the posteromedial and posterolateral reach directions may utilize more practiced motor patterns and are thus less susceptible to change than anterior reach seen here. The most likely explanation for the better reach performance in the free arm movement condition relates to the mechanical effects of outstretching the arms. Specifically, greater dispersion of body mass in the sagittal plane from a vertical line perpendicular from the base of support increases the moment of inertia, which should theoretically increase stability of the postural control system. Additionally, free arm movements may generate restoring torque to aid dynamic postural control (Patel et al., 2014).

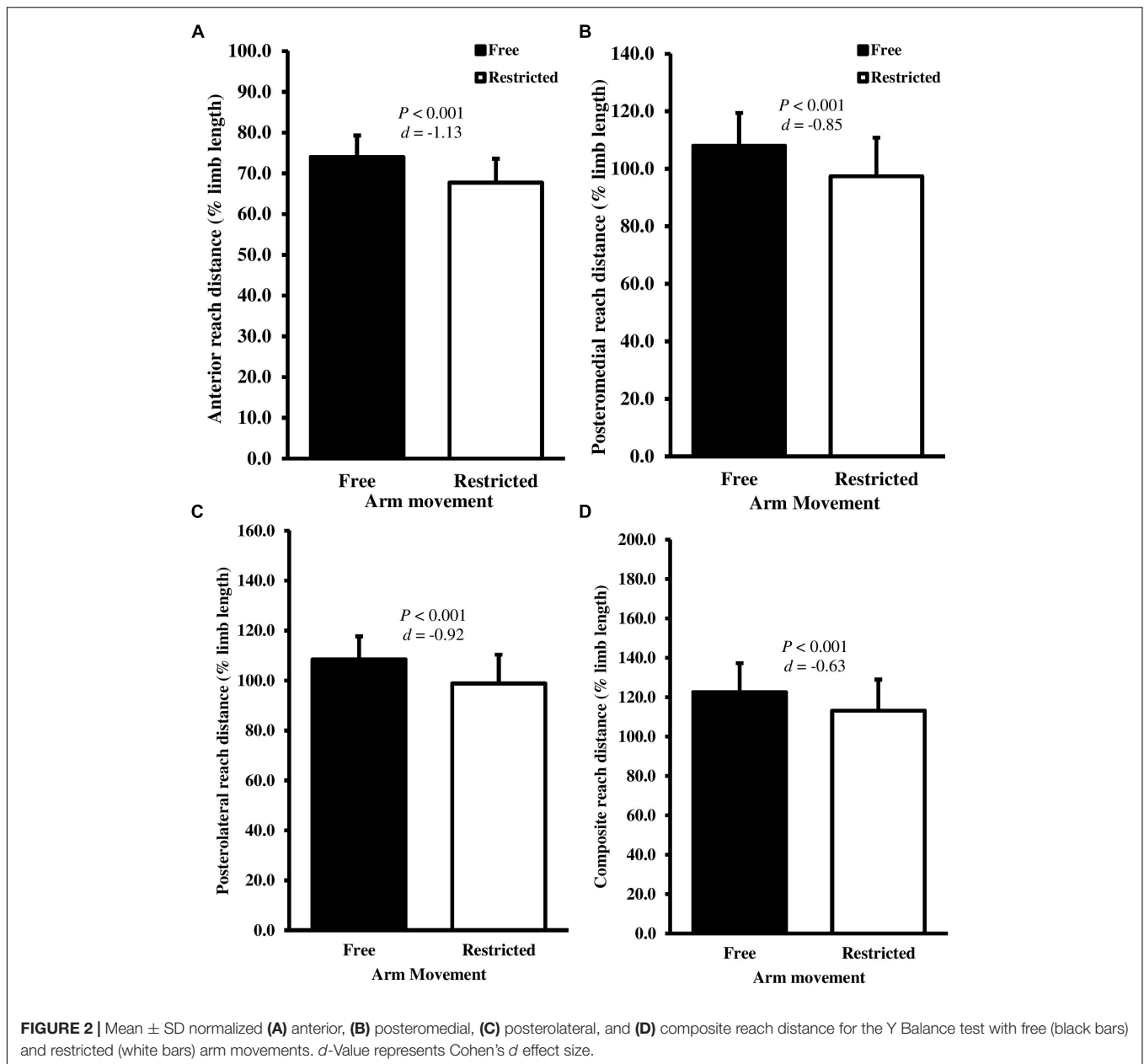
Data indicated the timed beam walk was suitable as a quantitative assessment of dynamic stability based on its sensitivity and discriminatory capability in this sample. The timed tandem beam walk test lacks predictive validity/diagnostic cut-off values, therefore, the 1.5 s mean difference in performance is harder to practically interpret. However, this finding suggests that the arms beneficially contribute to one or more of the tasks of stepping up, stepping down, turning or walking across a narrow beam. With respect to the later, Boström et al. (2018) recently examined movements of the upper and lower body during tandem beam walking. They reported that when the task became more difficult (i.e., for narrower beam width), the contribution of upper body movements to balance maintenance increased, while the lower body contribution remained the same. Taken together,

these findings suggest that the arms hierarchically compliment the lower body during dynamic balance scenarios.

In the present study, the DPSI was the only postural control metric which did not show significant changes with restricted arm movement. This finding was not expected. Outstretching the arms has been shown to reduce postural sway during quiet tandem standing (Patel et al., 2014). It is possible that ankle, knee and hip neuromuscular response strategies would effectively respond to the directionality of this task (i.e., anterior jump). Thus, improvements in performance with the arms stretched out to the side may only be evident under task constraints which challenge postural control in the frontal plane (i.e., tandem walk, or lateral jumps) (Milosevic et al., 2011; Patel et al., 2014; Hébert-Losier, 2017). A further possibility for the non-significant improvement in DPSI seen here reflects a “ceiling effect” in the restricted arm movement condition. Specifically, the task of jumping forward and landing on a single limb may have already been close to optimal, and therefore using the arms freely does now allow for any noticeable improvement in balance. Further, much like the Y Balance test and the tandem walk, the DPSI is quantitative in nature. Therefore, it is possible that postural control strategies helped absorb the vertical ground reaction force from contact to stabilization of the vertical displacement of the COM during landing.

## Implications

There are several important implications to be garnered from the present study. Firstly, this study provides the first direct evidence that the arms play a functionally relevant role in certain dynamic postural tasks among children. In agreement with previous research (Milosevic et al., 2011; Hébert-Losier, 2017) we support the recommendations that future research should clearly define and describe arm placement and movement



to avoid misinterpretation of dynamic balance tests and to facilitate experimental replication. Similarly, studies that adopt either a restricted or free arm movement should not be used interchangeably. The intent of this study was not to simply provide recommendations for arm placement, as this will depend upon the aims of the clinician/therapist. Instead, this proof of concept study intended to elucidate the importance of the use of the arms as a critical factor in postural stability strategies to better inform clinicians, physical therapists, researchers and practitioners for the purposes of identifying impairments, planning individualized interventions and evaluating change over time. We suggest that permitting arm movements is more functionally relevant to typical activities of daily living, but it is difficult to control the variability and dynamic nature of how

individuals use the arms. In contrast, restricting arm movements is likely to provide a more definite and standardized assessment of lower limb function. More specifically, this task controls for the differential use of the arms to overcome a lack of postural control demonstrated by the ankle, knee, and hip postural control mechanisms. Thus, assessing the difference in performances scores during restricted and non-restricted protocols may provide a better understanding of the extent to which people use their arms to further improve balance even when their lower body postural control is well-developed. These findings might also provide the impetus to develop training interventions to enhance postural control by employing constraint-based strategies with the arms in activities of daily living. Specifically, the observed improvement in performance with free arm movement suggests

that initially allowing arm movements may be valuable in acting as a starting point as part of a continuum of balance training to progress to more challenging programs (i.e., restricted arm movements). In contrast, it may also be appropriate to restrict arm movements to decrease the moment of inertia to promote more effective control of the COM by focusing on ankle, knee and hip coordinative development strategies. Such proximal-distal strategies may promote a more sensitive anticipatory and/or recovery postural response mechanism.

## Limitations

A few limitations in this work should be acknowledged. Firstly, we were unable to measure or control arm movements (i.e., kinematics) during either of the free or restricted conditions. As no quantitative movement analysis was undertaken we are aware that this study cannot comprehensively contribute to understanding upper body strategies used for movement. We are conscious that, due to the demands placed on participants and their age, we were unable to also measure ankle, knee, and hip postural control mechanisms. Future research would, however, be welcome which addresses this issue. Secondly, we calculated the DPSI from a forward jump. Given the important contribution of arm movements to lateral postural control, future studies should examine the effects of arm movement on a lateral jump (Wikstrom et al., 2005; Sell, 2012). A more detailed analysis of dynamic stability should also include the anteroposterior and mediolateral stability index (Wikstrom et al., 2006). We did not calculate the mediolateral stability index because it has previously been shown to have poor test-retest reliability ( $r = 0.38$ ) and a high standard error of measurement as a percentage of the mean score (26.1%) compared to the DPSI (3.7%) (Wikstrom et al., 2005). Another limitation was that we only examined Y Balance test performance on the dominant limb (stance limb). Therefore, we are precluded from calculating asymmetry between the dominant and non-dominant leg. It is likely that the detrimental effects of arm restriction on Y Balance test performance would be more pronounced on the non-dominant leg. Finally, we did not measure reactive balance. Several studies have reported that arm movements play a functional role in trip recovery in response to perturbations during standing (Allum et al., 2002; Maki and McLroy, 2006) and walking (Marigold et al., 2003; Roos et al., 2008; Pijnappels et al., 2010) scenarios.

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## CONCLUSION

In summary, this is the first study to demonstrate evidence of a marked degradation in the performance of the balance beam walking and Y Balance tests when arm movements are restricted among children. Overall, it appears feasible for clinicians and practitioners to test the effectiveness of arm usage in balance and mobility tasks by calculating difference scores between restricted and non-restricted arm conditions. The results highlight that the difference score between the restricted and non-restricted conditions provide a robust quantitative assessment that may identify individuals that rely more heavily on lower limb postural stability mechanisms. Future research should expand on these findings by exploring which individuals rely most on arm movements for postural control (i.e., potential moderator variables).

## AUTHOR CONTRIBUTIONS

MH, MW, AP, and MD conceived and designed the research. MH, AP, and MW conducted the experiments. MH performed the analyses and wrote the manuscript. AP, MD, MW, and DS revised the manuscript. All authors read and approved the final manuscript.

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# Ventilatory Limitation of Exercise in Pediatric Subjects Evaluated for Exertional Dyspnea

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**Purpose:** Attribution of ventilatory limitation to exercise when the ratio of ventilation ( $\dot{V}_E$ ) at peak work to maximum voluntary ventilation (MVV) exceeds 0.80 is problematic in pediatrics. Instead, expiratory flow limitation (EFL) measured by tidal flow-volume loop (FVL) analysis – the method of choice – was compared with directly measured MVV or proxies to determine ventilatory limitation.

**Methods:** Subjects undergoing clinical evaluation for exertional dyspnea performed maximal exercise testing with measurement of tidal FVL. EFL was defined when exercise tidal FVL overlapped at least 5% of the maximal expiratory flow-volume envelope for > 5 breaths in any stage of exercise. We compared this method of ventilatory limitation to traditional methods based on MVV or multiples (30, 35, or 40) of FEV<sub>1</sub>. Receiver operating characteristic curves were constructed and area under curve (AUC) computed for peak  $\dot{V}_E$ /MVV and peak  $\dot{V}_E$ /X·FEV<sub>1</sub>.

**Results:** Among 148 subjects aged 7–18 years (60% female), EFL was found in 87 (59%). Using EFL shown by FVL analysis as a true positive to determine ventilatory limitation, AUC for peak  $\dot{V}_E$ /30·FEV<sub>1</sub> was 0.84 (95% CI 0.78–0.90), significantly better than AUC 0.70 (95% CI 0.61–0.79) when 12-s sprint MVV was used for peak  $\dot{V}_E$ /MVV. Sensitivity and specificity were 0.82 and 0.70 respectively when using a cutoff of 0.85 for peak  $\dot{V}_E$ /30·FEV<sub>1</sub> to predict ventilatory limitation to exercise.

**Conclusion:** Peak  $\dot{V}_E$ /30·FEV<sub>1</sub> is superior to peak  $\dot{V}_E$ /MVV, as a means to identify potential ventilatory limitation in pediatric subjects when FVL analysis is not available.

**Keywords:** flow-volume curve, flow limitation, ventilation, dyspnea, exercise, children

## INTRODUCTION

The ventilatory response to exercise changes through childhood and adolescence (Cooper et al., 1987). Younger children have higher ventilatory equivalents (Armstrong et al., 1997; Prioux et al., 1997) such that they achieve levels of ventilation ( $\dot{V}_E$ ) near maximum voluntary ventilation (MVV) at peak work (Godfrey, 1974); but this morphs through adolescence until peak  $\dot{V}_E$ /MVV ratio reaches typical adult levels (Rowland and Cunningham, 1997; Giardini et al., 2011). Pre-pubertal children develop significant expiratory flow limitation (EFL) during exercise (Nourry et al., 2006;

Swain et al., 2010; Borel et al., 2014) with the prevalence of EFL decreasing post-puberty due at least in part to lower ventilatory requirement (Emerson et al., 2015; Smith et al., 2015). It behooves one to understand EFL during exercise as it may limit  $\dot{V}_E$ , worsen dyspnea, or reduce capacity (Dempsey et al., 2008; Babb, 2013).

Cardiopulmonary exercise testing is used clinically to investigate exertional dyspnea. The traditional method of assessing ventilatory limitation is based on breathing reserve (Ross, 2003):

$$100\% \cdot [1 - \text{peak} \dot{V}_E / \text{MVV}]$$

This approach is problematic as it ignores the fact that the maximal voluntary ventilation (MVV) maneuver does not mimic the breathing pattern or respiratory mechanics that occur during exercise (Klas and Dempsey, 1989; Agostoni et al., 2011). Prevailing wisdom in pediatric exercise medicine maintains that children achieve “near to or slightly less than 70% of their MVV at maximal ventilation” (Orenstein, 1993; Takken et al., 2017) but this observation has never been directly tested. Furthermore, proxy measures for MVV based on multiples of FEV<sub>1</sub> are often used rather than direct measurement of MVV but *only* 35·FEV<sub>1</sub> has been examined in a pediatric population. Specifically, Fulton et al. (1995) found that MVV was similar to the proxy measure of MVV viz 35·FEV<sub>1</sub> in healthy African-American, adolescent girls. Furthermore, a recent study found that conclusions from test results depend on which surrogate for MVV is chosen only compounds interpretation challenges (Colwell and Bhatia, 2017). Taken together, it is not at all clear whether directly measured MVV or multiples of FEV<sub>1</sub> as proxies for MVV reflect EFL and therefore ventilatory limitation during exercise in children and adolescents.

Thus, the central question of this study is whether a simple, reproducible test that incorporates confounding and inter-related variables such as age, sex, and height, enables one to confidently identify whether a subject would exhibit EFL during exercise. Tidal flow-volume loop (FVL) analysis was used as the method of choice to confirm EFL during exercise and compare with directly measured MVV vs. multiples of FEV<sub>1</sub> as proxies for MVV. We hypothesized that a proxy measure of MVV based on FEV<sub>1</sub> predicts development of EFL and ventilatory limitation during exercise in pediatric subjects undergoing testing for investigation of exertional dyspnea.

## MATERIALS AND METHODS

### Subjects

Medical records of children and adolescents up to 18 years of age seen at Mayo Clinic from 2007 to 2014, who underwent clinically indicated, maximal exercise tests with FVL analysis as part of clinical evaluation of exertional dyspnea were audited retrospectively. Subjects with exercise-induced laryngeal obstruction that was diagnosed by continuous laryngoscopy during exercise were excluded. The cohort was comprised of patients with known asthma who still complained of exertional dyspnea despite aggressive therapy, patients with disease in other

organ systems affecting the respiratory system, and subjects evaluated for exertional dyspnea or chest pain in which no cause was found and thus had no specific medical diagnosis. Diagnosis of asthma required a history of compatible symptoms plus evidence of airway hyperreactivity or bronchodilator responsiveness. Patients with asthma were clinically stable when tested. Informed consent was not required as testing was conducted for clinical indications. Minnesota statute permits retrospective chart review for an IRB approved protocol. Mayo Clinic Institutional Review Board approved the study with waiver of consent.

### Pulmonary Function Tests

Routine instructions for pulmonary function testing (PFTs) included avoiding short-acting bronchodilator for at least 4 h and long-acting bronchodilators for at least 12 h. All subjects performed PFTs immediately prior to exercise on the same MedGraphics system used for the exercise test (see below) according to ATS/ERS criteria (Miller et al., 2005). Subjects performed PFTs while seated on the cycle ergometer and their largest maximum expiratory flow-volume envelope was used for analysis. PFTs were expressed as percent of predicted (Knudson et al., 1983). MVV was measured in all subjects by the 12-s sprint method on a Jaeger MasterScreen, on the same day as exercise or within the same week (median [IQR] time 0 [0–4] days) in a subset of subjects undergoing bronchoprovocation challenge (to complete workup for exertional dyspnea). Post exercise PFTs were performed only at the discretion of the triage physician, as all testing was done for clinical indications.

### Exercise Test

Subjects were instructed to fast 2 h before the test. They performed a maximal exercise test on a Corival V3 cycle ergometer. We employed James' protocol consisting of three programs for three ranges of body surface area starting at 200 kg m min<sup>-1</sup> with increments of 100 or 200 kg m min<sup>-1</sup> every 3 min depending on body surface area prior to 2008 (James, 1981). All subsequent tests were done using Godfrey protocol (Godfrey, 1974) starting at 10 to 25 W, with step increments of 10 to 25 W min<sup>-1</sup> based on subject's height and sex, in order to obtain test duration of 10 ± 2 min. Patients were strongly encouraged to exercise to volitional fatigue in order to achieve criteria (e.g., gas exchange ratio > 1.1, HR > 190 bpm) implying maximal effort. Heart rate and SpO<sub>2</sub> were monitored continuously with a 12-lead ECG and pulse oximetry, respectively. Blood pressure was measured every other workload.

### Ventilatory Measurements During Exercise

Ventilation and gas exchange were measured breath-by-breath via MedGraphics CPX/D (Breeze software) that employs a Pitot tube to measure flow, electronically integrated to yield volume. The software corrects for drift that occurs when inspiratory and expiratory volumes differed. Exhaled gasses



were measured by mass spectrometry. System calibration was done prior to every exercise test. The Breeze© program measures EFL according to method described by Johnson et al. (1995) at Mayo Clinic. In short, the degree of EFL was obtained by aligning a tidal breath during exercise within the maximum expiratory flow-volume curve. Alignment was achieved by having subjects perform an inspiratory capacity (IC) maneuver from end-expiratory lung volume. The program permits review of individual FVLs on a breath by breath basis, and automatically computes percent overlap of tidal breath with maximum expiratory FVL. ERV expiratory reserve volume was calculated by subtracting IC from forced vital capacity. IC maneuvers were rehearsed prior to exercise until subjects demonstrated acceptable consistent maneuvers. Once exercise began, an IC maneuver was repeated during a 3-min warm-up at the first workload, then every other load (alternating with blood pressure) until the respiratory compensation point, after which most subjects were able to perform only 1–2 more loads before blood pressure check at peak exercise.

## Definition of EFL

Consensus definition of EFL has not yet been formulated. Nourry et al. (2005) defined EFL to occur when “part of the exercise FVL met the boundary of the expiratory portion of the maximum expiratory FVL determined before exercise” and considered subjects to be flow limited when EFL was observed over  $\geq 5\%$  exercise tidal volume, maintained up to peak work. Swain et al. (2010) similarly defined EFL when intersection of the exercise tidal loop and maximal FVL was  $>5\%$  for any breath. We defined EFL as  $\geq 5\%$  tidal volume overlap with the maximum expiratory flow-volume envelope for  $>5$  breaths during sub-maximal or peak exercise.

## Statistical Analysis

Comparisons between subjects with and without EFL were evaluated using two-sample  $t$ - or  $\chi^2$  tests as appropriate. Different multiples ( $x$ ) of FEV<sub>1</sub> were calculated and predictive ability using area under curve (AUC) was computed from ROC curves plotted for peak  $\dot{V}_E/MVV$  and peak  $\dot{V}_E/x \cdot FEV_1$ , using presence of EFL as the method of choice to determine exercise limited by ventilation. Optimal cutoff was chosen as the value with highest sensitivity and specificity using the point closest to perfect separation. This analysis was done using R software v. 3.2.3, with significance set at  $p < 0.05$ . We dichotomized subject into flow limited and non-flow limited, such that EFL was a fixed factor and workload was fitted as a within-subjects factor, to analyze behavior of operating lung volume during exercise between subjects with vs. without EFL. Changes in operating lung volume were calculated as change from rest in expiratory reserve volume (ERV) and IC as fractions of a one's VC; i.e., ERV/VC and IC/VC. As these were not measured at every workload, they were binned according to relative exercise intensity: 8–19, 20–55, and 55–90% peak workload for analysis. A mixed effects regression model was fit to assess the effect of EFL on ERV/VC and on IC/VC at different workloads using Stata v.14.0.

## RESULTS

### Subjects

The final sample comprised 148 subjects: mean  $\pm$  SD age  $14.3 \pm 2.6$  years, height  $164 \pm 13$  cm, and weight  $59.3 \pm 15.5$  kg. Eighty-six (58%) subjects presented as exertional dyspnea (DoE) with no underlying disorder, and 38 (26%) had asthma. The remaining 24 (16%) subjects (“other”) comprised congenital heart disease ( $n = 7$ ), pectus excavatum ( $n = 4$ ), colitis ( $n = 2$ ), bronchiectasis ( $n = 2$ ), cardiac dysrhythmia ( $n = 2$ ), plus one subject each with mediastinal fibrosis, postural tachycardia syndrome, CF-related metabolic syndrome, scoliosis with fused ribs, weakness, angioedema, and post-ARDS, presenting with dyspnea or chest pain on exertion. Lung function data are shown in **Table 1**.

### EFL During Exercise

Expiratory flow limitation occurred at some point during exercise in 87 (59%) subjects, over  $49 \pm 21\%$  (mean  $\pm$  SD) of tidal volume. Onset of EFL during exercise occurred in lighter exercise in those with more obstruction (greater concavity of resting maximum expiratory FVL) but not until peak exercise in those with normal spirometry (Pianosi, 2018b). Subjects with EFL achieved higher values for peak  $\dot{V}O_2$  with concomitant higher minute volume and O<sub>2</sub> pulse at peak exercise (**Table 2**). No subjects exhibited SpO<sub>2</sub>  $< 94\%$ .

All subjects augmented tidal volume at onset of exercise by decreasing ERV. The effect of work level on ERV/VC did not show a linear trend as the largest change occurred between rest and light exercise, mirrored by a rise in IC/VC. Those who developed EFL maintained this strategy, causing IC/VC to rise further; whereas other subjects averted EFL by subsequently raising end-expiratory lung volume back to near resting levels, such that IC/VC remained relatively stable despite rising exercise intensity. Indeed, ERV/VC was consistently higher (all  $p$ -values  $< 0.002$ ) and IC/VC consistently lower in (all  $p$ -values  $< 0.003$ ) non-EFL subjects once exercise began (**Figure 1**). The addition of an interaction term for EFL\*Work did not add significantly to the model fit ( $p = 0.27$ ).

**TABLE 1** | Pulmonary function test results, means (SD), split by EFL.

	No EFL	EFL	$p$ -value*
FVC (%pred)	105 (15)	101 (17)	0.16
FVC (L)	3.94 (1.05)	3.85 (1.20)	0.65
FEV <sub>1</sub> (%pred)	103 (15)	94 (17)	0.0007
FEV <sub>1</sub> (L)	3.97 (1.06)	3.32 (0.59)	0.09
FEV <sub>1</sub> /FVC	0.86 (0.07)	0.88 (0.08)	$<0.0001$
FEF <sub>50</sub> (%pred)	81 (23)	64 (22)	$<0.0001$
FEF <sub>50</sub> (L·s <sup>-1</sup> )	4.09 (1.20)	3.39 (1.26)	0.0008
MVV (L·min <sup>-1</sup> )	93 (28)	98 (37)	0.37

\*Unpaired  $t$ -tests.

**TABLE 2** | Peak exercise results in each sex stratified by EFL.

Mean $\pm$ SD	Females		Males	
	No EFL (N = 43)	EFL (N = 46)	No EFL (N = 18)	EFL (N = 41)
Age (years)	14.9 $\pm$ 2.0	14.7 $\pm$ 2.8	13.6 $\pm$ 3.5	13.7 $\pm$ 2.6
Height (cm)	163.4 $\pm$ 7.6	160.7 $\pm$ 12.1	165.7 $\pm$ 20.2	166.2 $\pm$ 15.6
Weight (kg)	58.3 $\pm$ 10.7	57.0 $\pm$ 13.4	59.5 $\pm$ 19.7	62.8 $\pm$ 19.9
BMI (kg·m <sup>-2</sup> )	21.8 $\pm$ 3.5	21.8 $\pm$ 4.0	21.1 $\pm$ 4.6	22.3 $\pm$ 4.6
BSA (m <sup>2</sup> )	1.62 $\pm$ 0.16	1.59 $\pm$ 0.23	1.65 $\pm$ 0.37	1.69 $\pm$ 0.32
Work (W)	144 $\pm$ 37	160 $\pm$ 56	173 $\pm$ 70	185 $\pm$ 69
Work (%pred*)	88 $\pm$ 23 <sup>†</sup>	102 $\pm$ 24 <sup>†</sup>	77 $\pm$ 19	90 $\pm$ 25
$\dot{V}O_2$ (mL·kg <sup>-1</sup> ·min <sup>-1</sup> )	33.1 $\pm$ 6.1 <sup>†</sup>	36.2 $\pm$ 7.5 <sup>†</sup>	39.0 $\pm$ 7.3	39.5 $\pm$ 9.2
$\dot{V}O_2$ (%predicted*)	89.5 $\pm$ 18.0	99.1 $\pm$ 19.4	85.4 $\pm$ 16.1	88.3 $\pm$ 17.5
HR (beat·min <sup>-1</sup> )	184 $\pm$ 14	185 $\pm$ 11	185 $\pm$ 15	185 $\pm$ 13
O <sub>2</sub> pulse (mL·beat <sup>-1</sup> )	10.4 $\pm$ 2.3	11.1 $\pm$ 3.3	12.3 $\pm$ 4.1	13.2 $\pm$ 4.3
RR (breath·min <sup>-1</sup> )	47 $\pm$ 9 <sup>‡</sup>	53 $\pm$ 9 <sup>‡</sup>	55 $\pm$ 16	54 $\pm$ 9
VT (L)	1.55 $\pm$ 0.33	1.58 $\pm$ 0.52	1.75 $\pm$ 0.91	1.77 $\pm$ 0.66
$\dot{V}_E$ (L·min <sup>-1</sup> )	69.7 $\pm$ 16.1 <sup>□</sup>	82.3 $\pm$ 25.0 <sup>□</sup>	84.5 $\pm$ 32.2	93.1 $\pm$ 34.6
$\dot{V}_E / \dot{V}O_2$	37.6 $\pm$ 5.9	40.2 $\pm$ 4.8	37.3 $\pm$ 5.8	38.1 $\pm$ 6.1
RER	1.15 $\pm$ 0.08	1.20 $\pm$ 0.09	1.16 $\pm$ 0.12	1.19 $\pm$ 0.10
PetCO <sub>2</sub> (mmHg)	35 $\pm$ 4	34 $\pm$ 4	35 $\pm$ 4	36 $\pm$ 4

\*Godfrey for Work Godfrey (1974) and Cooper et al. (1984). <sup>†</sup> $p \leq 0.006$ , <sup>‡</sup> $p = 0.001$ , <sup>□</sup> $p = 0.01$  (t-test).

## Relationship to Symptoms

Prevalence of EFL among the three groups is shown in **Table 3**. EFL was less common in subjects with DoE compared with the asthma or “other” groups ( $p = 0.038$ ). Dyspnea, alone or with leg fatigue, was cited by half the subjects as the reason for inability to continue at peak work, at similar rates in subjects with or without EFL ( $p = 0.80$ ).

## MVV vs. FVL

Maximum voluntary ventilation was similar in EFL vs. non-EFL subjects, but only slightly better than chance at identifying subjects who developed EFL during exercise with an AUC of only 0.69 (95% CI 0.61–0.78). AUC for multiples of FEV<sub>1</sub> as surrogate measures for MVV are shown in **Table 4**. Having shown no difference in AUC among FEV<sub>1</sub> multiples, **Table 5** shows combinations of sensitivity and specificity for peak  $\dot{V}_E/30 \cdot \text{FEV}_{1.0}$ . The optimal cut-point from the AUC (**Figure 2**) was 0.853 (95% CI 0.764–0.894), significantly better ( $p < 0.001$ ) than MVV.

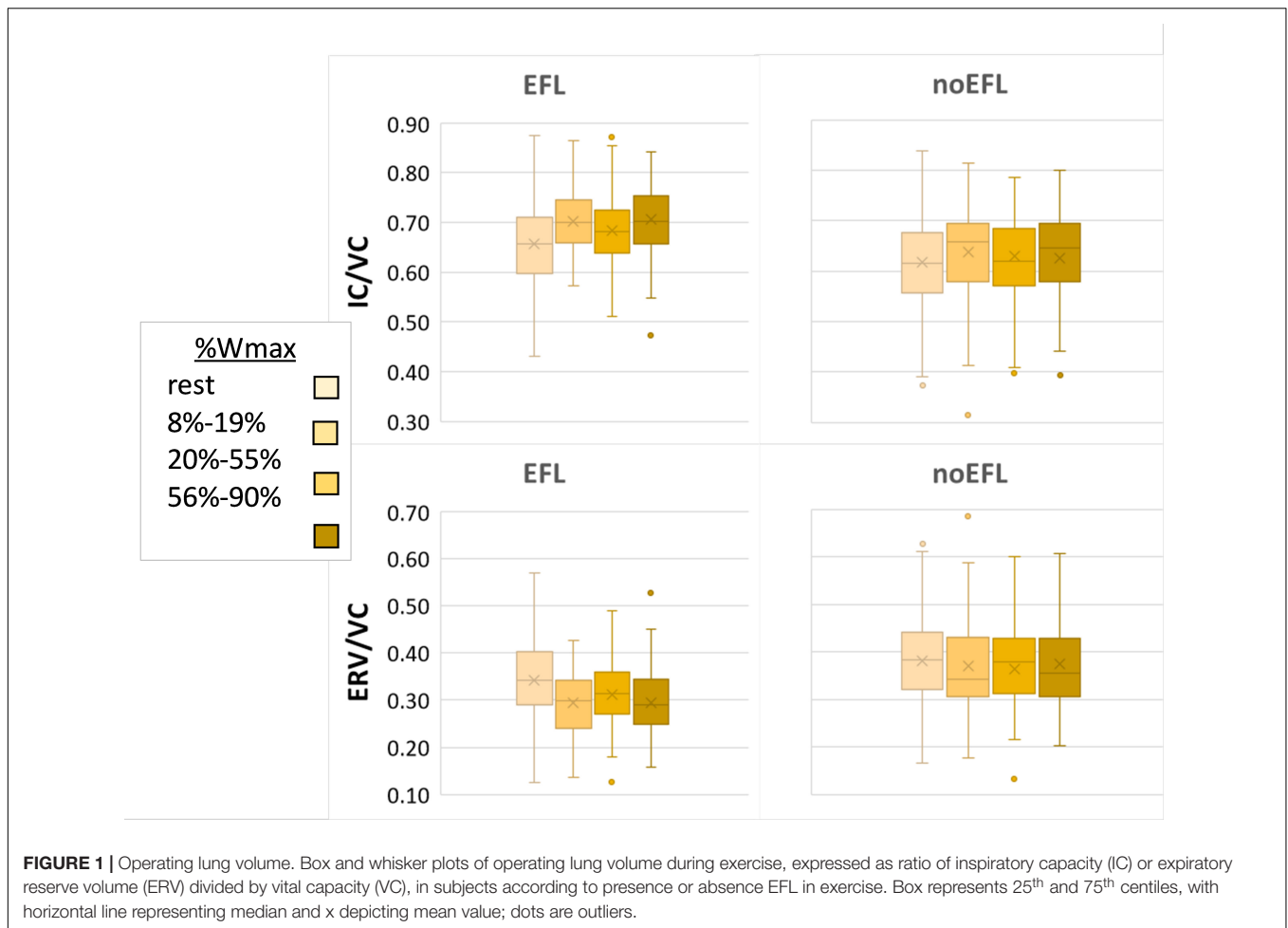
## DISCUSSION

Measured MVV was only marginally better than chance at identifying subjects with ventilatory limitation whereas FEV<sub>1</sub> was superior to 12-s sprint MVV for estimation of maximum breathing capacity on exercise compared to EFL demonstrated by tidal FVL analysis. Use of MVV as the benchmark for maximal exercise is problematic in children. Healthy pre-pubertal children often achieve levels of peak  $\dot{V}_E$  at or very close to MVV. Godfrey (Figure 4.2 of his book) speculated this observation was due to a mix of relatively high ventilatory requirements of younger children combined with their inability to properly

perform an MVV maneuver (Godfrey, 1974). A recent study indeed found that 26% of children were unable to properly perform MVV maneuver (MacLean et al., 2016). ROC curve analysis confirmed that measured MVV is not particularly useful for concluding ventilatory limitation to exercise compared to FVL analysis. Ventilatory reserve is defined by peak exercise  $\dot{V}_E$  as a fraction of MVV with the lower limit set at 15% (American Thoracic Society and American College of Chest Physicians, 2003). This cut-off is reasonable based on 95% confidence limits of adult norms but is not independent of fitness or aging (Ross, 2003). Newer textbooks on pediatric exercise medicine state “one can infer that ventilatory reserve increases with age, at least for males” (Bar-Or and Rowland, 2004); and children achieve “near to or slightly less than 70% (of MVV) at maximal ventilation” (Fawkner, 2007). We submit that tidal FVL demonstrating EFL is the preferred method for this designation, but one can be confident without said analysis if the ratio of peak  $\dot{V}_E/30 \cdot \text{FEV}_{1.0}$  exceeds 0.85. Our data adds wanting scientific rigor and validity to current interpretation standards. This index provides a target peak  $\dot{V}_E$  from which one can judge maximal effort; is similar to  $\dot{V}_E$  sustainable by adolescents during eucapnic voluntary hyperpnea (Van der Eycken et al., 2016); and to a surrogate measure for MVV in exercising CF patients (Stein et al., 2003).

## EFL and Ventilatory Limitation vs. Exercise Limitation

Numerous studies have been published using FVL analysis as a means of demonstrating ventilatory constraint to exercise despite limitations of the technique (Calverley and Koulouris, 2005). Exercise FVL have been used more often in adult studies but there is growing acceptance if not tacit recognition of its merits



and limitations to detect EFL during exercise. EFL is common in pre-pubertal children (Nourry et al., 2006; Swain et al., 2010), obese adolescents (Gibson et al., 2014), and more common in trained vs. untrained pediatric subjects (Nourry et al., 2005); but its prevalence falls after puberty from 90 to 45% in boys and from 90 to 20% in girls (Emerson et al., 2015; Smith et al., 2015) whose mean ages ranged from 14 to 15 years. Dysanaptic lung growth may contribute to this changing prevalence of EFL (Smith et al.,

2014). Nearly 60% of our subjects aged  $14.3 \pm 2.6$  years had EFL during exercise indicating an enriched group with EFL among subjects evaluated for dyspnea. The incidence of EFL in males and females was ~69 and 52%, respectively, which was higher than previously reported in the smaller cohort of post-pubescent adolescents (Emerson et al., 2015). This discrepancy is likely due to the inclusion of both pre, peri, and post-pubescent pediatric subjects in the present study; a caveat being gas compression

**TABLE 3 |** Stated cause for exercise cessation among the three diagnostic groups.

EFL	No (N = 61)	Yes (N = 87)	Total (N = 148)	p-value
<b>Diagnostic group</b>				
DoE	43 (70%)	43 (49%)	86 (58%)	0.038*
Asthma	11 (18%)	27 (31%)	38 (26%)	
Other	7 (12%)	17 (20%)	24 (16%)	
<b>Reason to stop</b>				
Leg fatigue	17 (28%)	20 (23%)	37 (25%)	0.6311
Dyspnea	24 (39%)	30 (35%)	54 (36%)	
Fatigue	11 (18%)	23 (26%)	34 (23%)	
Leg Fatigue +Dyspnea	9 (15%)	14 (16%)	23 (16%)	

\*Pearson's  $\chi^2$  test.

**TABLE 4** | Area under curve (AUC) results using MVV and different multiples of  $\dot{V}_{E1}$  in denominator.

Peak $\dot{V}_E/x \cdot \text{FEV}_1$ $x =$	AUC (95% CI)	Best cutoff to maximize sensitivity and specificity	Sensitivity	Specificity
0.30	0.840* (0.776, 0.905)	0.819	0.816	0.705
0.35	0.840	0.702	0.816	0.705
0.40	0.840	0.614	0.816	0.705
MVV	0.695 (0.614, 0.785)	0.850	0.623	0.617

\*95% CI identical for different multiples of  $\text{FEV}_1$ .

**TABLE 5** | Exploration of cut-off values for peak  $\dot{V}_E/30 \text{ FEV}_1$  compared to current interpretation standard.

Cut-point peak $\dot{V}_E/30 \cdot \text{FEV}_1$	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
>0.80	86	61	72	79
>0.85	76	78	80	74
>0.90	63	86	84	67
MVV*	62	62	71	54

PPV and NPV, positive and negative predictive values, respectively.

\*Using value > 0.850 for ratio of peak  $\dot{V}_E/\text{MVV}$ .

artifact that may overestimate EFL (see below). In addition, we found that females that exhibited EFL had greater peak  $\dot{V}_{O_2}$ , ventilation, and respiratory rate compared to females that did not exhibit EFL. These data suggest that girls who achieve higher workloads and metabolic rates are more likely to exhibit EFL during exercise similar to women (McClaran et al., 1998).

Mechanical constraints to  $\dot{V}_E$  affect operating lung volume and breathing pattern during heavy exercise in subjects who experience EFL (McClaran et al., 1999). Babb postulated that onset of dynamic airway compression may be just as critical as EFL in evoking adjustments to minimize degree of EFL during exercise (Babb, 2013). Such strategic changes may demand more perfusion of the respiratory muscles potentially depriving working leg muscles (Harms et al., 1997), and leg muscle fatigue resulting from high-intensity exercise is at least partly due to increased inspiratory muscle work (Dempsey et al., 2006). Unloading the respiratory muscles by Heliox breathing resulted in small but statistically significant boost in performance at heavy exercise (Wilkie et al., 2015); whereas mechanical unloading with proportional-assist ventilation resulted in clinically significant improvement in performance at 90% peak  $\dot{V}_{O_2}$  (Harms et al., 2000). Hyperinflation attenuates stroke volume at rest and response to exercise in healthy controls (Stark-Leyva et al., 2004; Cheyne et al., 2018). There is likely a hierarchy of blood flow distribution during exercise between respiratory vs. locomotor musculatures and muscle afferent feedback influence fatigue and dyspnea (Sheel et al., 2018).

## Exertional Dyspnea

Half the subjects cited breathing as the reason for exercise test cessation though no particular limiting symptom was cited more often among those with vs. without EFL. Some subjects continued to exercise despite developing EFL before finally stopping but one should not be surprised by an uncoupling ventilatory constraint

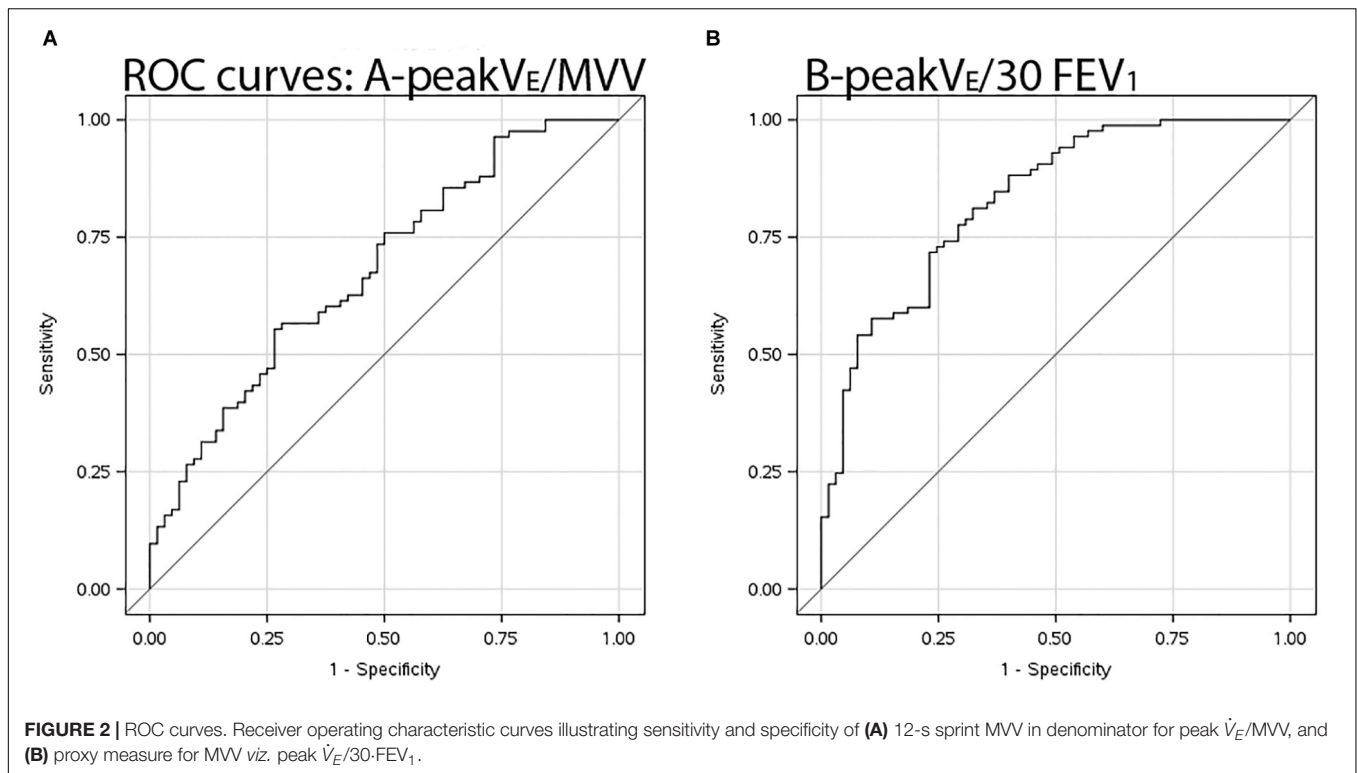
and exercise limited by dyspnea. The most likely explanation is that some subjects raised end-expiratory lung volume during heavy exercise to counter impending or evolving EFL, which would have generated greater elastic work of breathing as lung compliance falls at higher lung volumes. One might then cease exercise due to dyspnea without EFL manifest. Subjects in this report chose their breathing strategy early during exercise, in virtually all subjects before EFL developed. Examination of **Figure 2** suggests that subsequent behavior may perhaps have mitigated EFL but those with EFL clearly had lower end-expiratory lung volume than did those without EFL by heavy exercise. Results of studies in children offer disparate findings with respect to dyspnea in presence vs. absence of EFL (cf. Nourry et al., 2006; Swain et al., 2010) and studies in adults show similar discordance (Lovering et al., 2014; Wilkie et al., 2015). The reality is that our understanding of mechanisms of dyspnea in pediatrics is rudimentary (Pianosì, 2018a).

## Clinical Significance

Triage of exertional dyspnea in pediatric populations is hindered by lack of data, forcing clinicians to rely on empiricism derived from adult subjects. Abu-Hasan and Weinberger concluded respiratory limitation from “restrictive” physiology as responsible if a subject’s exercise ventilation fell within specified ranges of tidal volume, breath rate, and breathing reserve, based on extrapolation from adult studies (Abu-Hasan et al., 2005). Mahut et al. (2014) similarly concluded that the most frequent cause of exertional dyspnea in adolescents was “physiologic,” defined as normal aerobic power and ventilatory response. The present study creates a novel paradigm for attribution of exertional dyspnea in pediatric subjects using data obtained from pediatric subjects and suggests a possible explanation. Just as Dominelli et al. (2015) stated some healthy individuals are more likely to exhibit mechanical constraints during exercise, one may postulate the respiratory system is culpable in pediatric subjects evaluated for exertional dyspnea, particularly females who push themselves (**Table 2**). We believe it is no coincidence that exercise enhances every component of the  $O_2$  transport system except the lungs. Ergo, the lungs may contribute to the limitation of peak  $\dot{V}_{O_2}$  (Wagner, 2005).

## Limitations

There are caveats when using exercise tidal FVL to determine EFL (Guenette et al., 2010, 2013; Dominelli and Sheel, 2012). First, among alternative methods, only negative expiratory pressure (NEP) technique has been employed in pediatrics – and only in infants (Jones et al., 2000). We did not measure



IC at peak exercise, meaning placement of tidal breaths at peak exercise was based on IC maneuvers done at work up to 90% peak work. However, Nourry et al. (2006) showed minimal change in end-expiratory lung volume from this range to peak work. Second, the best acceptable maximum flow-volume curve at rest (pre-exercise) was used to place the exercise FVL within its maximum envelope. Swain et al. (2010) noted only ~3% difference between pre- and post-test maximum expiratory flow-volume curves. We essentially doubled this inherent variability when choosing our EFL threshold. Moreover, said factors could affect determination of EFL in *either* direction, and any bias should be nullified given the sample size, as these pitfalls could positively or negatively influence EFL adjudication. The effect of thoracic gas compression on FVLs when volume is measured at the mouth may affect identification of EFL during exercise, but there are no data concerning this in children and adolescents; and any presumption that adult studies apply to children or adolescents should be viewed with skepticism in view of the high prevalence of EFL in children. Though certainly present, we truly do not know the volume gas compression in pediatric subjects during exercise. During routine spirometry, its magnitude is unpredictable (Coates et al., 1988) but, with few exceptions, one would expect it to be small in the population reported herein, most of whom had normal spirometry. Our subject sample did not include patients with cystic fibrosis. Indeed, Stein et al. (2003) proposed an equation for calculating MVV based on FEV<sub>1</sub> in CF patients which performed nearly as well as an FEV<sub>1</sub>-based proxy measure, and which is very similar to our selection of 30·FEV<sub>1</sub>. Finally, dyspnea ratings were not

recorded because existing dyspnea scales had dubious validity in children when testing began in 2007. We recently published our validation studies in pediatric subjects (Pianosi et al., 2014, 2015), and found remarkable similarity in dyspnea ratings during exercise among healthy controls, subjects with asthma, or cystic fibrosis.

## CONCLUSION

30·FEV<sub>1</sub> is superior to MVV for assessing potential ventilatory limitation to exercise defined as >5% overlap of exercise (tidal FVL with maximal expiratory FVL) in pediatric subjects if tidal FVL analysis is unavailable and could be used to decide whether a child or adolescent experiences exercise limitation due to the respiratory pump. ROC analysis for breathing reserve < 0.15 yields positive predictive value of nearly 80% and negative predictive value of 75% for detecting EFL.

## Future Directions

Defining EFL is still a moving target which will be subject to change as our understanding of, and limitations to, using tidal FVL analysis to determine ventilatory limitation. There are caveats when using exercise tidal FVL to determine EFL, chief among which are understanding how thoracic gas compression during a forced expiratory maneuver or during active exhalation such as may occur during heavy exercise may affect flow rate at any given lung volume, so more research is necessary in this domain. Ideally, this should be explored by plotting iso-volume pressure-flow diagrams, or employing NEP technique

(Eltayara et al., 1996), to quantify how thoracic gas compression affects FVL during exercise in pediatric subjects.

## ETHICS STATEMENT

The study meets the guidelines for ethical conduct and report of research, and was approved the Mayo Clinic IRB study ID# 14-009335.

## AUTHOR CONTRIBUTIONS

PP supervised exercise testing, collated and data, and wrote initial draft. JS revised the manuscript.

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# Applying the New Teaching Methodologies in Youth Football Players: Toward a Healthier Sport

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At early ages (6–12 years), the levels of physical activity developed in sports initiation and Physical Education often fall short of optimal levels. Ecological models of education seek, among other things, to make up for this deficit by modifying the structural elements of sport, bringing play closer to the child's developmental characteristics. In this sense, Nonlinear Pedagogy is a model of active pedagogy that seeks the integral development of young players through a sport more in line with their abilities, and that for this is based on a system of constraints on the environment, the task and the player himself. However, there are no studies that analyze the effects of these methodologies on the parameters of physical activity at such an early age. The aim of this study was to analyze the impact of a learning methodology based on Nonlinear Pedagogy on health-related levels of physical activity (heart rate) in young football players (U-11). A quasi-experimental study was developed in which three tasks were applied using structural modifications of the football elements related to Nonlinear Pedagogy (modification of the number of players related to situations of inferiority, equality and numerical superiority; dimensions of the field of play). The sample studied was composed of football players, U-11  $n = 32$ , age:  $10.35 \pm 0.54$  years; years of experience:  $2.14 \pm 0.768$  years. The players carried out each task for 10 min. Physical activity levels were measured by controlling heart rate using heart rate monitors (Polar Team2). The results showed very high levels of vigorous and very vigorous physical activity in all the tasks designed. These data show that the use of these new teaching methodologies has an impact on levels of physical activity in accordance with the recommended parameters.

**Keywords:** Nonlinear Pedagogy, children, physical activity, physiology, soccer, sports initiation, rule modification

## INTRODUCTION

Educational needs have changed over time, and so have the educational methods used (Van Wert et al., 2018). In the discipline of sport, traditional teaching styles that do not satisfy the demands and needs of sport have been replaced by Pedagogical Models that attend to the integral development of youth athletes (Araújo et al., 2006; Jones et al., 2017; Casey and MacPhail, 2018).



Within these ecological models, Nonlinear Pedagogy is a step forward in teaching for the integral development of children (Tan et al., 2012; Chow, 2013). Nonlinear Pedagogy is a methodology of active pedagogy that is characterized by establishing a system of constraints on three aspects in the tasks: on the environment, in which all climatic, environmental, and spatial factors are taken into account (Chow et al., 2007, 2009); on the task, in terms of the modification of the number of players, playing area, playing surface or goals (Davids et al., 2007); these constraints on the task are closely related to those proposed by the Teaching Games for Understanding model (Thorpe and Bunker, 1989; Werner et al., 1996); or on the player, degree of physical development or evolutive moment (Hopper, 2002). These constraints interact with the three principles of Nonlinear Pedagogy to optimize the teaching process: (a) variability, generating tasks with a multitude of actions and movements (Davids et al., 2003); (b) self-organization, developing the capacity to react to these stimuli in order to respond successfully (McGarry et al., 2002); (c) decision-making, inasmuch as choosing the most suitable alternative for each situation is the main conditioning factor of sporting success (Araújo et al., 2006). From this idea, technical skills lose importance in relation to perceptual mechanism and decision-making (Tallir et al., 2012). These approaches are based on the need to train actions in a real context. Actions in team sports are neither predictable nor random, due to the fact that the cooperation-opposition relationship is complex (Miller et al., 2016; Moy et al., 2016; Santos et al., 2017). Several studies have demonstrated the effectiveness of this model of active pedagogy for the development of decision making and technical-tactical execution in maintaining possession of the ball and making progress in the game in football (Práxedes et al., 2018; Pizarro et al., 2019).

Among the different didactic proposals most used in Nonlinear Pedagogy is the use of small-sided games (SSGs), situations in which coaches reduce different structural elements of sports. In this sense, Clemente et al. (2014), have shown that SSG significantly improve the physical condition in the sport of football.

In this line of integral child development, health-oriented sport is one of the objectives of the ecological models of sports initiation. In them, the use of new technologies plays a powerful role in terms of a more adapted sport and greater control of the health of youth players (Williamson, 2015), based on objective records. The benefits of physical activity have been widely documented in terms of improving body composition and preventing overweight, as well as metabolic and cardiovascular health (Kriemler et al., 2011; Gunter et al., 2012). Moreover, these benefits extend to psychological factors such as reduced levels of depression, stress, anxiety, and improved self-esteem and confidence (Olmedilla and Ortega, 2009; Biddle and Asare, 2011; Morgan et al., 2012). These benefits have an impact at all ages, but are especially relevant in children for proper and healthy development, prevention of disease, and improvement of cognitive functions (Hills et al., 2015; Donnelly et al., 2016).

However, on many occasions, training, and competition in collective sports are far removed from the needs and preferences of children (García-Angulo et al., 2017; Ortega et al., 2017;

García-Angulo and García-Angulo, 2018). Similarly, it has been found that the time of physical exercise at healthy intensity levels is far from the recommendations in extracurricular sports activities for children (Moral, 2018). While it has been shown that adapted sports programs in school children improve parameters related to healthy body composition (Manjula et al., 2018).

In football, several studies have analyzed physiological parameters in certain training stages (Eniseler et al., 2017; Randers et al., 2017; Olthof et al., 2018), and especially in high performance sports (Owen et al., 2011). However, there are no studies that analyze the influence of the modification of the structural variables of football tasks on the parameters of physical activity in the early stages of training, when the child most requires adequate control of the loads for their development. For all the above, the aim of this study is therefore to analyze the impact of a learning methodology based on Nonlinear Pedagogy on health-related levels of physical activity (measured by heart rate) in youth football players.

## MATERIALS AND METHODS

### Design

A quasi-experimental study was developed with the objective of analyzing the incidence of three tasks (small-sided games) based on Nonlinear Pedagogy on the levels of physical activity in youth football players. For this purpose three SSGs were designed in which the constraints of the task on the number of players proposed by Nonlinear Pedagogy. This study respected the ethical principles established by the UNESCO Declaration on Bioethics and Human Rights. The study was approved for development by the ethics committee of the University of Murcia (Spain) (ID 1944/2018). Following the Declaration of Helsinki the players voluntarily participated in the study and the participants' parents/guardians signed their written consent for the development of this study.

### Participants

The sample under study was made up of players in the U-11 category ( $n = 32$ ) who belonged to four different teams participating in federated sports initiation leagues in Spain (U-12). For the SSGs in equality and numerical superiority the sample consisted in 32 players, while for the SSGs in numerical inferiority the sample consisted in 24 players. They were  $10.35 \pm 0.54$  years old, and  $2.14 \pm 0.768$  years of practice.

### Instruments and Materials

For the control, monitoring and recording of the heart-rate was used the Polar Team 2® system (Polar Electro, Finland), designed for the control and monitoring of both group physical activity and collective sports, was used to control and monitor heart rate parameters. Base station frequency is 2400 to 2483.5 MHz, maximum 100 mW. For the recording of the heart rate signal, Polar® bands (Polar Electro, Finland) were used, which sent the data to the Polar Team 2® dock using Bluetooth technology. The control, monitoring and recording system used in this study has been validated (Goodie et al., 2000) and has been used in

others studies as a reference system to validate other systems (Molina-Carmona et al., 2018).

## Procedure

Three SSGs were developed in which the structural elements of the sport itself were manipulated, such as the game area and the number of players. Different numerical situations of game were introduced: (I) in situations of numerical equality, (II) in situations of numerical superiority, and (III) in situations of numerical inferiority.

The game area was modified according to the number of players participating in each SSG. For this purpose, the Regulations on sports and leisure facilities (NIDE) of the Superior Sports Council of Spain (2017) of Spain were followed, which determines the dimensions of the game spaces. Taking into account that for this age, maximum dimensions of 2925 m<sup>2</sup> (65 m × 45 m) and minimum dimensions of 1500 m<sup>2</sup> (50 m × 30 m) for 8-a-side football were established. The most common playing field size within these measures was taken as a reference, which is 2400 m<sup>2</sup> (60 m × 40 m), which represents a playing space of 156.2 m<sup>2</sup> per player. According to this game space per player and the number of players playing in each SSGs the game space in each of them was calculated. Thus the dimensions of the first SSG was 1562.5 m<sup>2</sup>, and the dimensions in the second and third SSG were 1406.25 m<sup>2</sup>.

Each of the SSGs was developed by the players in a standardized session for all groups. Before the session started, a standardized warm-up of 20 min was performed by all players. The warm-up consisted of: continuous run (3'); individual technique exercises (7'); dynamic stretching (3'); and technical and tactical situations in the official field of superiority, equality and offensive inferiority (7').

The order of the SSGs was the same for all the teams, we started with the 5 vs. 5 numerical equality, then we made a 7' break to rehydrate and recover the basal heart levels of the players, then we developed the second SSG of 5 vs. 4, we made a second 7' break to rehydrate and recover the basal levels. Finally, the third SSG was developed in a 4 vs. 5 situation.

Each of the SSGs had a duration of 10'. All the SSGs were played by the same players except when the teams played in inferiority, that a player was eliminated, and that it was always the same. At the beginning of each SSG a draw was made to establish the field and the team that made the kick-off.

The standardized sessions in which the different SSGs were played at the same time for all teams and in similar weather conditions.

The levels of physical activity in each of them were recorded and analyzed using the players' heart rate. The tasks (small-sided games) designed were as follows:

Task 1: 4+goalkeeper vs. 4+goalkeeper (5 vs. 5), for 10 min, with a space per player of 160 m<sup>2</sup> (40 m × 40 m) and using the goals and balls of the category. Objective of the task: to provide the player with situations similar to the real competition (see **Figure 1**).

Task 2: 3+goalkeeper vs. 4+goalkeeper (4 vs. 5), for 10 min, with a space per player of 155 m<sup>2</sup> (35 m × 40 m) and using

the goals and balls of the category. Objective of the task: to facilitate defensive behavior and counter-attack situations in the game (see **Figure 2**).

Task 3: 4+goalkeeper vs. 3+goalkeeper (5 vs. 4), for 10 min, with a space per player of 155 m<sup>2</sup> (35 m × 40 m) and using the goals and balls of the category. Objective of the task: to facilitate and encourage the emergence of offensive actions (see **Figure 3**).

During the modified matches the young players wore heart rate monitors (Polar Team2). Five zones were established in accordance with the American College of Sports Medicine [ACSM] (2014), very low intensity (light): % Heart Rate Reserve (%RFC) ≤ 30, % maximum heart rate (%HRmax) ≤ 57, %VO<sub>2max</sub> ≤ 37-4; low intensity (light): RFC 30-40; %HRmax 58-64; %VO<sub>2max</sub> 37-45; moderate intensity: %RFC 40-59; %HRmax 65-76; %VO<sub>2max</sub> 46-63; vigorous: %RFC 60-89; %HRmax 77-95; %VO<sub>2max</sub> 64-90; very vigorous: %RFC ≥ 90; %HRmax ≥ 96; %VO<sub>2max</sub> ≥ 91.

For each of the three tasks performed, the following variables were analyzed for each of the five heart rate zones established by the American College of Sports Medicine [ACSM] (2014): (1) Time in seconds (total time that the players remained during the tasks with a heart rate within the determined work zone); (2) Percentage of total work time (percentage of work within each of the zones established by the ACSM as a percentage of total work time).

The heart-rate of the 32 players was recorded in the situation of numerical equality (5 vs. 5) and in the situations of numerical superiority (in the situations of 5 vs. 4 and 4 vs. 5 when those players were in the team that had 5 players). Of those 32 players, 24 of them were also analyzed in the situation of inferiority (in the situations of 5 vs. 4 and 4 vs. 5 when their teams were formed by 4 players).

## Statistical Analysis

To assess possible differences between the three task analyzed, it was used an analysis of variance with repeated measures. First, the homoscedasticity of the data was checked. It was appreciated that the data met this criterion. The sphericity of Mauchly test was used, from which, it was decided to use the Trace of Pillai, or assumed sphericity (the Huynh-Feldt correction test was used, in case the principle of sphericity was not met). For *post hoc* analysis, the *post hoc* of Bonferroni was used, with a significance level of  $p < 0.05$ . Finally, the effect size is calculated ( $\eta^2$  or D of Cohen). For the statistical analysis the SPSS software was performed in his 24.0 version.

## RESULTS

In **Table 1**, the mean values and standard deviation of the variables under study (working time, percentage of work) are recorded for each of the heart rate bands in the Task 1 (5 vs. 5), that is to say, in a situation of numerical equality.

The data in **Table 1** showed that in zone four the youth players work the most time in the Task 1 (5

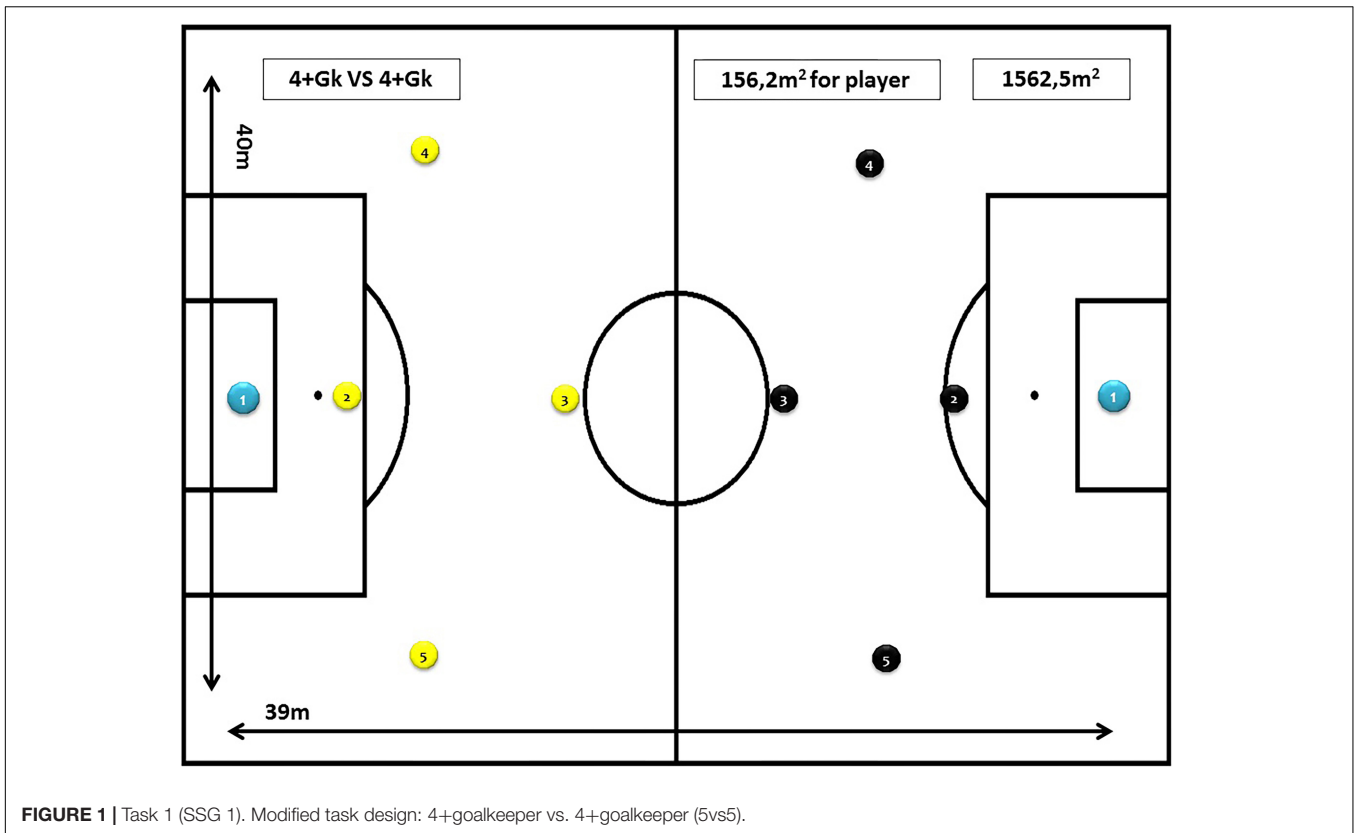


FIGURE 1 | Task 1 (SSG 1). Modified task design: 4+goalkeeper vs. 4+goalkeeper (5vs5).

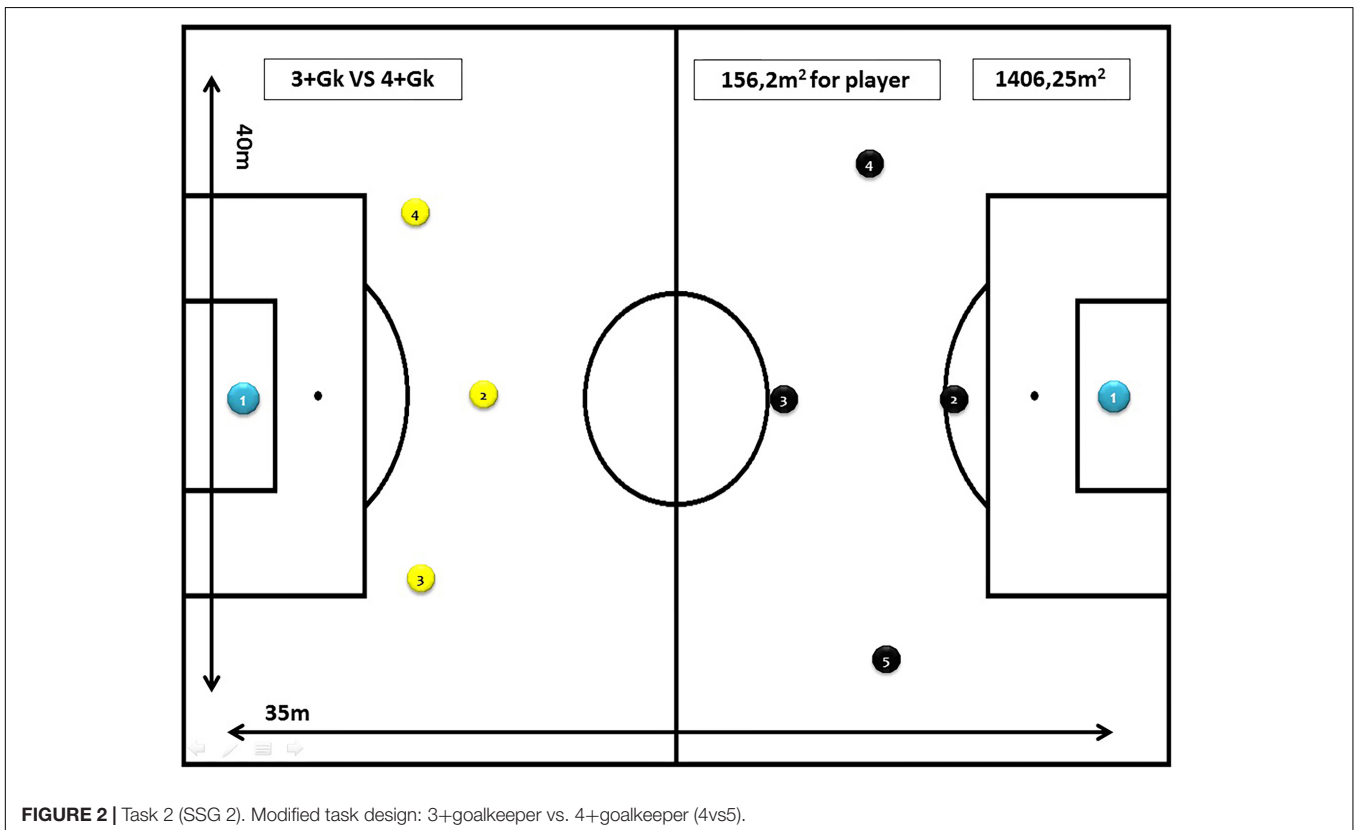
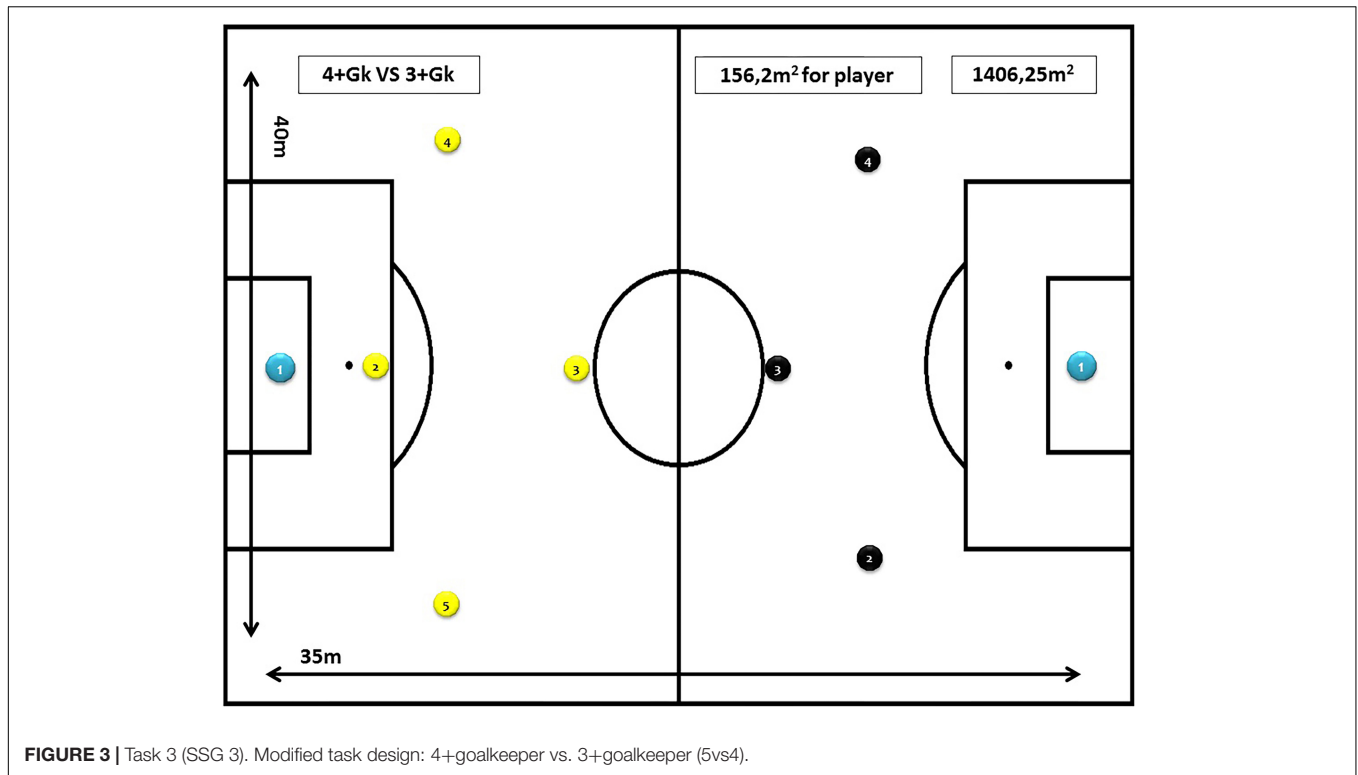


FIGURE 2 | Task 2 (SSG 2). Modified task design: 3+goalkeeper vs. 4+goalkeeper (4vs5).



**FIGURE 3 |** Task 3 (SSG 3). Modified task design: 4+goalkeeper vs. 3+goalkeeper (5vs4).

vs. 5, situation of numerical equality). Between zone 4 and zone 5 the young players during the 5 vs. 5 tasks (numerical equality) are more than 80% of the total working time.

Table 2 shows the means and standard deviation of the variables under study (working time, percentage of work) in each of the heart rate and cardiac frequency bands for the Task 2 and Task 3 (4 vs. 5 and 5 vs. 4) for players who were in a situation of numerical superiority.

The data in Table 2 shows that zone 4 is where the youth players who were in numerical superiority work the most time in the Task 2 and Task 3 (4 vs. 5 and 5 vs. 4), followed by zone 3. Between zone 4 and zone 5, youth players spend more than 84% of their total working time.

In Table 3, the mean values and standard deviation of the variables under study (working time, percentage of work) are recorded for each of the heart rate and cardiac frequency bands in the Task 2 and Task 3 (4 × 5

**TABLE 1 |** Mean and standard deviation of working frequencies in the Task 1 (5 vs. 5).

Intensity zone	Variable	Mean	Standard deviation
Zone 1 (0–57% HR <sub>max</sub> )	Time in seconds	12,22	29,27
	Percentage of total work time	2,02	4,87
Zone 2 (58–64% HR <sub>max</sub> )	Time in seconds	18,53	38,35
	Percentage of total work time	3,06	6,37
Zone 3 (65–76% HR <sub>max</sub> )	Time in seconds	96,25	105,80
	Percentage of total work time	15,93	17,50
Zone 4 (77–95% HR <sub>max</sub> )	Time in seconds	442,25	137,32
	Percentage of total work time	74,17	22,49
Zone 5 (96–100% HR <sub>max</sub> )	Time in seconds	33,94	80,77
	Percentage of total work time	5,68	13,50

Players in a situation of numerical equality (I) (n = 32).

**TABLE 2 |** Mean and standard deviation of working frequencies in Task 2 and Task 3 (4 vs. 5 and 5 vs. 4).

Intensity zone	Variable	Mean	Standard deviation
Zone 1 (0–57% HR <sub>max</sub> )	Time in seconds	3,59	8,58
	Percentage of total work time	0,57	1,34
Zone 2 (58–64% HR <sub>max</sub> )	Time in seconds	8,16	21,29
	Percentage of total work time	1,28	3,25
Zone 3 (65–76% HR <sub>max</sub> )	Time in seconds	83,78	94,86
	Percentage of total work time	13,57	15,07
Zone 4 (77–95% HR <sub>max</sub> )	Time in seconds	474,69	105,79
	Percentage of total work time	77,94	18,97
Zone 5 (96–100% HR <sub>max</sub> )	Time in seconds	44,50	102,18
	Percentage of total work time	6,64	14,41

Players in a situation of numerical superiority (II) (n = 32).

and 5 vs. 4) for players who were in a situation of numerical inferiority.

**Table 3** shows that zone 4 is where the youth players who were in numerical inferiority work the most time in the Task 2 and Task 3 (4 vs. 5 and 5 vs. 4), followed by zone 3. Between zone 4 and zone 5, youth players spend more than 88.9% of their total working time.

The data from **Tables 1–3** show that when comparing the time in seconds between the situations of equality, superiority and inferiority, no statistically significant differences were found even in zone 2 ( $F_{2,22} = 2.538$ ,  $p = 0.102$ ,  $\eta^2 = 0.187$ ), neither in zone 3 ( $F_{2,22} = 0.864$ ,  $p = 0.435$ ,  $\eta^2 = 0.073$ ), nor in zone 4 ( $F_{2,22} = 1.730$ ,  $p = 0.201$ ,  $\eta^2 = 0.136$ ), nor in zone 5 ( $F_{2,22} = 1.069$ ,  $p = 0.360$ ,  $\eta^2 = 0.089$ ). However, trends to significance were observed in zone 1 ( $F_{1,10,25,45} = 4.093$ ,  $p = 0.050$ ,  $\eta^2 = 0.151$ ), specifically, trends to significance were observed between the situation of equality and inferiority ( $p = 0.099$ ,  $d = 0.554$ ).

Similarly, there were no statistically significant differences in the percentage of total working time between the situations of equality, superiority and inferiority, nor in the heart rate zone 2 ( $F_{2,22} = 2.513$ ,  $p = 0.104$ ,  $\eta^2 = 0.186$ ), neither in heart rate zone 3 ( $F_{2,22} = 0.832$ ,  $p = 0.448$ ,  $\eta^2 = 0.070$ ), nor in heart rate zone 4 ( $F_{2,22} = 1.388$ ,  $p = 0.270$ ,  $\eta^2 = 0.112$ ), nor in heart rate zone 5 ( $F_{2,22} = 0.851$ ,  $p = 0.440$ ,  $\eta^2 = 0.072$ ). However, trends to significance were observed in the heart rate zone 1 ( $F_{1,05,25,42} = 4.038$ ,  $p = 0.052$ ,  $\eta^2 = 0.149$ ), specifically, trends to significance were observed between inferiority and equality ( $p = 0.099$ ,  $d = 0.550$ ).

## DISCUSSION

The aim of this paper is to analyze the impact of a learning methodology based on Nonlinear Pedagogy on health-related levels of physical activity (measured by heart rate) in youth football players. For this purpose, the constraints related to

equality, superiority or inferiority in the number of players in these levels of physical activity were analyzed. It's a novel study in terms of the age of the participants. The vast majority of researches that has analyzed the degree of physical activity in various tasks in football has focused on higher ages, adult subjects and high performance levels. This paper presents data on physiological parameters related to the intensity of physical activity at very early ages, specifically in the U-11 category. Studies of this type at this age are very rare, and most of those that have been carried out have been in school Physical Education (Davids et al., 2007; Slingerland and Borghouts, 2011; Fröberg et al., 2017; Moral, 2018). In formative football, these studies have focused on decision-making and execution (Práxedes et al., 2018), or on middle ages higher than those proposed in this paper, such as U-13 (Olthof et al., 2018); U-15 (Olthof et al., 2018); U-17 (Eniseler et al., 2017); U-18 (Sánchez-Sánchez et al., 2017), and U-19 (Özcan et al., 2018). As well as recreational adult levels (Randers et al., 2017), and higher competitive levels (Owen et al., 2011). In this sense, studies of technical-tactical actions conclude that the use of SSG helps to improve learning teaching processes (González-Villora et al., 2011; Silva et al., 2014). On the other hand, studies that analyze physiological indicators in older soccer players to those of the object of study again indicate the usefulness of SSG for improving physical condition. In this way, the great utility of the SSG has been demonstrated, but no studies have been found in the ages object of study, nor approaches that analyze the differences or similarities between the SSG in equality, superiority, and numerical inferiority from the perspective of the heart-rate. This study allows to know the influence of the modification in the tasks in the ages not studied until now and to link the formative process with other studies that have analyzed the levels of physical activity in the tasks in soccer in higher ages.

The data found in this study showed very high levels of vigorous and very vigorous activity in the Task 1 (5 vs. 5), Task 2 (4 vs. 5) and Task 3 (5 vs. 4), with low values of moderate activity in the tasks (less than 20%) and very low values of low or very low activity (below 10%). These results may be due to the reduction in the number of players and the size of the field of play, along with the situations of numerical superiority and inferiority that are proposed in the tasks. From the player's point of view the higher levels of physical activity may be due to the greater space for action and a greater number of interventions. Similarly, higher levels of physical activity when players are in superiority over when they are on an equal playing level may be due to the player's moral responsibility to take advantage when his coach gives him an advantage in the task. Other studies also have analyzed the incidence of training modifications in young football players (Hill-Haas et al., 2009; Katis and Kellis, 2009). These studies have found that the reduction in the number of players in various tasks affects an increase in the physiological load, technical, and tactical parameters and the perception of the effort of the youth player in training (Hill-Haas et al., 2009; Katis and Kellis, 2009). Other studies indicate that increasing the size of the playing space has repercussions on the physiological demands of the player (Casamichana and Castellano, 2010; Belozo et al., 2018). In this same line of investigation, it has been found that the generation of numerical superiorities or inferiorities by means

**TABLE 3** | Mean and standard deviation of working frequencies in Task 2 and Task 3 (4 vs. 5 and 5 vs. 4).

Intensity zone	Variable	Mean	Standard deviation
Zone 1 (0–57% HR <sub>max</sub> )	Time in seconds	0,71	2,03
	Percentage of total work time	0,12	0,34
Zone 2 (58–64% HR <sub>max</sub> )	Time in seconds	2,83	6,43
	Percentage of total work time	0,47	1,07
Zone 3 (65–76% HR <sub>max</sub> )	Time in seconds	63,25	88,70
	Percentage of total work time	10,51	14,74
Zone 4 (77–95% HR <sub>max</sub> )	Time in seconds	484,17	108,01
	Percentage of total work time	80,25	17,96
Zone 5 (96–100% HR <sub>max</sub> )	Time in seconds	52,42	96,04
	Percentage of total work time	8,66	15,92

Player in a situation of numerical inferiority (III) ( $n = 24$ ).

of supports influences the internal and external burden of youth players (Sánchez-Sánchez et al., 2017). The data in this study reinforce the data found in other studies on the importance of changes in the rules and methodology of teaching for optimal sports practice at the physical activity level (Eniseler et al., 2017; Özcan et al., 2018).

The results of this paper show high levels of vigorous and very vigorous activity in all tasks. However, there are no statistically significant differences between the three SSGs based on the Nonlinear Pedagogy, although there is a slight tendency to differences in the amount and percentage of mild physical activity. This small difference is probably due to the fact that in the 5 vs. 5 task, probably due to a question of organization, and that the number of opponents and opponents is the same, the players perceive that it is not necessary to start with a high intensity and therefore the task is started with a lower intensity, although the data indicate that it is a very short time. On the other hand, in the rest of the frequencies, the working time is very high, mainly in zones 3 and 4, and very similar in the three modalities. These data show that the reduction in the number of players is the variable that causes this high participation in medium and high intensities, although it is not so decisive if the reduction gives rise to situations of equality, superiority, or inferiority. These data show that these modifications bring the training closer to the physical activity recommendations proposed by American College of Sports Medicine [ACSM] (2014). These results are relevant as many studies indicate that levels of physical activity in training sport are below the minimum recommendations (Lonsdale et al., 2013; Hollis et al., 2016). Along the same lines, studies indicate that football generates more vigorous activity than other sports in the formative stage (Leek et al., 2011). When comparing the data from studies in which a physical activity is analyzed in an extracurricular context (sports initiation), with the data from Physical Education studies, there are large differences in favor of activities in sporting contexts (?). These differences are most likely due to the fact that extracurricular sports activities are chosen by athletes and therefore have a significant degree of motivation. However, Physical Education classes are compulsory, so that in some cases the participants do not have a motivation for the activity, and therefore the intensity of the sessions is low (Hollis et al., 2016). Physical Education teachers have a major challenge in this regard. In addition to improvements in the parameters related to physical activity with respect to Physical Education, recent studies indicate that Nonlinear Pedagogy is not limited only to improving the parameters of physical activity of young players, but is a methodology that improves decision making and technical-tactical execution in the maintenance of possession of the ball and in the advancement of the game in football (Práxedes et al., 2018; Pizarro et al., 2019).

This fact, together with the modifications proposed in this work, could be a basis to guarantee a healthy practice for children. These data reveal the need for coaches to be more aware of workloads in the different tasks they propose to their youth players. Several studies indicate that the same task may involve very different levels of workload when modified or not modified (Hill-Haas et al., 2009; Casamichana and Castellano, 2010; Belozo et al., 2018). The adequate control of the physiological loads

to which the child is subjected will allow an optimization of the short training time in the formative stages and of greater benefits for the health of the child (Sánchez-Sánchez et al., 2015). Therefore, it is necessary for trainers to control the influence of the modifications introduced and their repercussion on the tasks in order to keep the tasks in activity times and intensities within the parameters recommended by the American College of Sports Medicine [ACSM] (2014), both in professional teams and mainly in teams in training stages.

The results of this study indicate that the proposed modifications maintain optimal levels of activity in all tasks. The slight differences found indicate that control and mastery of these variables by coaches is a necessary requirement for a more age-appropriate practice and for maintaining adequate levels of physical activity. However, the data should be taken with caution as it is a very small sample with very specific characteristics and ages. It is possible that an increase in the sample size generated differences between the tasks of 5 vs. 5 and the rest, in the lighter intensity bands, due surely to the fact that the players start with a moderate predisposition, since they are in situations of equality. When the players are in situations of inferiority or superiority, they perceive that it is necessary from the beginning to generate a greater intensity of work to reach the objectives. In any case, this reflection must be analyzed with caution.

The impact of the proposed methodologies on levels of healthy physical activity at other ages should be assessed. It would also be necessary to extend the study to players in the formation of elite clubs, to compare whether the impact of the proposed methodology has the same results. Future studies should assess the impact of modifying other variables, such as the inclusion of wildcards or the size and number of goals, to those already proposed and their impact on levels of healthy physical activity. In the same way it would be very interesting to evaluate the impact of the rest of the constraints on the environment and the player who proposes the Nonlinear Pedagogy on the parameters of physical activity, are the heart-rate parameters different when the child plays at different times of the day? Are the levels of physical activity different when the child plays in an unusual environment? These questions may be useful for future research in this area. These data can serve as a basis for prescribing a sport closer to the levels of healthy physical activity and optimizing sports practice time.

## CONCLUSION

The most relevant conclusions of this study indicate that the use of the Nonlinear Pedagogy model's own modifications based on the modification of the game space and the number of players in the sports initiation, can approximate the physical activity parameters to the levels recommended by the American College of Sports Medicine [ACSM] (2014). The effectiveness of the proposed modifications in this category (U-11) may allow coaches to design training tasks according to the physical activity needs of their youth players.

These data can help overcome the low levels of very vigorous and vigorous physical activity that other studies have found in formative sport and school Physical Education.

## AUTHOR CONTRIBUTIONS

All authors participated in the design, documentation, development, and writing of the manuscript. This paper was reviewed by all authors and all of them are responsible for its contents and providing they are responsible for the final version.

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# Body Composition in Children and Adolescents Residing in Southern Europe: Prevalence of Overweight and Obesity According to Different International References

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The objective was to analyze body composition in children and adolescents of Southern Europe to identify prevalence of overweight and obesity. This investigation involved 512 girls and 488 boys between 7-to 19-years. Variables evaluated were Body Mass Index (BMI) and Fat Mass (FM; electrical bioimpedance). The references used to establish prevalence according to BMI were those of the World Health Organization (WHO) and the International Obesity Task Force (IOTF); in the case of FM, the Child Growth Foundation (CGF) reference was used. There were significant differences ( $p < 0.05$ ) in the prevalence of overweight and obesity between the three classifications (32.3% according to IOTF, 37.3% according to WHO, and 39.8% according to CGF), being higher in males. WHO-IOTF concordance was substantial ( $\kappa = 0.793$ ), whereas concordances WHO-CGF ( $\kappa = 0.504$ ) and IOTF-CGF ( $\kappa = 0.447$ ) were moderate. The authors recommend evaluating overweight and obesity not only with BMI, but also with FM, and always specify the references used.

**Keywords:** fat mass, BMI, nutritional status, WHO, IOTF, CGF

## INTRODUCTION

Overweight and obesity can be defined as an abnormal or excessive accumulation of fat that can be harmful toward one's health (World Health Organization [WHO], 2017). Body mass index (BMI) is a simple indicator of the connection between weight and height that is frequently used to indirectly identify overweight and obesity (World Health Organization [WHO], 2017). The simplicity and low cost of BMI has made it a popular indicator to identify overweight and obesity in science and practice (McCarthy et al., 2006). However, BMI does not distinguish between increased mass in the form of fat, lean tissue or bone, and consequently, it may lead to significant misclassification. Therefore, due to excess fat being a pathology that defines obesity, it would be ideal to also evaluate total fat mass (McCarthy et al., 2006). The evaluation of fat mass *per se* allows one to obtain important information about the state of the health of the population under study, as well as identify

those at risk of certain diseases (Albuquerque Sendín, 2008). For example, excessive fat mass has been shown to be associated with Type 2 Diabetes Mellitus (Abdullah et al., 2010), cancer (Renehan et al., 2008), coronary heart disease and associated risk factors (Bogers et al., 2007), depression (Luppino et al., 2010), and early mortality (Flegal et al., 2013), to list just a few.

Hence, it is clear when studying the prevalence of overweight and obesity, that it is highly recommended to evaluate not only BMI, but also the percentage of fat mass. In addition, consideration must be given to the cut off points used to classify children and adolescents, an aspect dealt with in previous studies (Wang and Wang, 2002; De Onis and Lobstein, 2010; Shields and Tremblay, 2010; Espín Ríos et al., 2013; Bergel et al., 2014; Lasarte-Velillas et al., 2015; Polo Martín et al., 2015). These studies investigated the prevalence of overweight and obesity but focused only on the cut-off points for BMI and not on cut-off points for fat mass. The present article adds to this literature by comparing cut-off points for BMI but also for fat mass, studying the three main international references: World Health Organization (WHO), International Obesity Task Force (IOTF), and Child Growth Foundation (CGF).

The main objective of this research is to evaluate the BMI and fat mass of children and adolescents residing in Southern Europe, studying the prevalence of overweight and obesity according to common international references of these two indicators, and observing the degree of concordance that these different classifications present. This will provide updated data on BMI, fat mass, and prevalence of overweight and obesity in children and adolescents residing in Southern Europe. Moreover, this study will provide evidence about differences between common international references when classifying children and adolescents weight status.

## MATERIALS AND METHODS

### Sample

A total of thirteen schools from Southern Europe, nine from Southern Italy (regions of Lazio and Calabria) and four from Southern Spain (region of Murcia) were included in this study. The final sample was made up of 1,000 children and adolescents (512 female and 488 male) between the ages of 7 and 19 years. Excluded from the study were those children and adolescents who did not fulfill any of the recommendations for an adequate analysis of electric bioimpedance, described below. The estimated maximum sampling error at 95% confidence level ( $p \leq 0.05$ ) for a sample size of 1000 is  $\pm 3.1\%$  (Wimmer, 2011).

This research project was carried out according to the International Code of Medical Ethics (Declaration of Helsinki) for experiments with human beings and, was approved by the Research Ethics Commission of the University of Murcia (No. 03/02/2012). Moreover, the parents/legal guardians of all the participants signed an informed consent form for their children and adolescents to take part. Also, children and adolescents provided assent. The children and adolescents were coded individually, and the details treated anonymously.

### Analysis of Body Composition

The measurements were carried out at school, in an indoor hall prepared for the occasion during the morning timetable. Height was measured with the portable height rod of Tanita model Leicester HR 001 (Tanita, Tokyo, Japan), with the precision of 0.1 cm and with the subjects standing up and barefooted. Weight and total fat mass were measured with the Tanita BC-418-MA Segmental Body Composition Analyzer (Tanita, Tokyo, Japan), with the corresponding correction for the weight of the clothes (underwear or short sleeve). The procedure required the subjects to be standing with bare feet on the places marked on the analyzer, at the same time as they held onto the handles, one in each hand. The analysis through electric bioimpedance lasted approximately 30 s per subject. BMI was calculated with the formula  $\text{Weight (kg)} / \text{Height}^2 \text{ (m)}$ . Even though the Tanita BC-481-MA Analyzer provides separate measurements for the fat in the torso and the inferior and superior extremities, only the percentage of total fat mass was taken into consideration to analyze the prevalence of overweight and obesity in the study sample. As indicated by McCarthy et al. (2006), the equations used for this model are based on bioimpedance, weight, height and age, and were obtained through calibration and validation studied with Dual-energy X-ray absorptiometry (DXA) and BodPod, having a standard error of 2.7% for the body mass of boys and of 2.8% for girls. The validity of this method was also established by the studies carried out by Merritt and Ballinger (2003) and Prefontaine and Ballinger (2003). The software used to pass the data to the computer was Suite Biologica 7.1. Moreover, all recommendations to collect electric bioimpedance data were followed (Sgroi and De Lorenzo, 2011; TANITA, 2016). First, a letter was given to parents, teachers and participants explaining in detail procedures that had to be followed before data were collected, such as no excess of food and drink the day before, no intense exercise in the last 12 h, no alcohol consumed in the last 12 h, no metallic objects, no pace-makers, not done during the menstrual cycle and not during pregnancy. These aspects were also checked by researchers asking participants prior to data collection. Data were collected at 11.00 am, before lunch, to ensure that data were collected more than 3 h after participants woke, and to avoid participants eating and drinking 3 h prior to measurement. Finally, prior to data collection the participants were asked to urinate to follow standard procedure for bioimpedance measurement.

### Studies Used as References

The references used to establish the prevalence of overweight and obesity according to the BMI were from the WHO (De Onis et al., 2007) and the IOTF (Cole et al., 2000; Lobstein et al., 2004; Cole and Lobstein, 2012); in the case of FM, CGF was used as a reference (McCarthy et al., 2006).

### Statistical Analysis

First, the normality of continuous variables was assessed through the Kolmogorov-Smirnov test. The median values and the standard deviation (SD) of the BMI were calculated, along with the percentage of fat mass, globally, by gender and age. A gender

comparison was carried out with the *t*-test for independent samples. Furthermore, the size of the effect was calculated using Cohen's *d* (Cohen, 1988).

In addition, the prevalence of overweight and obesity were calculated, by gender and age for the three references. The significant differences between references were calculated (Franklin, 2007) as well as the degree of concordance between each pair using the kappa coefficient (Cohen, 1960; Landis and Koch, 1977; Cerda and Villarroya, 2008). Traditionally, values <0 indicate no agreement, 0–0.20 slight, 0.21–0.40 fair, 0.41–0.60 moderate, 0.61–0.80 substantial, and 0.81–1 almost perfect agreement. Finally, the comparison of the prevalence of overweight and obesity between gender was carried out using the chi-square test ( $\chi^2$ ). The significant value used was  $p < 0.05$ . The statistical package SPSS-22.0 (Statistical Package for the Social Sciences) and Microsoft Office Excel were used to carry out the analysis.

## RESULTS

**Table 1** shows the medium values and SD corresponding to the BMI and the percentage of fat mass of the 1000 children and adolescents from the sample. The results are shown organized in function to age and gender (**Supplementary Figures S1, S2**).

It is noteworthy that girls show higher medium values of fat mass than boys ( $p < 0.0001$ ;  $d = 0.886$ ). The fat mass in the female gender was higher in all age groups, showing significant differences at 7 years of age and from 13 to 19 years of age.

**Tables 2, 3** show the prevalence of “overweight/excess fat” and “obesity” by gender, age and globally by the three references which have been studied: WHO, IOTF, CGF. They also show the significant differences found between references. Moreover, **Supplementary Figures S3–S8** show graphically the different

prevalences found when classifying the sample with the 3 references used.

Significant differences ( $p < 0.05$ ) were found in the prevalence of overweight and obesity between the three classifications. The total prevalence of overweight and obesity was of 32.3% according to IOTF, 37.3% according to WHO and 39.8% according to CGF. The WHO-IOTF concordance was substantial (kappa = 0.793), meanwhile the WHO-CGF (kappa = 0.504) and IOTF-CGF (kappa = 0.447) concordances were moderate.

The  $\chi^2$  test showed that there is no statistical significance when making a global comparison of the prevalence of overweight and obesity in function to gender. However, it was observed that male demonstrate a greater prevalence independent of the classification used.

## DISCUSSION

The three classifications used found a high prevalence of overweight and obesity in the studied sample. There were significant differences between the classifications: 32.3% according to IOTF, 37.3% according to WHO and 39.8% according to CGF. The WHO-IOTF concordance was substantial (kappa = 0.793), meanwhile the WHO-CGF (kappa = 0.504) and IOTF-CGF (kappa = 0.447) concordances were moderate.

The results of this study contrast with those found by Espín Ríos et al. (2013) who evaluated the BMI of 178,894 students (91,517 boys and 87,377 girls) of ages 2 to 14 in the region of Murcia (Spain), classifying them according to the criteria provided by the WHO and IOTF. Espín Ríos et al. (2013) found a greater prevalence of overweight and obesity in girls in contrast to the present study: 42.1% according to the WHO and 33.2% according to IOTF, in comparison to the 33.6 and 29.9% in this study. In boys, they observed a prevalence of 45.2% according

**TABLE 1** | Comparison of means of BMI and FM% ( $N = 1000$ ).

Age (years)	BMI					FM %				
	Females ( $n = 512$ )	Males ( $n = 488$ )	Sig.	95% CI	<i>d</i>	Females ( $n = 512$ )	Males ( $n = 488$ )	Sig.	95% CI	<i>d</i>
7 ( $n = 57$ )	18.21 (3.08)	16.49 (2.19)	0.021*	0.267 to 3.161	0.632	26.45 (5.79)	21.27 (4.05)	0.000*	2.477 to 7.884	1.116
8 ( $n = 24$ )	19.73 (2.92)	19.19 (3.24)	0.676	2.082 to 3.149	0.173	29.79 (5.93)	25.38 (6.42)	0.094	0.821 to 9.644	0.715
9 ( $n = 21$ )	19.79 (4.40)	19.66 (3.43)	0.947	3.703 to 3.947	0.030	26.08 (6.37)	24.86 (5.42)	0.659	4.465 to 6.897	0.202
10 ( $n = 79$ )	19.96 (5.46)	19.49 (2.90)	0.617	1.405 to 2.352	0.116	26.75 (5.75)	25.04 (6.13)	0.218	1.034 to 4.459	0.286
11 ( $n = 98$ )	19.83 (3.59)	20.24 (3.36)	0.575	1.842 to 1.027	0.116	25.72 (5.65)	23.99 (5.98)	0.150	0.638 to 4.098	0.299
12 ( $n = 77$ )	20.49 (2.98)	21.68 (3.10)	0.096	2.600 to 0.2183	0.394	25.52 (6.48)	22.95 (5.65)	0.079	0.307 to 5.439	0.416
13 ( $n = 76$ )	21.42 (5.01)	20.59 (3.88)	0.455	1.404 to 3.081	0.180	28.71 (8.54)	21.40 (7.50)	0.000*	3.539 to 11.065	0.986
14 ( $n = 88$ )	21.62 (3.32)	21.39 (3.20)	0.751	1.163 to 1.607	0.068	27.94 (7.29)	19.74 (6.95)	0.000*	5.172 to 11.222	0.871
15 ( $n = 53$ )	21.80 (3.53)	24.01 (4.86)	0.062	4.534 to 0.116	0.525	27.59 (6.27)	21.38 (7.44)	0.002*	2.428 to 9.991	1.162
16 ( $n = 82$ )	22.11 (3.63)	23.19 (5.35)	0.277	3.061 to 0.889	0.249	26.56 (6.94)	17.19 (8.28)	0.000*	5.982 to 12.755	0.933
17 ( $n = 77$ )	22.10 (2.66)	22.81 (2.76)	0.255	1.938 to 0.521	0.262	26.21 (6.18)	17.30 (6.01)	0.000*	6.144 to 11.681	0.937
18 ( $n = 153$ )	22.80 (4.74)	23.59 (3.71)	0.250	2.149 to 0.564	0.187	27.81 (7.04)	18.98 (6.60)	0.000*	6.653 to 11.011	0.646
19 ( $n = 115$ )	23.29 (5.20)	24.05 (4.54)	0.399	2.568 to 1.031	0.158	29.08 (7.77)	18.39 (6.51)	0.000*	8.054 to 13.329	0.755
Total ( $n = 1000$ )	21.34 (4.23)	21.76 (4.22)	0.122	0.938 to 0.111	0.098	27.15 (6.77)	20.71 (6.99)	0.000*	5.585 to 7.293	0.886

Values are Mean (SD). \*Significant differences between sexes.

**TABLE 2** | Prevalence (%) of overweight and obesity of females by age (N = 512).

Age	N	Overweight			Obesity			Overweight + Obesity		
		BMI WHO	BMI IOTF	FM % CGF	BMI WHO	BMI IOTF	FM % CGF	BMI WHO	BMI IOTF	FM% CGF
Females	512	22.3	21.9	17.6	11.3 <sup>3</sup>	8.0 <sup>3</sup>	18.2 <sup>1,2</sup>	33.6	29.9 <sup>3</sup>	35.8 <sup>2</sup>
7	31	32.3	22.6	25.8	22.6	22.6	35.5	54.9	45.2	61.3
8	12	16.7	25.0	8.3	50	41.7	58.3	66.7	66.7	66.6
9	13	30.8	38.5	23.1	23.1	15.4	23.1	53.9	53.9	46.2
10	31	29.0	29.0	12.9	19.4	12.9	22.6	48.4	41.9	35.5
11	59	33.9 <sup>3</sup>	30.5	16.9 <sup>1</sup>	13.6	6.8	10.2	47.5 <sup>3</sup>	37.3	27.1 <sup>1</sup>
12	47	25.5	29.8	14.9	10.6	4.3	12.8	36.1	34.1	27.7
13	27	18.5	25.9	7.4	22.2	11.1 <sup>3</sup>	33.3 <sup>2</sup>	40.7	37.0	40.7
14	46	23.9	21.7	17.4	6.5	4.3 <sup>3</sup>	17.4 <sup>2</sup>	30.4	26.0	34.8
15	28	21.4	21.4	35.7	7.1	3.6	10.7	28.5	25.0	46.4
16	51	15.7	15.7	5.9	5.9	5.9	15.7	21.6	21.6	21.6
17	39	17.9	15.4	25.6	0.0	0.0	5.1	17.9	15.4	30.7
18	75	16.0	13.3	18.7	4.0 <sup>3</sup>	4.0 <sup>3</sup>	14.7 <sup>1,2</sup>	20.0	17.3 <sup>3</sup>	33.4 <sup>2</sup>
19	53	15.1	17.0	18.9	11.3	9.4	22.6	26.4	26.4	41.5

BMI, body mass index; FM %, fat mass percentage; WHO, world health organization; IOTF, international obesity task force; CGF, child growth foundation. Significant differences between references ( $p < 0.05$ ) indicated with superindex: WHO = 1; IOTF = 2; CGF = 3.

**TABLE 3** | Prevalence (%) of overweight and obesity of males by age (N = 488).

Age	N	Overweight			Obesity			Overweight + Obesity		
		BMI WHO	BMI IOTF	FM % CGF	BMI WHO	BMI IOTF	FM % CGF	BMI WHO	BMI IOTF	FM % CGF
Males	488	25.8	26.4	22.1	15.4 <sup>2,3</sup>	8.4 <sup>1,3</sup>	21.9 <sup>1,2</sup>	41.2 <sup>2</sup>	34.8 <sup>1,3</sup>	44 <sup>2</sup>
7	26	11.5	15.4	30.8	15.4	7.7	23.1	26.9 <sup>3</sup>	23.1 <sup>3</sup>	53.9 <sup>1,2</sup>
8	12	25.0	33.3	25.0	33.3	25.0	41.7	58.3	58.3	66.7
9	8	37.5	37.5	25.0	25.0	12.5	37.5	62.5	50.0	62.5
10	48	35.4	39.6	33.3	27.1 <sup>2</sup>	4.2 <sup>1,3</sup>	25.0 <sup>2</sup>	62.5	43.8	58.3
11	39	33.3	33.3	33.3	23.1 <sup>2</sup>	5.1 <sup>1</sup>	17.9	56.4	38.4	51.2
12	30	50.0	43.3	36.7	23.3	10.0	20.0	73.3	53.3	56.7
13	49	24.5	20.4	24.5	12.2	8.2	20.4	36.7	28.6	44.9
14	42	33.3	35.7 <sup>3</sup>	16.7 <sup>2</sup>	11.9 <sup>2</sup>	0.0 <sup>1,3</sup>	21.4 <sup>2</sup>	45.2	35.7	38.1
15	25	32.0	32.0	24.0	20.0	20.0	32.0	52.0	52.0	56.0
16	31	12.9	12.9	16.1	12.9	12.9	16.1	25.8	25.8	32.2
17	38	21.1	21.1	10.5	5.3	5.3	10.5	26.4	26.4	21.0
18	78	20.5	20.5	14.1	9.0 <sup>3</sup>	7.7 <sup>3</sup>	24.4 <sup>1,2</sup>	29.5	28.2	38.5
19	62	16.1	19.4	16.1	11.3	11.3	21.0	27.4	30.7	37.1

BMI, body mass index; FM %, Fat mass percentage; WHO, world health organization; IOTF, international obesity task force; CGF, child growth foundation. Significant differences between references ( $p < 0.05$ ) indicated with superindex: WHO = 1; IOTF = 2; CGF = 3.

to the WHO and 30.9% according to IOTF, in comparison to the 41.2 and 34.8% in this study. In terms of both genders, there was a prevalence of 43.7% according to the WHO and of 32% according to IOTF, percentages that somewhat differ to the current study: 37.3 and 32.3%, respectively. These observed discrepancies may be explained by differences in samples sizes and the different populations studied (Spain and Spain/Italy). For example, Spain and Italy are countries of Southern Europe with a similar Mediterranean diet, however, the percentage of total fat in the diet of Spanish adolescents has been found to be higher than in the diet of Italian adolescents (Cruz, 2000).

The National Health Survey 2011–2012 carried out by the National Institute of Statistics “INE” (Spain), in children and

adolescents aged 2 to 17 years (3,580,100 female and 3,883,500 male), determined the BMI of all the participants and obtained the following percentages for overweight, obesity and excess weight: girls (16.94% overweight, 9.56% obesity, 26.50% excess weight), boys (19.46% overweight, 9.57% obesity, and 29.03% excess weight). The percentage of excess weight obtained by the Instituto Nacional de Estadística [INE] (2012) in girls (26.50%) is lower than those obtained in the present study (33.6% according to the WHO, 29.9% according to IOTF, and 35.8% according to CGF). Moreover, in boys the percentage of excess weight which was obtained by the INE (29.03%) is lower than the percentages obtained in the present study (41.2% according to the WHO, 34.8% according to IOTF, and 44% according to CGF).

When only children and adolescents from the region of Murcia were considered, the data of the INE determined the following percentages for overweight, obesity and excess weight: girls (9.77% overweight, 16.75% obesity, 26.52% excess weight), boys (19.16% overweight, 9.46% obesity and 25.62% excess weight). The percentage of excess weight obtained in Murcia by the Instituto Nacional de Estadística [INE] (2012) in girls is lower than those obtained in the present study (33.6% according to the WHO, 29.9% according to IOTF, and 35.8% according to CGF). Moreover, in boys the percentage of excess weight which was obtained in Murcia by the INE (25.62%) is lower than the percentages obtained in this study (41.2% according to the WHO, 34.8% according to IOTF, and 44% according to CGF). This suggests that excess weight is increasing in the region of Murcia, Spain.

In the study by the National Institute of Statistics “ISTAT” (Italy) in 2010, in those aged 6 to 17 years (3,368,000 female and 3,558,000 male), the BMI of the participants was determined, and the following percentages of excess weight were obtained, according to IOTF criteria: 23.2% of excess weight in girls and 28.9% of excess weight in boys. The percentage of excess weight obtained by Instituto Nacional de Estadística [INE] (2012) for girls (23.2%) was lower than the estimate obtained in this study by IOTF (29.9%). Moreover, in boys the percentage of excess weight obtained by the ISTAT (28.9%) is lower than the percentage obtained in the present study by the IOTF (34.8%). When only children and adolescents from the regions of Lazio and Calabria were considered, the data of the ISTAT determined the following percentage for excess weight: 27.0% of excess weight in Lazio and 30.4% of excess weight in Calabria. This suggests that excess weight is increasing in the regions of Lazio and Calabria.

Previous studies (Wang and Wang, 2002; Shields and Tremblay, 2010; Espín Ríos et al., 2013; Bergel et al., 2014; López-Sánchez et al., 2015) with different samples (schoolchildren from Ibero-American countries, Canada, United States, Russia and China) also found important differences when using different classifications to evaluate the prevalence of overweight and obesity according to BMI, in the same way as the current study. However, the present study also found significant differences with the cut-off points for fat mass, which is why it is essential to specify always the methods and references used until an agreement is reached.

The main strength of the present study is the comparison between cut-off points for BMI and fat mass in a sample of children and adolescents residing in Southern Europe. However, the present study is not without limitations, the sample was composed of 1000 children and adolescents from distinct geographic areas/countries and, although all the recommendations for an adequate analysis of electric bioimpedance were followed, the electrical bioimpedance is not the gold standard method to evaluate fat mass.

## CONCLUSION

The present findings suggest that there is a high prevalence of overweight and obesity in children and adolescents residing in

Southern Europe. However, prevalence estimates of overweight and obesity differ by methods and reference cut points. Higher prevalence was obtained with the classification of CGF (fat mass), followed by the classifications of WHO and IOTF (BMI).

## PRACTICAL APPLICATIONS

A precise definition of overweight and obesity is needed, as well recommended methods for evaluation, and accurate cut-off points. The authors of this study recommend the evaluation of overweight and obesity not only by BMI, but also through the percentage of fat mass, and to always specify the references used to classify the sample.

It would also be convenient to carry out regular assessments in schools, which could be carried out by the Physical Education teacher because of the direct connection between the subject and overweight and obesity. This simple practice would provide updated reference values in any geographical location.

Finally, due to the high prevalence of overweight and obesity found in this study, it would be interesting to carry out intervention programs in children and adolescents, through physical activity and a dietary improvement, to reduce their fat mass and BMI values and develop healthy lifestyle habits. To control the dietary habits during these intervention programs, researchers should evaluate regularly the quality of the diet of the children and adolescents participating in the intervention. Future researchers should also consider the psychological factors of motivation and perceived motor competence, and the family influence, when carrying out intervention programs to ensure that the children and adolescents will adhere to any program put in place.

## AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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# The Effect of Physical Activity on Cognitive Performance in an Italian Elementary School: Insights From a Pilot Study Using Structural Equation Modeling

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When compared to the previous generations, younger generations have become sedentary on a global level. Physical activity positively contributes to human growth and development, causing, indeed, both physiological and psychological benefits. The aim of the current study was examining the relationship between physical activity and school achievement in a sample of 80 Italian elementary (viz. primary) school last year responding children ( $11.0 \pm 0.3$  years,  $1.46 \pm 0.09$  m,  $39.5 \pm 7.9$  kg). Such an aim was fulfilled by investigating eventual correlations between physical tests results and school marks and by disclosing eventual mutual relationships between socio-demographics, family context, lifestyle (including physical activity), and school performance information using a structural modeling approach. Children were assessed for lower/upper limbs muscle strength and running/agility performance. Pearson's correlation between physical tests and school performance was studied. We found that agility correlated with English, Italian, mathematics, music, and sport marks, whereas jump correlated with English, mathematics, sport, and technologies marks. Sprint correlated with mathematics, sport, and technologies marks. All correlation coefficients were moderate, except for correlations between sport marks and physical tests (strong correlation). From the structural model, we found that socio-demographics and lifestyle significantly impacted on school achievement. In particular, lifestyle was found to fully moderate the impact of the family context on school achievement. Schools and households represent important settings for improving children physical and psychological-cognitive health and status, offering physical activities opportunities.

**Keywords:** physical activity, cognitive effect, elementary school, exercise for health, structural modeling

## INTRODUCTION

When compared to the previous generations, the younger ones have become sedentary on a global level, experiencing an increasingly less physically demanding life (Owen et al., 2010), and being, as such, more vulnerable to developing overweight and obesity and suffering from their long-term health consequences, like dysmetabolic disorders and tumors (Mitchell and Byun, 2014).

Work sites, time spent using digital media and virtual social contacts are creating a relevant time debt for sport and related physical activity (Corder et al., 2015), leading people to adopt sedentary behaviors (Owen et al., 2010). Physical activity, on the contrary, positively contributes to human growth and development, preparing children for the mental and physical challenges of adolescence and emerging adulthood (Prakash et al., 2015; Felfe et al., 2016). There is a huge body of evidence indicating that physical activity produces, indeed, both physiological and psychological benefits, being associated with better mental health and enhancement of brain function and cognition (Donnelly et al., 2016).

In a series of meta-analyses (Ahn and Fedewa, 2011; Jackson et al., 2016), small but significant improvements in mental health and in the domain of executive functioning (in particular, inhibitory control) were reported. These findings were confirmed by the meta-analysis performed by de Greeff et al. (2018), which documented improvements of executive functions as well as of attention. Sibley and Etnier (2003) found that even physical activity of low intensity or for a short term is related to improvements in executive and neurocognitive functioning, being the relationship not strictly dose-dependent, but both quantitative and qualitative (Pesce, 2012). Jäger et al. (2014) investigated the effects of an acute 20-min physical activity intervention on executive functions in a sample of 52 young elementary school children, aged 6–8 years old versus other 52 children assigned to a resting control condition. Authors found statistically significant cognitive effects induced by acute sports intervention.

Acute physical activity significantly improves attention. Concerning the link between physical activity and subsequent future school performance, a systematic review of the literature established a long-term relationship between the two variables under scrutiny (Singh et al., 2012). Besides the above-mentioned psychological effects, authors offered several plausible biological mechanisms underlying the reason(s) why exercise might be beneficial for cognition, including (1) increased oxygenation to the brain (Ide and Secher, 2000); (2) increased levels of neurotransmitters, including norepinephrine, epinephrine, and serotonin, associated with memory, information processing and other neuropsychological skills (Meeusen and De Meirleir, 1995); (3) release of molecules like endorphins, endogenous opioid neuropeptides, which result in stress reduction (Goldfarb and Jamurtas, 1997; Yang and Chen, 2017); and (4) increased levels of growth factors, such as brain-derived neurotrophic factor (BDNF), growth hormone, and insulin growth factor 1 (IGF-1), associated with cellular development, angiogenesis, neurogenesis, and synaptogenesis (Alesi et al., 2015; De Giorgio et al., 2018).

Given these speculated processes, it is not unexpected that being involved in physical activities predicts better classroom engagement and participation. A recently published meta-analysis by de Greeff et al. (2018) quantitatively confirmed the relationship between sports and academic achievements. However, despite many researches being conducted on this topic, studies exhibit a high degree of variability and inconsistency concerning both study quality and results (Howie and Pate, 2012). The topic of the link between physical activities and school performance remains urgent and of crucial importance, given the convergence of an increasing focus on academic achievements and the decreasing physical activities opportunities in schools, worldwide (Howie and Pate, 2012).

The aim of the current study was to examine the relationship between physical activity and school achievement by 1) investigating eventual correlations between physical tests results and school marks and 2) disclosing eventual mutual relationships between socio-demographics, family context, lifestyle, and school performance information using a structural modeling approach. We used a structural model since this method enables to overcome the shortcomings that plague a cross-sectional study, showing reciprocal causal relationships among variables and having both explanatory and predictive power. Furthermore, it can estimate the multiple and interrelated dependences among variables in a single analysis (Jeon, 2015). The use of structural modeling equations and the exploration of different variables, including family context, represent the main novelties of the present study.

## MATERIALS AND METHODS

### Participants

Specifically for correlational analysis sample size calculation purposes, a pilot study that included a sample of 50 children was performed *a priori* and gave a statistical power greater than 0.81. Therefore, one hundred and six voluntary children (80 responders,  $11.0 \pm 0.3$  years,  $1.46 \pm 0.09$  m,  $39.5 \pm 7.9$  kg; further details in **Tables 1, 2**) were recruited to participate in this study. The criteria of inclusion were: (i) attending the last year of the Italian primary school. The *criteria* of exclusion were: (i) not having completed 5 years as students of the same school in which the present investigation was carried out, and (ii) suffering from neurological, cardio-vascular or orthopedic disorders that might impair their abilities to execute a battery of explosive exercises. Twenty-six children were excluded, on the basis of the first exclusion *criterion*, whereas none was excluded due to the second *criterion*.

Before participation in this study, children and their parents were given a letter that included written information about the study and a request for consent from parents to allow their children to take part into the study. Parental informed consent was obtained after thorough explanation of study objectives, procedures, risks, and benefits. This study was conducted according to the Declaration of Helsinki. The study also conformed to the University of Split, Human Research Ethics



**TABLE 1** | Socio-demographics and family context of the recruited sample.

Parameter	Value
<b>Socio-demographics</b>	
Body Mass Index (mean $\pm$ standard deviation)	18.45 $\pm$ 2.66
Citizenship	
Italian (n; %)	70 (87.5%)
Non Italian (n; %)	10 (12.5%)
Gender	
Female (n; %)	46 (57.5%)
Male (n; %)	34 (42.5%)
<b>Family context</b>	
(living at) Floor	
Ground floor (n; %)	32 (40.0%)
First floor (n; %)	30 (37.5%)
Second floor (n; %)	12 (15.0%)
Third floor (n; %)	6 (7.5%)
Number of family (parents + offspring) members	
Three members (n; %) Four members (n; %)	14 (17.5%) 48 (60.0%)
Five members (n; %)	18 (22.5%)
Number of family members practicing sport (median)	2
Presence of a dog (n; %)	28 (35.0%)

**TABLE 2** | Lifestyle and school performance of the recruited sample.

Parameter	Value
<b>Lifestyle (i.e., daily activities)</b>	
Walking the dog (n; %)	16 (57.1%)
Using the lift (n; %)	2 (2.5%)
Practicing sport (n; %)	72 (90.0%)
Number of sport disciplines practiced	
One sport (n; %)	26 (36.1%)
Two sports (n; %)	24 (33.3%)
Three sports (n; %)	8 (11.1%)
Four sports (n; %)	6 (8.3%)
Five sports (n; %)	8 (11.1%)
Practicing team sports (n; %)	32 (44.4%)
Practicing individual sports (n; %)	62 (86.1%)
Weekly hours of sport practice (mean $\pm$ standard deviation; median)	3.50 $\pm$ 3.21 (2.5)
Hours spent playing video games (mean $\pm$ standard deviation; median)	5.84 $\pm$ 6.61 (3.25)
Hours spent watching TV (mean $\pm$ standard deviation; median)	9.99 $\pm$ 7.19 (10.0)
<b>School performance</b>	
Absences from school (days, mean $\pm$ standard deviation; median)	7.93 $\pm$ 7.21 (6.0)
Italian (mean $\pm$ standard deviation; median)	7.74 $\pm$ 1.03 (7.9)
Maths (mean $\pm$ standard deviation; median)	7.63 $\pm$ 1.06 (7.6)
English (mean $\pm$ standard deviation; median)	8.03 $\pm$ 0.84 (8.2)
Sciences (mean $\pm$ standard deviation; median)	7.66 $\pm$ 0.93 (7.6)
History (mean $\pm$ standard deviation; median)	7.77 $\pm$ 0.79 (8.0)
Geography (mean $\pm$ standard deviation; median)	7.70 $\pm$ 0.82 (7.7)
Technology (mean $\pm$ standard deviation; median)	7.82 $\pm$ 0.71 (7.9)
Arts (mean $\pm$ standard deviation; median)	7.95 $\pm$ 0.81 (7.9)
Sport (mean $\pm$ standard deviation; median)	8.35 $\pm$ 0.57 (8.4)
Music (mean $\pm$ standard deviation; median)	7.99 $\pm$ 0.57 (8.2)

Committee guidelines. The Human Research Ethics Committee of the University of Split approved the experimental protocol.

## Protocol

The primary outcome of the present investigation was the academic performance for different disciplines (namely, Italian language and literature, mathematics, English language and literature, sciences, history, geography, technology, arts, sport, and music).

Grades were obtained directly from the school board for each term based on examinations prepared and administered according to the ministerial guidelines. Grades were on a numeric scale and ranged from 1 to 10.

A battery of physical tests validated for children, including standing long jump and medicine ball throw to assess muscle strength for lower/upper limbs, and sprint/agility test to assess running/agility performance, was administrated (Duncan et al., 2018; Scheuer et al., 2019). Such tests (repeated after 1 week to assess reliability of the measures) were randomly administered paying particular care to minimize any potential interference with usual children schedule: more in detail, physical tests were performed at the same time of day each day (at 11.30 a.m.), in the school gym.

A standardized warm-up consisting of jogging, dynamic stretching (Chaouachi et al., 2017), and a series of increasing-intensity sprints was performed before testing, in order to better prepare the children to the execution of the physical tests. For each test, three repetitions were performed with 1–2 min of passive recovery in-between. The best performance obtained was used in our statistical analysis. For each test the time spent was 20 min.

## Long Jump

Participants stood stationary with toes aligned with the start line, and were instructed to push off vigorously and to jump forward as far as possible. Participants were allowed to make use of a counter-movement with arms and body swing (Padulo et al., 2014). Distance jumped from the start line to position of heel at touchdown was measured in centimeters using a metal measuring tape. The test was repeated three times and the maximum distance achieved was used for further statistical analysis.

## Free Ball

Children were asked to sit on a chair with their shoulders in contact with the chair-back. Then, they were asked to throw forward the medicine ball as far as possible for three times with 1 min of passive recovery in-between. The used medicine ball weighed 2 kg (Padulo et al., 2014). The distance from participants' shoulders to the point of impact of the ball with the ground was measured and the longest distance was used for further analysis. The throw distance was measured by means of the measuring tape.

## Sprint

Running ability was evaluated using a maximal 10-m sprint. Children were instructed to run as quickly as possible along a 10-m distance after a standing start. Time was automatically

recorded using photocell gates (Brower Timing Systems, Salt Lake City, UT, United States; accuracy of 0.01 s) placed 0.4-m above the ground (Hammami et al., 2014). Subjects started the sprint when ready while standing just behind the first timing gate. Subjects performed two trials with at least 2 min of rest between them. The fastest run was used for further analysis.

### Agility Test

The used agility test was 4 × 9-m shuttle run test (Kibele and Behm, 2009). Children stood behind a starting line and started the electronic clock by passing through the first timing gate. At the end of the first 9-m section, subjects were asked to step with one foot beyond a marker while reversing the running direction and sprinting back to the start where a same reversal was required. After the fourth 9-m section, participant passed through the second timing gate to stop the electronic clock. For time measurement, this test was conducted using the same equipment as in the sprint tests.

### Statistical Analysis

To quantitatively assess the relationship between physical tests and school performance, Pearson's correlation was computed. Based on obtained *r* coefficient values, qualitative interpretation of correlation strength was carried out using the following rule of thumb proposed by Cohen (1988). Namely, values up to 0.100, between 0.100 and 0.300, and above 0.300 indicated weak, moderate, and strong relationship, respectively.

In order to properly investigate the impact of physical activity on school performance, using partial-least squares structural equation modeling analysis (PLS-SEM), a conceptual model obtained reviewing the existing scholarly literature was used. This model related to the relations between latent variables (LVs) and their corresponding manifest variables (MVs).

In particular, the conceptual model was based on 27 MVs, which have been subsequently grouped into 4 LVs (first three exogenous and fourth endogenous) named: socio-demographic variable (consisting in ethnicity/citizenship, age, gender, and body mass index or BMI), family context (consisting in the number of family members, in the number of family components practicing sport, floor of the house where family lives, and presence of a dog), lifestyle behaviors (consisting in the use of a lift, walking the dog, number of sports disciplines practiced and their type, team or individual sports, weekly hours spent practicing sport in average, and years practicing sport) and school performance.

In PLS-SEM, the model is generally described by two components referred as (1) measurement model (or construct), which relates MVs with their corresponding LVs, and (2) structural model which shows the relationship between various LVs. These relations can be pictorially shown as a path diagram, in which LVs are usually drawn with an oval shape, while rectangular shaped elements represent MVs.

The commercial software SmartPLS was used to model data concerning physical activities and school performance. A 2-step procedure (measurement model plus structural model), as recommended by Henseler et al. (2009) was adopted.

Concerning the measurement model, in order to check for reliability of the measurement model at the indicator level, the standardized outer loading for each indicator and its corresponding structure was calculated and investigated. The acceptable levels of outer loading were values higher than 0.7, as recommended by Henseler et al. (2009).

Also, the validity of the measurement model was examined by using discriminant validity verifying the Fornell-Larcker criterion, the assessment of potential cross-loadings, and the heterotrait-monotrait *ratio* statistics. Moreover, in order to examine convergent validity, several indicators were used, such as Cronbach's alpha, Dijkstra-Henseler's  $\rho A$ , and average variance extracted (AVE). Usually, Cronbach's alpha greater than 0.7, Dijkstra-Henseler's  $\rho A$  greater than 0.7, and AVE greater than 0.5 indicate a good convergent validity.

To evaluate the structural section of the model, coefficient of determination ( $r^2$ ) and path coefficient were evaluated.  $r^2$  equal to 0.19 or lower were considered weak. To assess reliability of the measures, Intra-class correlation coefficient (ICC) was calculated (Hopkins, 2000). Standardized root mean square residual (SRMR) for both saturated and estimated model was computed.

Statistical significance was computed calculating *t*-statistics for fitting indices such as d\_ULS and d\_G using bootstrapping with 5,000 iterations.

## RESULTS

The main characteristics of the recruited sample are described in **Table 1** (socio-demographics, and family context variables) and 2 (lifestyle and school performance parameters).

Of 106 eligible subjects, 80 fully answered the questionnaire (75.5% responding rate); 46 (57.5%) were females, whereas 34 (42.5%) were males; 70 (87.5%) were Italians, whereas only 10 (12.5%) were not Italians. Average BMI was  $18.45 \pm 2.66$  kg/m<sup>2</sup>. Most families lived on ground or first floor, whereas only 7.5% of households lived on third floor. In 60% of cases, family comprised four members and, generally, two members practiced sport activities. Only 28 (35.0%) families had a dog; in 57.1% of cases, children took part in walking the dog. Interestingly, majority of children (90% of the sample) practiced at least one sport; 32 (44.4%) practiced team sports, whereas 62 (86.1%) practiced individual sports.  $3.50 \pm 3.21$  (median 2.5) hours *per week* were spent practicing sport activities, whereas  $5.84 \pm 6.61$  (median 3.25) and  $9.99 \pm 7.19$  (median 10.0) hours *per week* were spent playing video games or watching TV, respectively.

The initial structural model is shown in **Figure 1**, while the final structural model obtained with 2-step procedure is depicted in **Figure 2**.

Fitting parameters (shown in **Table 3**) indicated a good fit of the model, with SRMR 0.046 and 0.049 for saturated and estimated models, respectively (being lower than the cut-off value of 0.080–0.100). d\_ULS and d\_G computed from bootstrapping were not significant, as expected.  $r^2$  of lifestyle and school performance variables resulted in 0.288 (adjusted

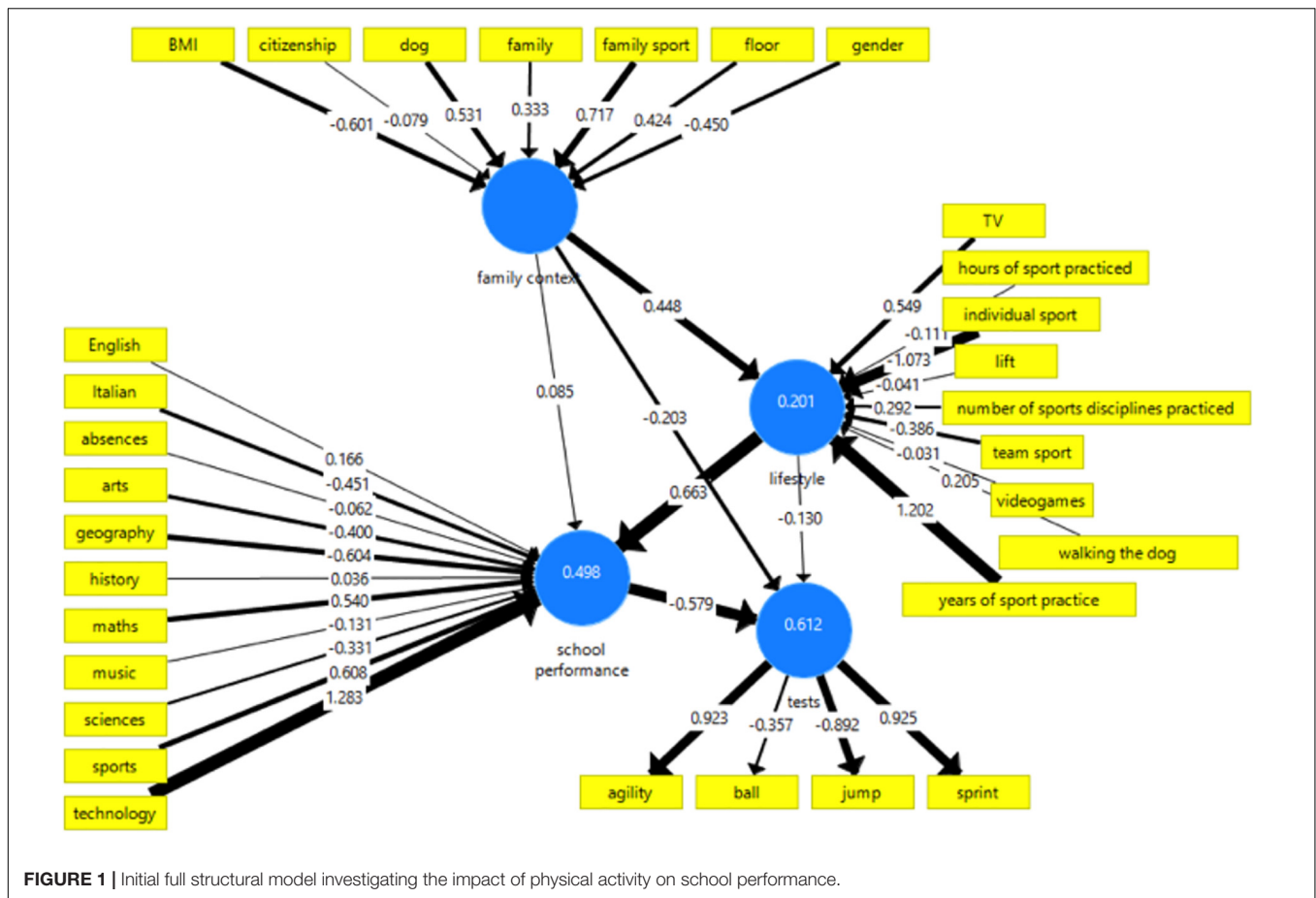


FIGURE 1 | Initial full structural model investigating the impact of physical activity on school performance.

0.250) and 0.394 (adjusted 0.343), respectively, well above the cut-off of 0.190.

Table 4 reports averages scores for agility test, free ball, long jump, and sprint, together with their ICCs, showing a good reliability of the administered physical tests.

Table 5 reports correlation coefficients between physical tests and school performance. Agility correlated with English, Italian, mathematics, music, and sports marks, whereas jump correlated with English, mathematics, sports, and technologies marks. Sprint correlated with mathematics, sports, and technologies marks. All correlation coefficients were moderate, except for correlations between sports marks and physical tests (strong correlation).

Socio-demographics and life-style significantly impacted on school performance. In particular, lifestyle was found to fully moderate the impact of family context on school performance, as shown in Figure 2.

More in detail (Table 6), path coefficient between family context (family members practicing sport activities) and lifestyle context (number of years spent by child practicing sport activities) was 0.537 ( $P = 0.000$ ), whereas the path coefficient between lifestyle context and school performance was 0.356 ( $P = 0.034$ ), with the path coefficient between family context and school performance yielding a value of 0.256 ( $P = 0.170$ , not statistically significant). Path coefficient between

socio-demographic variables and school performance resulted in 0.324 ( $P = 0.012$ ), whereas path coefficient between socio-demographic variables and lifestyle context was 0.032 ( $P = 0.815$ , not statistically significant).

## DISCUSSION

Improving school performance is an appropriate goal for educational policy-makers, as improving physical activity is an onus for health promotion and sports authorities. So, determining the factors that most affect the relationship between physical activity and school performance is really important for schools, sports, and health-care workers.

Our findings that gender, years of sport practice, and family context have a positive impact on academic achievements are in line with extant literature, even though we found that 90% of our sample practiced at least one sport, which is a percentage higher than those computed by other scholars. For instance, Fox et al. (2010), drawing data from the “Project EAT” (Eating Among Teens), an extensive survey of 4,746 middle and high school students, reported participation on at least 1 sports team in the past 12 months by 53–71% of all participants.

A growing number of articles has shown strong relationships among physical activity, cognitive functions, and academic

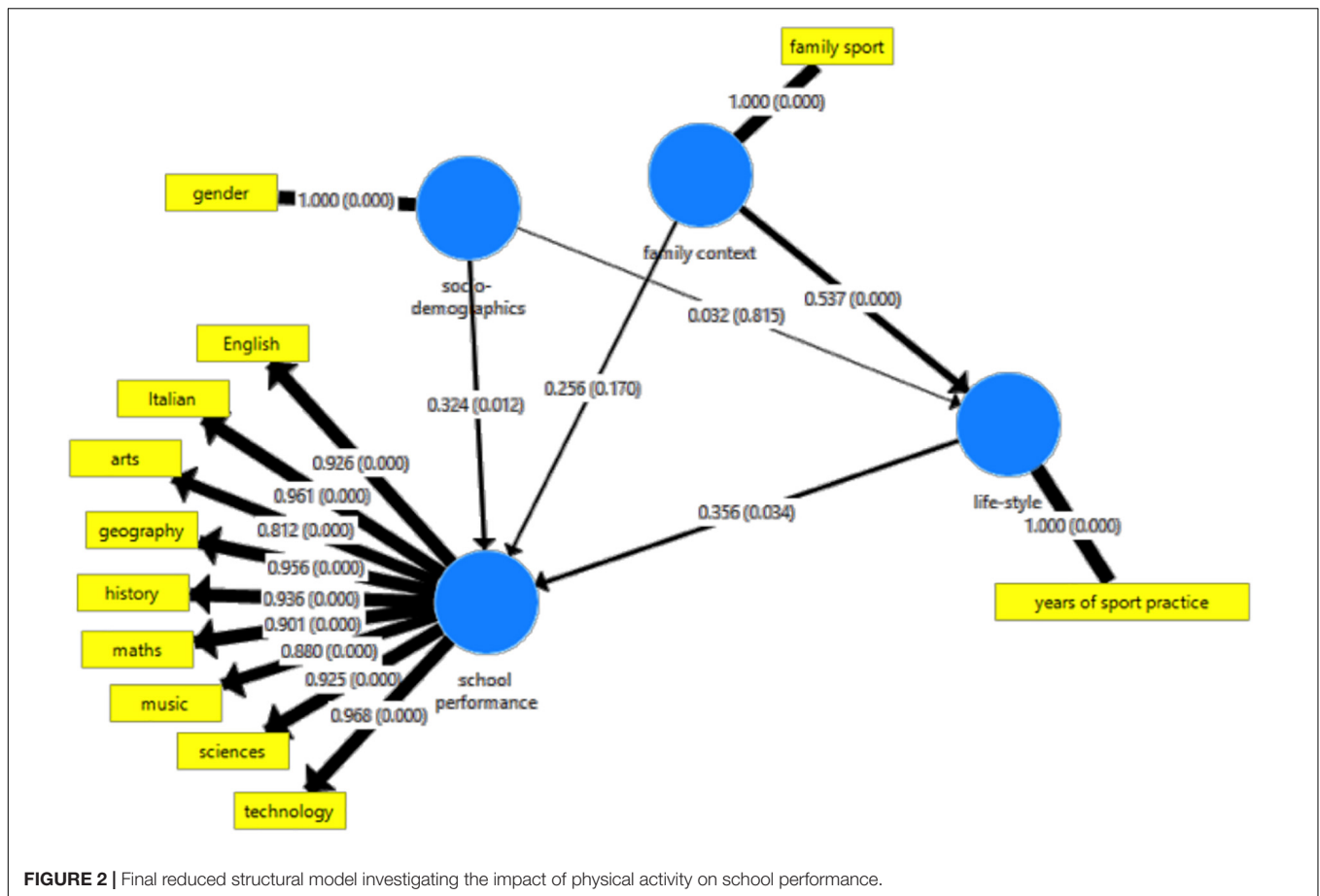


FIGURE 2 | Final reduced structural model investigating the impact of physical activity on school performance.

TABLE 3 | Fitting parameters of estimated structural model (bootstrapping has been performed with 5,000 iterations).

Fitting parameter	Saturated model	Cut-off value	Estimated model	Cut-off value		
SRMR	0.046	Should be <0.080–0.100	0.049	Should be <0.080–0.100		
Fitting parameter (bootstrapping)	Value	t statistics	P-values	Cut-off value		
d_uls	0.186	0.493	0.622	Should be ns		
d_g	0.879	0.989	0.323	Should be ns		
Latent variable	r <sup>2</sup>	t statistics	P-values	r <sup>2</sup> adjusted	t Statistics	P-values
Lifestyle	0.288	2.454	0.014	0.250	2.017	0.044
School performance	0.394	3.170	0.002	0.343	2.551	0.011

SRMR, standardized root mean residual.

achievements in children (Hillman et al., 2008; Vazou and Skrade, 2017). As aforementioned, under “physical activity” locution are intended both quantitative (e.g., intensity and duration) and qualitative (e.g., coordinative demands and cognitive engagement) characteristics of activities. Quantitative activities lead to enhance, for example, cardiovascular fitness, whereas qualitative activities can improve, for example, motor skills (Diamond, 2015). Without being exhaustive, several studies examined the relationships between physical activity or exercise, and cognitive performance, and various results were found (Trudeau and Shephard, 2008).

According to Landry and Driscoll (2012), exercise improves health, as well as psychological well-being, cognition, and school performance. Green (2016) studied whether healthy lifestyle factors impact on academic performance among university students and found that healthy lifestyle (being physically active, no smoker, no-binge drinker, and healthy diet) increases first-year students’ performance. Effect of physical education and activity levels on academic achievement in school-age children in different categories such as perceptual skills, intelligence quotient, achievement, verbal tests, mathematic tests, memory, and readiness was showed by Ahn and Fedewa (2011),

**TABLE 4** | Average scores of the different physical tests and their intra-class correlation coefficients (ICC) with their 95% confidence interval (CI).

Test	Mean	Standard Deviation
Agility test (s)	21.16	2.16
Free ball (m)	3.96	0.63
Long jump (m)	1.89	0.44
Sprint (s)	2.59	0.27

Test	ICC for single measures and 95% CI		ICC for average measures and 95% CI	
	Agility test	0.8687	0.7637–0.9290	0.9298
Free ball	0.7300	0.5413–0.8487	0.8439	0.7024–0.9182
Long jump	0.8469	0.7271–0.9167	0.9171	0.8420–0.9565
Sprint	0.7370	0.5519–0.8529	0.8486	0.7112–0.9206

**TABLE 5** | Correlational analysis between physical tests and school achievements.

Pearson's correlation	Agility	Free ball	Jump	Sprint
Arts	–0.084	–0.039	0.066	0.115
	0.6082	0.8089	0.6880	0.4815
Geography	–0.275	0.069	0.309	–0.249
	0.0863	0.6701	0.0520	0.1214
English	–0.400*	0.012	0.353*	–0.270
	0.0105	0.9415	0.0253	0.0915
Italian	–0.337*	–0.003	0.290	–0.176
	0.0333	0.9876	0.0693	0.2772
Maths	–0.423*	0.080	0.360*	–0.314*
	0.0065	0.6232	0.0226	0.0481
Music	–0.315*	0.125	0.295	–0.116
	0.0479	0.4423	0.0642	0.4769
Sciences	–0.203	–0.054	0.218	–0.182
	0.2091	0.7397	0.1762	0.2598
Sport	–0.622*	0.072	0.572*	–0.608*
	< 0.0001	0.6584	0.0001	< 0.0001
History	–0.143	0.005	0.144	–0.061
	0.3797	0.9738	0.3759	0.7066
Technologies	–0.381*	0.124	0.375*	–0.313*
	0.0154	0.4472	0.0171	0.0494

\*statistically significant.

**TABLE 6** | Path coefficients of the estimated structural model.

Path	Path coefficient	t statistics	P-values
Family context → lifestyle	0.537	4.469	0.000
Family context → school performance	0.256	1.366	0.172
Lifestyle → school performance	0.356	2.138	0.033
Socio-demographics → lifestyle	0.032	0.231	0.817
Socio-demographics → school performance	0.324	2.453	0.014

Carlson et al. (2008), Coe et al. (2006), de Greeff et al. (2018), Negi et al. (2016), and Sibley and Etnier (2003).

With regard to qualitative and/or quantitative characteristics of the physical activities, Beck et al. (2016) have demonstrated

that gross motor activity integrated into math lessons could improve children mathematical performance. Authors also investigated fine motor skills, showing that these abilities are not able to improve math performances. Children studied were 165 and were tested before, immediately after, and 8 weeks after an intervention lasted 6 weeks. Beck et al. (2016) highlighted that improvement was significantly greater after gross motor than fine motor activities and that physical activities exhibited a reduced effect 8 weeks after the intervention. Furthermore, increasing the level of engagement in physical activity in childhood can also improve scores in memory, attention, and problem-solving skills. For example, Davis et al. (2011) showed in overweight and obese children that after-school physical activity intervention through vigorous intensity was able to improve executive function scores. Authors highlighted significant improving of working memory.

Palmer et al. (2013) demonstrated that physical activity intervention leads to beneficial effect of sustained attention. It is also known that physical activity can lead to weight loss. This achievement is also very important, because, as it has been discussed in a recent review by Veronese et al. (2017), weight loss was associated with improvements in cognitive function among overweight and obese people. It is worth to mention that Schmidt et al. (2016) highlighted that effects of exercise are independent from its intensity and are able to increase executive functions after 6 weeks of physical activity intervention.

Regarding adulthood, a Saudi Arabian study by Al-Drees et al. (2016) reported a positive association between physical activity habits among medical students and high academic achievement.

Motor activity effects on brain are also studied. For example, greater gray matter hippocampal volumes but lower gray matter thickness in the frontal cortex are demonstrated after higher-fit activities in preadolescent children (Chaddock et al., 2010; Chaddock-Heyman et al., 2014, 2015). It was also shown that these brain modifications lead to superior cognitive and academic performance. In particular, physical exercise can cause a release of several biomarkers such as BDNF, nerve growth factor (NGF), and IGF-1 that have a positive impact on brain structures (Skriver et al., 2014).

These findings are also confirmed by several animal studies in which it was shown that neurotrophins influences neuroplastic processes related, also but not only, to the hippocampus and, then, to memory formation (Cotman et al., 2007). Interestingly, De Giorgio (2017) and Granato and De Giorgio (2014) discussed the role of motor activity in subjects with intellectual disabilities, showing that in an experimental model of intellectual disability the motor activity is able to activate neurotropic factors, resulting into a partial repair of the neural damage.

Finally, using data from the “National Educational Longitudinal Survey”, Eitle (2005) investigated whether there are gender and race differences in the effects of participation in a variety of sports on achievement in science, mathematics, reading, and history and found that achievement benefits of playing team sports and individual sports appear to be more relevant for white female participants than for others.

The main strength of our study lies in the fact that we exploited structural modeling and, as such, this allows drawing

causal conclusions from the data. On the other hand, the major shortcoming is that more running/agility performance measures would be needed to get a complete picture of children capabilities. Furthermore, future studies should replicate our findings utilizing larger sample sizes to consequently achieve greater statistical powers.

## CONCLUSION

Schools and households represent important settings for improving children physical and psychological-cognitive health and status, offering physical activities opportunities (Álvarez-Bueno et al., 2016, 2017).

In our investigation, we found that agility correlated with English, Italian, mathematics, music, and sport marks, whereas jump correlated with English, mathematics, sport, and technologies marks, and sprint correlated with mathematics, sport, and technologies marks. All correlations were moderate, except for correlations between sports marks and physical

tests (strong correlation). From the structural model, we found that socio-demographics and lifestyle significantly impacted on school achievement: lifestyle was found to fully moderate the impact of the family context on school achievement.

Our predictive model can be useful for different stakeholders, including educational policy-makers and workers in the field of health promotion, sport, and education.

## AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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# Repeated Sprint Ability and Muscular Responses According to the Age Category in Elite Youth Soccer Players

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The aim of this study was to analyse the influence of age category on the performance and muscle response after a Repeated Sprint Ability (RSA) test in elite youth soccer players. 62 soccer players from three different age categories (Under 14 [ $n = 21$ ], Under 16 [ $n = 20$ ], and Under 18 [ $n = 21$ ]) were selected to participate in this study. Players completed an RSA test (7 × 30 m) with a 20-s recovery between sprints. The muscular response to an electrical stimulus before and after the test of both the biceps femoris (BF) and the rectus femoris (RF) were evaluated using tensiomyography. A two-way ANOVA was used to analyse the differences in RSA parameters in each of the four distance-intervals (0–5; 5–25; 25–30; 0–30 m) between sprint and age category. The U14 age category ( $5.30 \pm 0.30$  s) showed higher mean sprint times than U16 ( $4.62 \pm 0.20$  s) and U18 ( $4.46 \pm 0.17$  s) throughout the entire test ( $p < 0.01$ ). U16 players revealed a worse best sprints time (RSA<sub>BEST</sub>) than U18 players ( $+0.12$  s, CI95%: to 0.01 to 0.24; ES: 1.09,  $p = 0.03$ ). The muscular contractile properties were similar in the three age categories analyzed ( $p > 0.05$ ), although the delay time (Td) of the muscle was significantly lower after the RSA test in U16 players ( $-1.53$  ms, CI95%:  $-2.607$  to  $-0.452$ ; ES: 0.38) and U18 players ( $-1.11$  ms, CI95%:  $-2.10$  to  $-0.12$ ; ES: 0.22). In conclusion, this study revealed an increase in physical performance and muscle response variability after a repeated sprint ability test in the U16's and over. The fatigue induced by the RSA test did not show differences depending on the age of the players, although muscle mechanical properties were altered after the RSA test in U16 and U18 soccer players. Physical performance and muscle response can be complementary variables in managing fatigue according to the age category in soccer players.

**Keywords:** RSA, soccer, age, tensiomyography, fatigue, muscle

## INTRODUCTION

During soccer practice, several capacities such as cardiovascular endurance and the ability to perform repeated sprint actions have shown to be decisive for players' performance (Rampinini et al., 2007). For youth players, maturity status is a key variable in their physical outcomes, especially in relation to their physical capacities and match running performances



(Buchheit and Mendez-Villanueva, 2014). Previous research has pointed out that the repeated sprint ability (RSA) is related to physiological parameters such as maximal oxygen uptake or muscle phosphocreatine degradation/resynthesis among others (Spencer et al., 2005). Recent research has reported percentile values of repeated sprint performance of young soccer player classified by maturity status (Selmi et al., 2018).

According to Falk and Dotan (2006), adults have a slower recovery from sprint exercise than children. This could be attributed to the fact that children's metabolism is less glycolysis dependent during prolonged recurrent sprints (Hebestreit et al., 1993). The sprint time decreases from the ages of 12–18 years, but at the beginning of adolescence the growth effect has more influence than at the end of this period (Mendez-Villanueva et al., 2011; Malý et al., 2015; Nikolaidis et al., 2016). The biological maturation varies substantially in duration and timing among individuals (Baxter-Jones et al., 2005). Recent studies have focused on the anthropometric and physical profiles (Perroni et al., 2018a; Selmi et al., 2018) but it is surprising that limited information exists on RSA in elite youth soccer players (Mujika et al., 2009), specifically about muscle responses of the players.

Fiber composition is the main factor that decides the speed of muscle contractions (Cormie et al., 2011). A skeletal muscle biopsy is the most accurate test used to collect information on the fiber type composition. Thus, to study the changes on the types of muscle fibers during a child growth it would be necessary to execute repeated biopsies from different muscles over the years. However, due to the great muscle damage that it induces and the ethical issues that might represent to do that in children, alternative methods such as tensiomyography (TMG) could be used to solve this problem.

Tensiomyography is a non-invasive method to assess the contractile properties of skeletal muscles (Valencic et al., 2001; Šimunič, 2012). The TMG applies a stimulation of electrical contraction in basal conditions on the skin, producing a displacement due to an involuntary muscular contraction that is measured with a digital transducer that is in direct contact with the muscular belly (Valencic et al., 2001). This tool has demonstrated a high short-term reliability and sensibility for detecting changes in muscular responses (Križaj et al., 2008; Rey et al., 2012); with a greater reliability in a fatigued state than in rested (Ditroilo et al., 2013). Moreover, this method has been demonstrated to predict 87% of the difference in the proportion of type I myosin heavy chain in the vastus lateralis (VL), and has provided reliable information on the fiber composition in children of maturation age, showing a transformation from slow to fast fibers between 6 and 10 years old (Simunic et al., 2017). Therefore, the TMG is a useful tool to compare muscle contractile properties according to age, and it could be used to fill the knowledge gap in the muscle response of young soccer players.

Among the tasks of physical trainers, is the quantification of training loads and the design of tasks according to the age, ensuring the highest efficiency of athletes' training, and optimizing the performance. However, there is a lack of knowledge on muscle performance and muscle contractile properties in young soccer players (Rumpf et al., 2011) after RSA test. Therefore, this study aims to analyse the influence of age

category on performance and muscle response after an RSA test in young elite soccer players. The hypothesis proposed in this study was that older soccer players would show lower total times in the RSA test. On the other hand, younger soccer players have a greater ability to recover between sprints and a better contractile response of the muscles of the lower body after a high intensity effort with incomplete recovery.

## MATERIALS AND METHODS

### Participants

A total of 62 elite youth soccer players ( $14.63 \pm 2.00$  years;  $167.3 \pm 10.5$  cm;  $58.75 \pm 12.52$  kg) from three age categories (U14 [ $n = 21$ ], U16 [ $n = 20$ ], and U18 [ $n = 21$ ]) participated in this study. Participants were recruited from an elite soccer academy, this academy signed an agreement to allow the researchers to carry out this study. A parent or guardian of each participant signed an informed consent allowing them to participate in the study. Test procedures and the possible risks of the procedures were explained in detail in the written consent. Moreover, all volunteers who presented at the medical examination, which was required to play soccer, players did not report any cardiopulmonary pathology or other diseases and did not take any medication during the study. The study protocol was approved by the Local Ethics Committee (Hospital of Toledo; CEIC61) and was conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

### Experimental Design

The data collection process took place during the third month of the regular season. The research team arranged with the soccer teams 2 days within the given period to allow the players to perform the proposed test. Participants agreed not to perform any exhausting activity 48 h before the trials. Prior to the beginning of the study, players completed a familiarization session with both the tests and the equipment included in the study protocol. The main part of the test consisted of a Repeated Sprint Ability Test (RSAt) whose effect was assessed through tensiomyographic measurements obtained right before and after the test. Testing was performed on the artificial turf pitches where participants regularly trained, in dry conditions (temperature:  $21 \pm 2^\circ\text{C}$ ; relative humidity:  $20 \pm 5\%$ ; wind speed: 0.0–0.5 m/s). All tests were carried out within each team's training schedule to avoid the results being affected by circadian rhythm.

### Experimental Protocol

Before completing the RSAt, participants carried out a standardized warm-up protocol consisting of 5 min of continuous running, 5 min of articulation mobility and three 30-meter sprints at increasing intensity. The warm-up concluded with two 30-meter sprints at maximum speed separated by 4 min of active recovery (participants had to walk during the resting time). The best time in these two sprints was used later as a control measure to guarantee players performed the RSAt at maximum intensity. If the time of the first sprint of the RSAt was higher (>5%) than the best individual sprint performed before,

the RSA<sub>t</sub> was not considered valid and the player had to repeat the test after 5 min of recovery.

### Repeated Sprint Ability Test (RSAt)

The RSAt included seven repetitive sprints of 30 min, with 20 s of active recovery between sprints. Four pairs of photoelectric cells (Witty, Microgate, Bolzano, Italy) were placed at 0, 5, 25, and 30 meters, they were used to assess the performance in this test. Time measurements in four distance-intervals (0–5; 5–25; 25–30; 0–30 meters) were obtained and compared between the groups (U14, U16, and U18). Also, the RSAt sprint time (RSA Time), the RSAt best sprint time (Best Sprint  $\times$  7 [RSA<sub>BEST</sub>]), the RSAt mean time (RSA<sub>MEAN</sub>), the RSAt percent decrement (RSA<sub>DEC</sub>) and the difference between the first and last sprint (RSA<sub>CHANGE</sub>) were estimated and compared between the groups.

### Tensiomyography (TMG)

Muscle response of both the rectus femoris (RF) and biceps femoris (BF) of the players' dominant leg was assessed through tensiomyography (TMG-100 System electrostimulator, TMG-BMC d.o.o., Ljubljana, Slovenia) before and after the RSAt. This mechanism provides information on the muscles' contractile properties as a response to an induced contraction caused by an external electric stimulus. The electric stimulus was induced through two self-adhesive electrodes (TMG electrodes, Ljubljana, Slovenia) and the muscle response was measured with a digital Dc-Dc transducer Trans-Tek<sup>®</sup> (GK 40, Ljubljana, Slovenia) placed perpendicular to the muscle belly and equidistant from the self-adhesive electrodes at a distance of 50–60 mm. The positions of the sensor and the electrodes were marked with a permanent marker to ensure that measurements before and after the RSAt were performed at the same point. The electric stimulus was 1 ms and they started with a stimulus of 20 mA which was increased by 10 mA each time until it reached 110 mA. A 15 s rest was left between measurements to minimize the effects of potentiation and fatigue. The variables assessed in this study were the maximum radial displacement of the muscle belly (Dm), contraction time (Tc), and delay time (Td). Križaj et al. (2008) previously reported a low error level (0.5 to 2.0 %) and a high reproductivity (ICC: 0.85–0.98) for these three parameters (Dm: 0.98; Tc: 0.97; Td: 0.94). No participants reported uncomfotableness during this test. The RF was assessed with participants in supine position with a 120° knee flexion. The BF was evaluated with participants in prone position and the knee flexed 5° (Šimunič, 2012). All measurements were carried out by the same expert technician.

### Statistical Analysis

Data are presented as mean  $\pm$  SD. The SPSS V21.0. software (SPSS Inc, Chicago, IL, USA) was used for data analysis and the level of significance was established at  $p < 0.05$ . The statistical analysis was divided into four parts. Firstly, a two-way ANOVA was used to analyse the differences in RSA sprint times at each of the four distance-intervals (0–5; 5–25; 25–30; 0–30 m) between the sprint (repeated measure: sprint 1 to 7) and the group (independent measure: U14, U16, and U18). Secondly, the differences between the groups (U14, U16, and U18) in

the rest of RSA variables (RSA<sub>MEAN</sub>, RSA<sub>BEST</sub>, RSA<sub>DEC</sub>, and RSA<sub>CHANGE</sub>) was evaluated by means of a one-way ANOVA. Thirdly, a two-way ANOVA was used to analyse the differences in the tensiomyography variables both in BF and RF when taking into account the moment (repeated measure: pre-post) and the group (independent measure: U14, U16, and U18). Bonferroni *post-hoc* analysis was used in all pairwise comparisons in the three previous ANOVA tests. The confidence interval of the differences (CI of 95%) was calculated to identify the magnitude of changes and the effect size (ES; Cohen's d). The ES was evaluated using the following criteria: 0–0.2 = trivial, 0.2–0.5 = small, 0.5–0.8 = moderate and  $>0.8$  = large (Cohen, 1992). Finally, a product-moment correlation (Pearson's  $r$ ) was established between the results of the RSA test and the values of the muscle response of the dominant leg of the soccer players. Correlations were evaluated following these criteria: 0–0.1 = trivial, 0.1–0.3 = small, 0.3–0.5 = medium, 0.5–0.7 = large, 0.7–0.9 = very large, and 0.9–1.0 = nearly perfect (Hopkins et al., 2009).

## RESULTS

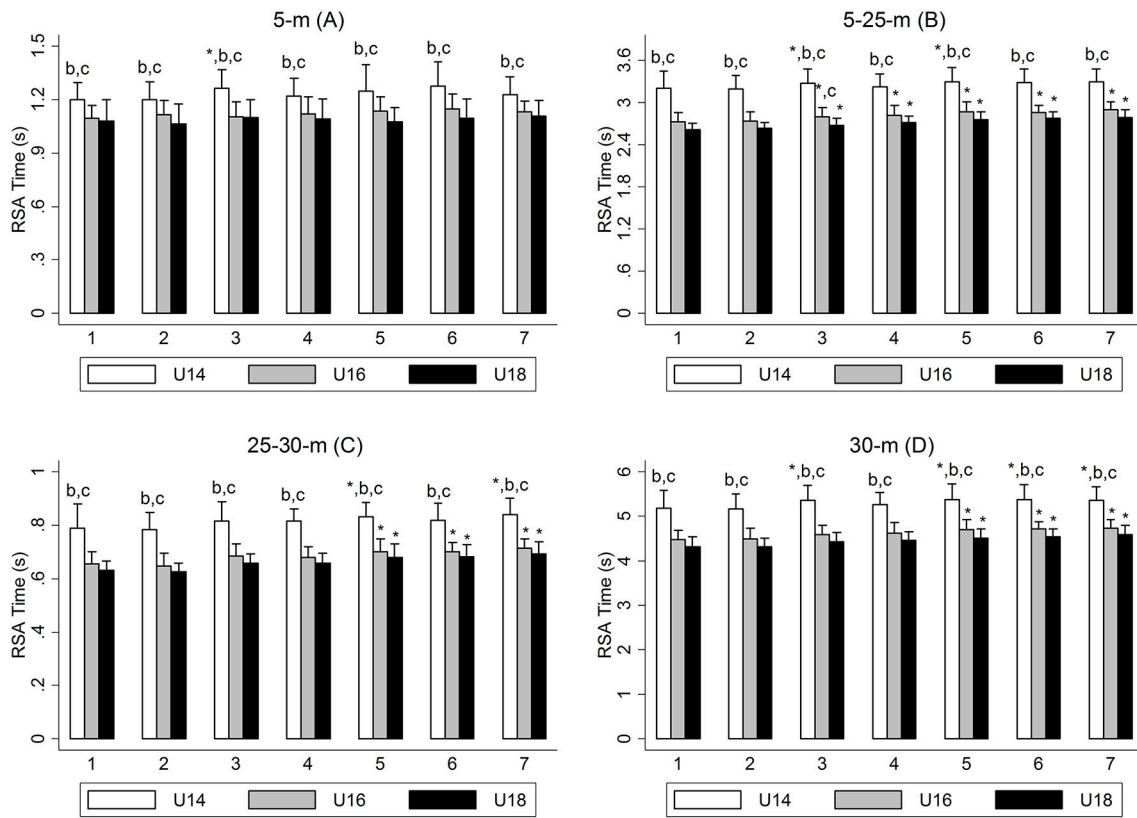
The RSA test results showed better sprint times in the U16 and U18 players in comparison with the U14 players in all the analyzed distance intervals (**Figure 1**,  $p < 0.05$ ), although the size of the difference was higher between the U14 and U18 players (from 1.11 to 4.08). On the other hand, the U16 players revealed a worse RSA<sub>BEST</sub> than the U18 players (+0.12 s, CI95%: to 0.01–0.24; ES: 1.09,  $p = 0.03$ ; **Table 1**). The performance decrement according to the first sprint in the RSA test (30 m) was marked from the fifth sprint in the age categories analyzed ( $p < 0.05$ ). However, RSA<sub>CHANGE</sub> and RSA<sub>DEC</sub> were similar between categories ( $p > 0.05$ ).

The tensiomyographic variables were similar among the soccer players of the three categories analyzed ( $p > 0.05$ , **Table 2**). The results after the RSA test in the RF revealed a significant reduction in the Td in U16 players (–1.53 ms, CI95%: –2.607 to –0.452; ES: 0.38,  $p = 0.006$ ) and U18 players (–1.11 ms, CI95%: –2.10 to –0.12; ES: 0.22,  $p = 0.03$ ), as well as a lower Dm in U16 players after the test (–1.82 mm, CI95%: –2.89 to –0.76; ES: 0.78,  $p = 0.001$ ).

Finally, the correlation analysis between the tensiomyographic variables and the performance in the RSA test revealed a significant relationship between the fatigue variables of the test (RSA<sub>DEC</sub> and RSA<sub>CHANGE</sub>) and the variation of the Dm in the RF of the sample analyzed ( $r = -0.41$  and  $r = -0.34$ ,  $p < 0.05$ , respectively, **Table 3**). The inclusion of the tensiomyographic values prior to the RSA test and its link with the performance in this test did not show any significant relationship ( $p > 0.05$ ).

## DISCUSSION

The purpose of this study was to analyse the influence of the age category on the 7  $\times$  30 meter RSA test performance in elite youth soccer players and the effect of this test on the mechanical behavior of the biceps femoris and rectus femoris of these players. The main results of this research were that U14



**FIGURE 1 |** 5 (A), 5–25 (B), 25–30 (C), and 30-m (D) time and performance deterioration profile for the RSA test (7 × 30 m) in U14 (n = 21), U16 (n = 20), and U18 (n = 21) soccer players. \*p < 0.05 significantly different from the 1st sprint for 5–25- and 30-m times; <sup>b</sup>p < 0.05 significantly different from U16; <sup>c</sup>p < 0.05 significantly different from U18. Data are presented as mean and SD.

**TABLE 1 |** Performance (RSA<sub>MEAN</sub>; RSA<sub>BEST</sub>) and fatigue (RSA<sub>DEC</sub>; RSA<sub>CHANGE</sub>) in a Repeated Sprint test (7 × 30 m) in U14, U16, and U18 soccer players.

	U14 (a)	U16 (b)	U18 (c)
RSA <sub>MEAN</sub> (s)	5.30 ± 0.30 <sup>b,c</sup>	4.62 ± 0.20	4.46 ± 0.17
RSA <sub>BEST</sub> (s)	35.54 ± 2.11 <sup>b,c</sup>	31.04 ± 1.44 <sup>c</sup>	29.80 ± 1.01
RSA <sub>DEC</sub> (%)	4.45 ± 1.79	4.21 ± 1.78	4.67 ± 1.97
RSA <sub>CHANGE</sub> (%)	9.42 ± 5.18	7.77 ± 2.99	8.80 ± 3.19

<sup>b</sup>p < 0.05 significantly different from U16; <sup>c</sup>p < 0.05 significantly different from U18. Data are presented as mean and SD (n = 62).

players presented a higher sprint time than the other two age categories, but no differences were found between the U16 and U18 players. However, the performance decrement during the RSA was not age-related. Regarding muscular responses to the electric stimulus, no differences were found among age category, but U16 and U18 players evidenced lower Td responses, Dm responses, and Td responses, respectively, on the rectus femoris after the RSA.

When analyzing the findings of previous studies on the influence of age in sprint performance, we can conclude that the sprint time improves from the ages of 12 to 18 years old, but

the age effect is larger at the beginning of adolescence (Mendez-Villanueva et al., 2011; Nikolaidis et al., 2016). Indeed, Mendez-Villanueva et al. (2011) found that the 15-year-old group ran 10 meters faster than the 13-year-old group (−0.13 s), but slower (+0.07 s) than the 17-year-old group. These assumptions are also evidenced in our research since U14 players run the 7 × 30 meter RSA test slower than U16 and U18 players; whereas U16 players presented a higher sprint time than U18 players for the RSA<sub>BEST</sub> (+0.122 s). Therefore, it seems that the biggest improvement in linear sprint performance happens at the end of the U14 player period. In contrast, the better RSA<sub>BEST</sub> in U18 players when compared to U16 players may be explained by the lower power and muscle mass of U16 players (Zafeiridis et al., 2005); suggesting that after the U16 age group, training is the most responsible for improving performance instead of maturation (Di Mascio et al., 2017). These findings are in line with Stratton et al. (2004) who showed that the peak height velocity in soccer players occurs from 13.8 to 14.2 years old. Nonetheless, sprint performance may be influenced by internal factors, such as biological maturity age or running technique (Buchheit and Mendez-Villanueva, 2014; Haugen et al., 2014).

The strong evidence that older adolescents run faster than younger ones (Mendez-Villanueva et al., 2011; Nikolaidis et al., 2016) may suggest that older teenagers also run faster during

**TABLE 2** | Results of the tensiomyography before and after the RSA test (7 × 30 m) for different categories (U14, U16, U18) in soccer players.

	U14		U16		U18	
	Pre	Post	Pre	Post	Pre	Post
<b>BF</b>						
Td (ms)	26.34 ± 2.84	24.91 ± 3.66	25.96 ± .76	26.04 ± 3.57	26.04 ± 3.77	24.92 ± 3.43
Tc (ms)	38.44 ± 17.66	35.20 ± 15.84	35.21 ± 13.97	33.48 ± 11.03	38.44 ± 14.96	35.30 ± 14.53
Dm (mm)	5.88 ± 2.59	5.38 ± 2.66	7.92 ± 4.83	6.85 ± 2.96	6.82 ± 2.66	6.24 ± 2.25
<b>RF</b>						
Td (ms)	30.19 ± 3.28	29.83 ± 3.19	28.17 ± 3.63	26.64 ± 4.44*	28.93 ± 5.10	27.82 ± 5.06*
Tc (ms)	42.99 ± 7.53	46.64 ± 7.94	40.57 ± 9.92	40.83 ± 9.47	42.49 ± 10.20	44.04 ± 10.34
Dm (mm)	8.85 ± 2.78	8.12 ± 2.27	10.61 ± 2.28	8.79 ± 2.58*	9.86 ± 2.38	9.97 ± 2.88

\*Differences between the pre and post tensiomyographic variables in U14 (n = 21), U16 (n = 20), and U18 (n = 21) soccer players (p < 0.05).

the RSA. Our findings partially support this hypothesis as U14 players achieved a lower sprint performance than the other two age groups (U16 and U18). However, U16 players did not evidence lower RSA performance than U18 players. These results are in line with those of Philippaerts et al. (2006) who evidenced a greater improvement in speed capacity in young players' right before and after their peak height velocity (13.8 ± 0.8 years old in male adolescents) whereas the anaerobic capacity of the soccer players showed a spurt at the time of peak height velocity. Moreover, Malina et al. (2004) reported an improvement in strength, power, and speed at this age. Therefore, these outcomes suggest that the speed training should be more specific from the U16 age category onwards. Whereas, the use of RSA protocols for talent identification should be applied once players surpass the peak height velocity period (Perroni et al., 2018b; Selmi et al., 2018). It is important to consider that the characteristics of each repeated-sprint ability test (recovery duration, number of sprints, distance, etc.) can influence the mean sprint time (Spencer et al., 2005). For instance, those protocols that include several sets of repeated sprints showed higher differences between the performance of the U16 and U18 players (Selmi et al., 2018). When comparing the RSA<sub>MEAN</sub> performance of this study with previous studies, we found that U14 and U18 soccer players selected for this study ran slower (+0.26 and +0.07 s, respectively) and presented a higher standard deviation (+0.02 and +0.05, respectively) than those chosen by Mendez-Villanueva et al. (2011). Whereas, U16 soccer players participating in the present study did not present a lower RSA<sub>MEAN</sub>, but a greater standard deviation (+0.03). Therefore, it is likely that our findings were affected by the lower repeated-sprint ability of the U14 and U18 groups included in our study. Perroni et al. (2018b) found a great variability in physical parameters like speed time even in young soccer players from the same category. Therefore, training loads should be in line with the individual maturity status of these players.

Regarding the capability of young soccer players to deal with incomplete recovery, previous studies have revealed that the ability to repeat short-term maximal efforts (linear and non-linear sprints) declines as age increases (Falk and Dotan, 2006; Ratel et al., 2006). In the present study, RSA<sub>DEC</sub> and RSA<sub>CHANGE</sub>

variables inform about the ability of players to sustain the sprint performance for a period of time (Glaister et al., 2008; Buchheit, 2010). Therefore, the lack of differences in these variables suggest that the high-intensity profile in U14 soccer players is slower than in U16 and U18 players, but they do not have different capacities to face incomplete recovery than the older groups. These findings coincide with those from Mujika et al. (2009) who found that repeated-sprint ability was not affected by age in elite soccer players aged 11–18 years old. Our findings reaffirm the findings of Bishop and Edge (2006), they revealed that repeated-sprint ability is positively correlated to aerobic fitness and negatively related with the anaerobic contribution of the first sprint. Thus, our expectation of higher RSA<sub>DEC</sub> and higher RSA<sub>CHANGE</sub> in U14 players than in U18 players was rejected. These findings suggest that the three age groups assessed in this study can face exercises that required the repetition of high intensity actions or sprints in a short period of time. However, coaches should control the fitness condition and body mass of their players, as the ability of young soccer players to deal with repeated sprinting actions are correlated to these variables (Perroni et al., 2018b).

On the other hand, the contractile properties of the muscles of the lower limb are related with the ability of these muscles to maintain or even increase performance in subsequent efforts and mitigate the injury risk especially in fatigued conditions (Rey et al., 2012; Malone et al., 2017; Simunic et al., 2017). In this study, the TMG has been used to assess the contractile responses of the rectus femoris and biceps femoris before and after a 7 × 30 meter RSA, as this tool has demonstrated a high short-term reliability and sensibility for detecting changes in muscular responses (Križaj et al., 2008; Rey et al., 2012); with a greater reliability in a fatigued state than in rested (Ditroilo et al., 2013). However, Wiewelhove et al. (2017) doubted that the sensibility of the TMG for detecting acute muscular fatigue; whereas the reliability of the measurement can be affected by the position of the electrodes and the digital displacement transducer, the research design or the recovery time between stimulus, among others (Rey et al., 2012). Therefore, care must be taken when considering these findings.

The lack of differences in basal values of Dm, Td, and Tc showed that the contractile properties of both biceps femoris

**TABLE 3** | Correlation coefficients between performance (RSA<sub>TT</sub>, RSA<sub>BEST</sub>, RSA<sub>MEAN</sub>) and fatigue (RSA<sub>DEC</sub>, RSA<sub>CHANGE</sub>) variables derived from the RSA test and the variation coefficients of the TMG parameters for the RF and BF.

	RSA <sub>TT</sub>	RSA <sub>BEST</sub>	RSA <sub>MEAN</sub>	RSA <sub>DEC</sub>	RSA <sub>CHANGE</sub>
<b>BF</b>					
Td <sub>CHANGE</sub>	0.06	0.05	0.06	0.04	0.08
Tc <sub>CHANGE</sub>	-0.04	-0.07	-0.04	0.10	0.11
Dm <sub>CHANGE</sub>	-0.07	-0.09	-0.07	0.09	0.12
<b>RF</b>					
Td <sub>CHANGE</sub>	-0.17	-0.17	-0.17	0.01	-0.12
Tc <sub>CHANGE</sub>	0.01	0.02	0.01	-0.07	-0.18
Dm <sub>CHANGE</sub>	0.12	0.19	0.12	-0.410**	-0.340*

\* $p < 0.05$ ; \*\* $p < 0.01$ ; Dm, maximum radial displacement of muscle belly; Tc, contraction time, Td, delay time, Ts, sustained contraction time; Tr, half-relaxation time; RF, rectus femoris; BF, biceps femoris ( $n = 62$ ).

and rectus femoris is not age-related in teenage soccer players (Simunic et al., 2017). When assessing the basal outcomes of this study we found that teenagers have a low number of type II fibers (Tc higher than  $> 30$  ms; Dahmane et al., 2001; Križaj et al., 2008). These findings go against the outcomes from adult soccer players who were shown to have a high number of fast fibers (Tc usually lower than  $< 30$  ms; Rey et al., 2012; Alentorn-Geli et al., 2015; García-García et al., 2017). On the other hand, teenagers have a low Dm value, which suggests that they have a high muscle tone (Dahmane et al., 2005), although these findings may be affected by factors such as fatigue or study design.

In TMG, fatigue is displayed by changes in electric muscle activity; the reduction in the capacity to sustain a determined level of strength during the contraction or the incapacity to reach an initial strength level in repeated contractions (Rodríguez-Matoso et al., 2010). In the present study, no differences before and after the RSA were found in U14 players; but in the rectus femoris after the RSA, U16 players showed lower values of Td and Dm, U18 players showed a lower Td. These findings may provide evidence that younger teenagers have a higher ability to recover from high-intensity exercise (Di Mascio et al., 2017); probably because the phosphocreatine (PCr) resynthesis is faster in children (Hebestreit et al., 1993; Taylor et al., 1997). However, further studies are required as our correlation analyses showed that the greater performance decrement of youth soccer players during the RSA is related to a lower variation in Dm values of the rectus femoris (moderate negative correlation [0.3–0.5] between players' fatigue [RSA<sub>DEC</sub> and RSA<sub>CHANGE</sub>] and the variation of Dm [Dm<sub>CHANGE</sub>] in the rectus femoris).

From a practical point of view, the results presented in this study suggest that repeated sprints in young soccer players should provide higher muscular adaptations from U16 category onwards, so training RSA actions should increase in importance in these categories (Mujika et al., 2009). However, training RSA actions in U14 players should not be avoided as they are able to cope with the demands of RSA actions like U16 and U18 players. Finally, despite no differences in performance deterioration are presented among the three age categories assessed in this study, it

is important to highlight that U16 and U18 show higher muscular responses to RSA test than U14. Coaches of U16 and U18 teams need to control the volume of training sessions that include repeated sprints to reduce the risk of muscular injuries.

Finally, it is necessary to consider that this study only assessed the acute response of players' rectus femoris and biceps femoris. Therefore, further analysis 24–48 h after the RSA would provide further information on how the RSA load varies according to the age and if the recovery strategies are different in these age groups. Moreover, the findings of this study belong to the third term of competition, so cautiousness must be taken when comparing them with other competition periods.

## CONCLUSION

This study revealed an increase in performance in repeated sprints from U16 players, at which time the ability to perform this type of action is equalized. In this sense, the deterioration of performance in the RSA test was homogeneous in the three age groups analyzed. Despite this, the contractile properties of the lower limbs in U14 soccer players remained unchanged after the RSA test, with values similar to those of the older players. Finally, the maximum radial displacement of the muscle belly showed a significant relationship with the decrease in the capacity to perform high intensity efforts with incomplete recovery in soccer players, muscle mechanical properties were altered after the RSA test in U16 and U18 soccer players. Physical performance and muscle response can be complementary variables to managing the fatigue according to the age category in soccer players. These data shows the need to adjust the training load according to the age of the soccer players, especially in the repeated-sprint ability which is considered as a relevant parameter for obtaining success in this sport.

## ETHICS STATEMENT

Parents and children were informed about the research goal and its procedure, as well as its possible risk. Children gave their consent verbally and their parents signed the written informed consent. The study protocols were approved by the ethical committee from the University of Castilla-La Mancha (Toledo, Spain) on 10 May 2015, according to the Helsinki Declaration about ethic principles of medical research in humans. All measurements were taken in the same condition, following the same actuation protocol with each participant. All evaluations were done from September to October 2016.

## AUTHOR CONTRIBUTIONS

JS-S and LG conceived the presented idea. EC and EH developed the background and performed the calibration of the different devices used in the tests. JG-U verified the methods section. All authors discussed the results and contributed to the final manuscript. JL-F, EC, and ML-J carried out the tests, JS-S wrote the manuscript with support from JL-F and EC. LG helped

supervise the project. Both JS-S and JG-U contributed to the final version of the manuscript. ML-J and JG-U contributed to the interpretation of the results and data analysis and they

drafted the manuscript and designed the figures and tables. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

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# Mechanical Efficiency at Different Exercise Intensities Among Adolescent Boys With Different Body Fat Levels

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This study investigated the mechanical efficiency (ME) and associated factors in obese, overweight, and normal-weight adolescent boys during incremental cycle exercise test to exhaustion. Forty-five sedentary adolescent boys (13–14 years old) were separated in three groups according to the percentage of fat mass as follows: 15 normal-weight (NW) (body fat:  $16.0 \pm 1.9\%$ ), 15 overweight (OW) (body fat:  $24.0 \pm 1.6\%$ ), and 15 obese (OB) (body fat:  $31.0 \pm 3.0\%$ ). All groups completed an incremental cycle exercise to exhaustion in which energy consumption (E, W), ME (%), lipid oxidation rate (LO, %), plasma epinephrine and norepinephrine concentrations were determined consecutively at rest and at three intensity levels corresponding to 50 and 75% of each participant's maximal heart rate (50%HRmax and 75%HRmax) and peak oxygen consumption ( $\dot{V}O_{2peak}$ ). During the incremental cycle exercise test, plasma epinephrine, and norepinephrine responses as well as ME determined at 50%HRmax, 75%HRmax, and at  $\dot{V}O_{2peak}$  stages were significantly lower in OB compared to NW and OW individuals ( $ps < 0.01$ ). Multiple linear regressions showed that body weight ( $\beta = -0.64$ ,  $p < 0.001$ ), energy consumption ( $\beta = -0.24$ ,  $p < 0.05$ ) and lipid oxidation ( $\beta = 0.69$ ,  $p < 0.01$ ) were significant predictors of ME at 50%HRmax. However, at 75%HRmax and  $\dot{V}O_{2peak}$ , significant predictors of ME were epinephrine ( $\beta = 0.34$ ,  $\beta = 0.49$ , respectively,  $ps = 0.01$ ), norepinephrine ( $\beta = 0.26$ ,  $\beta = 0.60$ , respectively,  $ps < 0.05$ ) and power output ( $\beta = 0.62$ ,  $\beta = 0.71$ , respectively,  $ps < 0.01$ ). These findings suggest that excess in body weight exerts a negative effect on ME at a low intensity by increasing energy consumption for obese and overweight adolescent boys, while at higher intensities (75%HRmax and  $\dot{V}O_{2peak}$ ) the lower ME could be better explained by the lower power output and catecholamine responses that were attenuated among obese and overweight adolescent boys.

**Keywords:** mechanical efficiency, energy consumption, obese, body mass, catecholamine, incremental cycle test to exhaustion, adolescent boys

## INTRODUCTION

Mechanical efficiency (ME) refers to the ability of an individual to transfer the energy consumed into performing external work (Weinstein et al., 2004). ME has recently been investigated as a potential factor underlying metabolic and mechanical adaptations to exercise not only among trained subjects (Boone et al., 2010) but also in special populations (Jabbour et al., 2013, 2017;



Jabbour and Iancu, 2015). In parallel to other “classical” variables such as cardiovascular risk factors, quality of life, maximal oxygen consumption, ME has been examined as a source of information regarding the effectiveness of exercise interventions (Villegas-Jaureguizar et al., 2018). With this growing interest in using ME for performance and health evaluations there is still a lot to know about underlying key factors.

ME has been proposed as an important measure relating to weight loss and obesity. Indeed, it was suggested that ME is influenced by body weight status (Butte et al., 2007) and metabolic milieu (Jabbour and Iancu, 2015; Laaksonen et al., 2018). A decreased ME may be considered as a limitation for physical activity (Layec et al., 2011; Jabbour and Iancu, 2015), where less efficiency for a given work output is attributed to higher energy consumption and energy cost of breathing during exercise (Layec et al., 2011; Jabbour and Iancu, 2015). For Lafortuna et al. (2006), the decreased ME reported in obese adults may be related to the increased proportion of glycolytic muscle fibers (Kriketos et al., 1997) which are substantially less efficient compared to type I fibers. The latter interpretation was proposed to explain the higher cycling energy cost (Coyle et al., 1992) found in obese adults as compared to normal weight and overweight adults. For Butte et al. (2007), the lower ME values observed for overweight children may be the consequence of the excess in body mass that may limit muscle efficiency. However, the study of Jabbour et al. (2013) conducted on 660 children showed that ME was not affected by body weight status. For these authors, the contradictory lower ME observed in previous studies may be simply related to the method used for ME calculation (net vs. crude value).

More recently, Laaksonen et al. (2018) have investigated the relationship between muscle metabolism and ME among 17 healthy recreationally active male subjects at an intensity corresponding to 45% of  $\text{VO}_{2\text{peak}}$ . Their findings suggested that the use of plasma fatty acids was higher in more efficient subjects and correlated significantly with ME. Nonetheless, no significant differences for blood glucose concentration were observed between the groups suggesting that plasma fatty acids may be an important determinant of ME during submaximal exercises. Furthermore, an interventional study of Jabbour and Iancu (2015) reported an increase in ME following a high intensity training program which was linked to improved homeostasis model assessment estimated insulin resistance and concomitant increases in power output. Interestingly, these improvements were reported at higher intensity stages of an incremental maximal cycling test corresponding to 60%, 80%, and 100% of peak power, respectively. As a factor of performance, ME may be involved in both aerobic and anaerobic performance (Kriketos et al., 1997; Jabbour and Iancu, 2015; Jabbour et al., 2017), therefore indicating that key factors underlying ME may diverge depending on the intensity and task performed.

Most studies on factors contributing to ME in obese individuals have looked into skeletal muscle adaptations and how they relate to metabolic improvements with training that have been associated for instance with muscle strengthening (Jabbour and Iancu, 2015). However, changes in ME may also be related to hormonal adaptations such as epinephrine and norepinephrine

responses. Human studies indicate that obese individuals have reduced catecholamine responses (Jabbour et al., 2011; Vettor et al., 1997) which could significantly affect exercise performance (Strobel et al., 1999). Therefore, the first aim of the present study was to compare ME, metabolic and physiological responses between normal weight, overweight and obese adolescent boys at different intensity levels of an incremental cycle exercise test to exhaustion. The second aim was to examine the relationship between ME and potential underlying factors amongst which plasma epinephrine and norepinephrine responses. We expect to find the common ME, metabolic and physiological differences as previously reported between the normal weight and obese groups during exercise. As for the relationship between ME and underlying factors, we hypothesize that it would be intensity-dependent and that efficiency would be positively correlated to epinephrine and norepinephrine responses during exercise.

## MATERIALS AND METHODS

### Participants

Forty-five healthy adolescent boys were recruited from several high schools in Lebanon. To prevent any maturation variability, only participants in the age range of 13–14 years who were at the same Tanner stage (Stage 3) (Tanner, 1962) were selected. Further inclusion criteria for participation included (i) being sedentary [participating in <1 h per week of structured exercise, as assessed by the International Physical Activity Questionnaire (Craig et al., 2003)], (ii) presenting no metabolic, cardiovascular or chronic health problems, (iii) having no history of drug consumption, or (iv) smoking. Health-related information was obtained from the participants' family physician prior to the study. Volunteers were separated into three groups based on the percent body fat (%body fat) criterion previously described by McCarthy et al. (2006): a normal-weight (NW) group ( $n = 15$ ; %body fat <22%), an overweight (OW) group ( $n = 15$ ; %body fat = 22–25%), and an obese (OB) group ( $n = 15$ ; %body fat >26%). Participants' physical characteristics and aerobic fitness level are presented in **Table 1**. Before the start of the experiment, a written informed consent was obtained from the parents and adolescents were familiarized with all testing equipment and procedures. The whole study was approved by the Ethical Committee on Human Research (ECHR) of the University of Balamand (Lebanon) according to the declaration of Helsinki.

### Protocol and Materials

After an overnight fast, participants reported once to the laboratory to perform the protocol that lasted 1 h on average. They were asked to refrain from strenuous exercise 24 h before the test. Anthropometric characteristics were firstly measured to assign participants to a weight category group, after which an incremental cycle exercise test followed.

### Anthropometric Measurements

Body mass was measured to the nearest 0.1 kg, with the participants wearing light clothing without shoes, using an electronic scale (MFB 150K100, Kern, Germany). Height was

**TABLE 1** | Physical characteristics and aerobic fitness of participants in the three groups: normal weight (NW), overweight (OW), and obese (OB) adolescents.

	NW (N = 15)	OW (N = 15)	OB (N = 15)	Group effect (df = 2)	
				F	p
Age (years)	13.6(0.1)	13.4(0.1)	13.6(0.3)	1.7	0.31
Height (cm)	162.9(6.2)	164.4(10.4)	168.9(9.6)	1.6	0.33
Body mass (kg)	50.5(5.2)	67.0(10.0) <sup>a</sup>	88.7(14.7) <sup>a,b</sup>	11.2	<0.0001
BMI (kg·m <sup>-2</sup> )	18.9(1.1)	24.5(1.5) <sup>a</sup>	30.8(2.3) <sup>a,b</sup>	19.1	<0.0001
FM (%)	16.0(1.9)	24.5(1.6) <sup>a</sup>	31.0(3.0) <sup>a,b</sup>	23.9	<0.0001
FFM (kg)	43.0(6.0)	52.0(9.0) <sup>a</sup>	62.0(8.0) <sup>a,b</sup>	21.4	<0.0001
$\dot{V}O_{2peak}$ (L·min <sup>-1</sup> )	2.10(0.12)	2.36(0.09) <sup>a</sup>	2.43(0.11) <sup>a</sup>	11.8	<0.0001

Values are presented as mean (standard deviation). BMI, body mass index; FM, fat mass; FFM, fat free mass;  $\dot{V}O_{2peak}$ , peak value of oxygen consumption. <sup>a</sup>Significant difference with NW ( $p < 0.01$ ). <sup>b</sup>Significant difference with OW ( $p < 0.01$ ).

determined to the nearest 0.5 cm with a measuring tape fixed to the wall. The body mass index (BMI, kg·m<sup>-2</sup>) was calculated as the ratio of body mass (kg) to height squared (m<sup>2</sup>). The %body fat, referred to here as fat mass (FM, %) was estimated from 3 skinfold thickness measurement sites (biceps, triceps, and subscapular) according to the validated method of Slaughter et al. (1988) for children and youth. The fat free mass (FFM, kg) was calculated by subtracting the fat mass from the body mass.

## Incremental Cycle Exercise Test to Exhaustion

Participants performed a maximal test on an upright cycle ergometer (Monark Ergomedic 839E, Monark, Sweden) to determine their peak oxygen consumption ( $\dot{V}O_{2peak}$ ). A breath-to-breath automated metabolic system (CPX, Medical Graphics, St-Paul, MN, United States) was used to collect gas exchange data. Prior to each test, the system was calibrated according to the manufacturer's instructions using standard gasses of known concentration as well as a calibration syringe for air flow. The laboratory environment was controlled where temperature and relative air humidity were maintained around 23°C and 60%, respectively. Heart rate was continuously measured using a heart rate monitor (Polar-F6, Polar, Finland). At the start of the test, participants remained seated for 5 min on the bicycle ergometer to measure their resting values. The test started at an initial power of 60 W and progressively increased by 20 W every 2 min until exhaustion. During the test, adolescents were instructed to pedal at a rate of 50–70 revolutions per minute that was monitored using an electronic counter (MEV 2000) embedded in the ergocycle. The test was terminated when adolescents could no longer maintain the required pedaling rate (<40 revolutions per minute) or requested to stop the exercise. At the end of the protocol, participants were asked to perform an active recovery of 5 min at 25 W.

At rest and at the end of each intensity level, a venous blood sample was collected from the antecubital vein in a vacutainer tube containing Ethylene Diamine Tetra Acetic Acid (EDTA). Plasma from the venous blood samples was separated by centrifugation at 3000 × g for 20 min (4°C) (ORTO ALRESA mod. Digicen.R, Spain). Aliquots were immediately frozen and

stored at –80°C for use in subsequent chemical analyses. At the end of incremental test and after a 3-min recovery period, fingertip capillary blood samples were collected and immediately analyzed for blood lactate concentration using a Lactate Pro portable device (Arkray, Japan). This procedure was done to verify one of the test termination criteria.

## Data Analysis

### Calculation of Metabolic and Physiological Variables

Gas exchange data were collected on a breath-to-breath basis with a continuous and synchronized measurement of heart rate (HR, beats·min<sup>-1</sup>). Mean values of HR, oxygen consumption ( $\dot{V}O_2$ , L·min<sup>-1</sup>), carbon dioxide production ( $\dot{V}CO_2$ , L·min<sup>-1</sup>), and respiratory exchange ratio (RER) were computed as the average of the last 20 s of each intensity level where a steady-state was reached.  $\dot{V}O_{2peak}$  was achieved when participants fulfilled at least three of the following criteria: a peak or plateau in  $\dot{V}O_2$  values despite an increase in exercise intensity, a RER greater than 1.1, a peak HR above 90% of the predicted maximal HR (220-age), a blood lactate concentration higher than 8.0 mmol·L<sup>-1</sup> and the apparent exhaustion of the subject (Spiro, 1979). In the present work, main variables were assessed at rest and at three stages corresponding to 50 and 75% of each participant's maximal heart rate (50%HRmax and 75%HRmax) and at the peak oxygen consumption ( $\dot{V}O_{2peak}$ ) level.

Substrate oxidation was determined at the submaximal aerobic intensity stages (50%HRmax and 75%HRmax) based on the corresponding mean values of the non-protein RER. Specifically, the percentage of lipid oxidation (%LO) contributing to energy was calculated using the method of McGilvery and Goldstein (1983) as follows: %LO = [(1 – RER)/0.29] × 100. The percentage of carbohydrate oxidation (%CHO) was then deduced by subtracting the %LO from 100.

### Calculation of Mechanical Efficiency

Net mechanical efficiency (ME<sub>net</sub>, %) was calculated using the formula developed by LaFortuna et al. (2006) as the ratio of work performed (W) to the rate of energy consumed (E, W) above resting level, that was in turn computed as follows:  $E = (4.94 \text{ RER} + 16.04) \times \dot{V}O_{2net} / 60$  (Garby and Astrup, 1987). Net  $\dot{V}O_2$  ( $\dot{V}O_{2net}$ , L·min<sup>-1</sup>) was calculated by subtracting the resting value

from the gross value at each intensity stage. The resting values of  $E$  ( $E_{\text{rest}}$ ) were also determined based on the equation using  $\dot{V}O_{2\text{rest}}$  values instead of the  $\dot{V}O_{2\text{net}}$  values.

### Blood Analyses

Plasma epinephrine and norepinephrine concentrations were measured using high-performance liquid chromatography (HPLC) (Chromsystems, Thermo finnigan, France), following the method of Koubi et al. (1991). Before the HPLC run, catecholamines were extracted by selective absorption from sodium bisulfite (Chromsystems-HPLC-Kit, Waters, Milford, MA, United States). 1 mL of plasma previously centrifuged was shaken up briefly with 250  $\mu\text{L}$  of sodium bisulfite (0.25%) and 50  $\mu\text{L}$  internal standard solution (600 pg dihydroxybenzylamine). After a three times wash (solution TRIS 1M EDTA, pH 8.8), catecholamines were eluted with 120  $\mu\text{L}$  buffer (10 mL ultra-pure water, 130  $\mu\text{L}$  acetic acid, 100  $\mu\text{L}$  bisulfate 0.25% and 25  $\mu\text{L}$  EDTA 10%). Eluant was centrifuged at 4000 rpm for 10 min (Thermo Fisher Scientific, Jouan. GR412), after which a 50  $\mu\text{L}$  of the sample eluant was injected into HPLC column (Column Waters reference 5007 alumina 20 mg) and eluted with a mobile phase. The flow rate was 1  $\text{mL}\cdot\text{min}^{-1}$  at 13.8 mPa and a potential of 0.60 V. The chromatogram was analyzed by computer integration (Baseline 815, Waters). The detection limit of catecholamines in the described method was 0.06 nM and the inter-assay coefficient of variation was 6.5%. The blood lactate concentration was determined enzymatically using a lactate analyzer (Microzyme, Cetrix, France). Plasma hormones and lactate values were corrected for plasma volume changes using the equation of Van Beaumont (1972).

### Statistical Analysis

Data are presented as mean and standard deviation (SD). After testing for normal distribution (Kolmogorov–Smirnov test), differences between the three groups (i.e., NW, OW, and OB) were analyzed using a one-way analysis of variance (ANOVA) performed on all dependent variables at rest, 50%HRmax, 75%HRmax, and  $\dot{V}O_{2\text{peak}}$ . A two-way ANOVA was performed to further test the interaction effect between the groups and the three relative exercise intensity levels on ME values. Each repeated measures ANOVA was preceded by a Mauchly's sphericity test, and if the test was significant (indicating a violation of the hypothesis of variance homogeneity), a Huynh-Feldt correction procedure was used to adjust the degrees of freedom. When needed, a *post hoc* analysis using Newman-Keul's test was performed for pairwise comparisons. A multiple regression analysis was conducted at each of the studied intensity level (i.e., 50%HRmax, 75%HRmax, and  $\dot{V}O_{2\text{peak}}$ ) to examine the relationship between the  $ME_{\text{net}}$  and various potential predictors (i.e., body weight, power output, energy consumption, lipid oxidation, epinephrine, norepinephrine). The analyses were performed using IBM SPSS Statistics 19 software (IBM SPSS Statistics for Windows, Version 24.0, Armonk, NY, United States: IBM Corp.). A value of  $p < 0.05$  was accepted as the minimal level of statistical significance.

## RESULTS

### Physical Characteristics and Aerobic Fitness Levels

The age and body height did not differ significantly between the groups of adolescents (Table 1). As expected, results indicated significantly different body mass, BMI and %FM between each of the three groups ( $ps < 0.01$ ). Furthermore, the FFM (kg) was significantly higher for OB in comparison to OW and NW as well as for OW in comparison to NW ( $ps < 0.01$ ). The maximal aerobic capacity (absolute  $\dot{V}O_{2\text{peak}}$ ,  $\text{L}\cdot\text{min}^{-1}$ ) was significantly higher for the OB and OW groups as compared to the NW group ( $ps < 0.01$ ).

### Mechanical Efficiency, Metabolic and Physiological Variables

#### Resting Values

Resting values of oxygen consumption were significantly higher for the OB group in comparison to the NW and OW groups, as well as for the OW as compared to the NW ( $p < 0.01$ , Table 2). However, no significant differences were revealed between groups for resting values of lactate concentration, heart rate, RER, and epinephrine and norepinephrine concentrations.

#### Submaximal Exercise Intensities

At the studied moderate intensity (i.e., 50%HRmax), most of the variables differed significantly between each of the three groups even though this relative intensity induced similar values of power output (PO), heart rate and lactate concentrations in all groups (Table 3). For instance, higher oxygen and energy consumption, RER and %LO and lower %CHO, epinephrine and norepinephrine concentrations were seen in the OB group as compared to the OW and NW groups ( $ps < 0.01$ ), as well as for the OW in comparison to the NW group ( $ps < 0.01$ ).

At 75%HRmax, the oxygen and energy consumption values as well as the heart rate, RER and lactate concentrations did not differ statistically between the three groups although power output was significantly higher for the NW group as compared to both OW and OB groups (Table 3). Moreover, the OB group showed a significantly lower increase in epinephrine and norepinephrine compared to the NW and OW groups ( $ps < 0.01$ ).

#### Peak Exercise Intensity Values

At  $\dot{V}O_{2\text{peak}}$ , all studied variables differed significantly between each of the three groups, except for heart rate and RER values (Table 4). Specifically, the NW group reached the highest power output  $\dot{V}$  and presented the lowest  $\dot{V}O_{2\text{peak}}$  and lactate concentration as compared to the OW and OB groups ( $ps < 0.01$ ). The OB and OW groups presented significantly different epinephrine and norepinephrine levels that were lower when compared to the NW groups ( $ps < 0.01$ ).

#### Mechanical Efficiency

The two-way ANOVA on  $ME_{\text{net}}$  revealed significant main effects of group [ $F(2, 28) = 102.95$ ,  $p < 0.001$ ,

**TABLE 2** | Metabolic and physiological responses at rest for the three groups: normal weight (NW), overweight (OW), and obese (OB) adolescents.

	NW (N = 15)	OW (N = 15)	OB (N = 15)	Group effect (df = 2)	
				F	p
$\dot{V}O_{2rest}$ (L·min <sup>-1</sup> )	0.19(0.01)	0.35(0.02) <sup>a</sup>	0.45(0.02) <sup>a,b</sup>	11.8	<0.0001
Lactate (mmol·L <sup>-1</sup> )	1.3(0.3)	1.3(0.5)	1.3(0.7)	1.3	1.53
HR (beats·min <sup>-1</sup> )	78.3(2.2)	76.2(9.1)	77.4(3.1)	2.9	0.44
RER	0.78(0.09)	0.79(0.09)	0.78(0.09)	2.2	0.22
E <sub>rest</sub> (W)	69(11)	79(17) <sup>a</sup>	99(15) <sup>a,b</sup>	6.6	<0.01
Epinephrines (nmol·L <sup>-1</sup> )	0.85(0.01)	0.81(0.02)	0.82(0.01)	1.9	0.24
Norepinephrines (nmol·L <sup>-1</sup> )	2.50(0.01)	2.40(0.04)	2.60(0.03)	3.9	0.74

Values are presented as mean (standard deviation).  $\dot{V}O_{2rest}$ : oxygen consumption at rest, HR: heart rate, RER: respiratory exchange ratio, E: energy consumption in Watts. <sup>a</sup>Significant difference with NW ( $p < 0.01$ ). <sup>b</sup>Significant difference with OW ( $p < 0.01$ ).

**TABLE 3** | Metabolic and physiological responses at the studied submaximal intensity levels (50%HRmax, 75%HRmax) of the incremental cycle exercise test to exhaustion for the three groups: normal weight (NW), overweight (OW), and obese (OB) adolescents.

	NW (N = 15)	OW (N = 15)	OB (N = 15)	Group effect (df = 2)	
				F	p
<b>At 50%HRmax</b>					
$\dot{V}O_{2net}$ (L·min <sup>-1</sup> )	1.32(0.02)	1.68(0.02) <sup>a</sup>	1.81(0.02) <sup>a,b</sup>	42.1	<0.0001
PO (W)	80(5)	80(10)	80(10)	1.3	0.31
Lactate (mmol·L <sup>-1</sup> )	3.9(1.9)	3.8(1.7)	4.1(1.1)	2.6	0.36
HR (beats·min <sup>-1</sup> )	104.7(2.1)	106.2(3.1)	102.9(6.1)	0.6	0.54
RER	0.78(0.05)	0.82(0.04) <sup>a</sup>	0.89(0.07) <sup>a,b</sup>	11.2	<0.01
%LO	48(2)	39(1) <sup>a</sup>	22(3) <sup>a,b</sup>	20.2	<0.0001
%CHO	52(2)	61(1) <sup>a</sup>	78(3) <sup>a,b</sup>	20.1	<0.0001
E(W)	458(11)	562(32) <sup>a</sup>	615(14) <sup>a,b</sup>	30.1	<0.0001
Epinephrines (nmol·L <sup>-1</sup> )	1.61(0.1)	0.98(0.2) <sup>a</sup>	0.82(0.1) <sup>a,b</sup>	8.6	<0.01
Norepinephrines (nmol·L <sup>-1</sup> )	5.4(0.3)	4.2(0.3) <sup>a</sup>	3.9(0.4) <sup>a,b</sup>	19.4	<0.001
<b>At 75%HRmax</b>					
$\dot{V}O_{2net}$ (L·min <sup>-1</sup> )	2.01(0.04)	2.02(0.02)	2.01(0.03)	2.1	0.22
PO (W)	140(5)	120(10) <sup>a</sup>	120(10) <sup>a</sup>	11.8	<0.001
Lactate (mmol·L <sup>-1</sup> )	6.9(1.9)	6.8(1.7)	6.1(1.1)	2.6	0.36
HR (beats·min <sup>-1</sup> )	144.2(2.2)	152.2(2.1)	148.9(2.1)	0.9	0.56
RER	0.89(0.05)	0.87(0.04)	0.87(0.07)	1.6	0.64
%LO	8(2)	9(1)	8(3)	11.2	0.46
%CHO	92(2)	91(1)	92(3)	3.1	1.1
E (W)	685(12)	684(12)	681(19)	2.1	2.2
Epinephrines (nmol·L <sup>-1</sup> )	2.01(0.1)	1.81(0.2)	1.51(0.4) <sup>a,b</sup>	6.6	<0.01
Norepinephrines (nmol·L <sup>-1</sup> )	7.4(0.2)	5.9(0.3) <sup>a</sup>	5.3(0.1) <sup>a,b</sup>	11.1	<0.001

Values are presented as mean (standard deviation).  $\dot{V}O_{2net}$ : oxygen consumption above the resting level, PO: power output, HR: heart rate, RER: respiratory exchange ratio, %LO: percent contribution of lipid oxidation to energy, %CHO: percent contribution of carbohydrate oxidation to energy, E: energy consumption. <sup>a</sup>Significant difference with NW ( $p < 0.01$ ). <sup>b</sup>Significant difference with OW ( $p < 0.01$ ).

$\eta^2 = 0.88$ ] and intensity [ $F(2, 28) = 137.03$ ,  $p < 0.001$ ,  $\eta^2 = 0.91$ ]. No significant interaction effect was found between factors. *Post hoc* comparisons showed that  $ME_{net}$  differed significantly between all groups and all intensities ( $ps < 0.01$ ) with the smallest values found for the OB group as well as for the lowest studied intensity level (Figure 1).

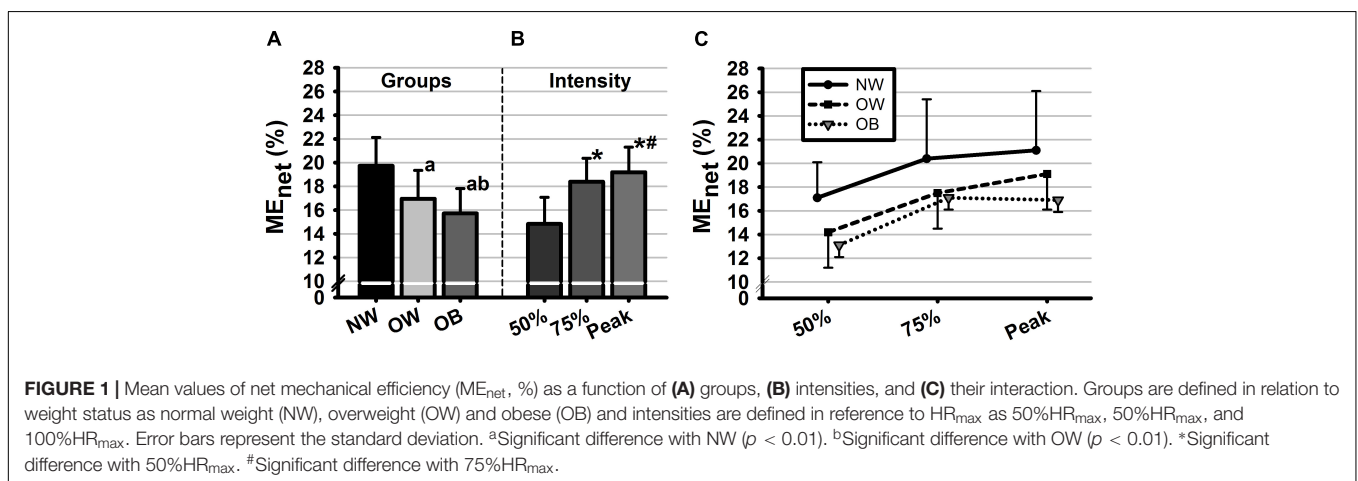
Multiple linear regressions were calculated to examine the degree to which studied variables predicted  $ME_{net}$  at each studied intensity level. Significant regression equations were

found at 50%HRmax [ $F(2,42) = 20.25$ ,  $p < 0.01$ ,  $R^2 = 0.47$ ], 75%HRmax [ $F(2,42) = 4.14$ ,  $p < 0.05$ ,  $R^2 = 0.19$ ] and  $\dot{V}O_{2peak}$  [ $F(3,35) = 11.01$ ,  $p < 0.01$ ,  $R^2 = 0.48$ ]. Specifically, at 50%HRmax, body weight ( $\beta = -0.64$ ,  $p < 0.001$ ), energy consumption ( $\beta = -0.24$ ,  $p < 0.05$ ) and lipid oxidation ( $\beta = 0.69$ ,  $p < 0.01$ ) were significant predictors of  $ME_{net}$ . At 75%HRmax, the analysis demonstrated that the epinephrine ( $\beta = 0.34$ ,  $p = 0.01$ ), norepinephrine ( $\beta = 0.26$ ,  $p = 0.01$ ) and power output ( $\beta = 0.62$ ,  $p < 0.01$ ) contributed significantly to  $ME_{net}$ . Finally, at  $\dot{V}O_{2peak}$ ,

**TABLE 4** | Metabolic and physiological responses at the peak intensity level ( $\dot{V}O_{2peak}$ ) of the incremental cycle exercise test to exhaustion for the three groups: normal weight (NW), overweight (OW), and obese (OB) adolescents.

	NW (N = 15)	OW (N = 15)	OB (N = 15)	Group effect (df = 2)	
				F	p
$\dot{V}O_{2peak}$ (L·min <sup>-1</sup> )	2.10(0.12)	2.36(0.09) <sup>a</sup>	2.43(0.11) <sup>a</sup>	11.8	<0.0001
PO (W)	180(19)	160(10) <sup>a</sup>	160(15) <sup>a</sup>	19.8	<0.001
Lactate (mmol·L <sup>-1</sup> )	8.4(4.4)	8.9(3.4)	9.8(3.3) <sup>a,b</sup>	21.32	<0.001
HR (beats·min <sup>-1</sup> )	202.9(4.1)	201.2(3.7)	201.3(4.1)	0.4	0.64
RER	1.22(0.05)	1.18(0.03)	1.20(0.04)	1.21	0.29
E (W)	780(38)	860(22) <sup>a</sup>	880(19) <sup>a</sup>	4.1	<0.01
Epinephrines (nmol·L <sup>-1</sup> )	2.78(0.3)	2.01(0.3) <sup>a</sup>	1.78(0.3) <sup>a,b</sup>	8.6	<0.01
Norepinephrines(nmol·L <sup>-1</sup> )	13.4(0.2)	12.1(0.2) <sup>a</sup>	11.3(0.2) <sup>a,b</sup>	19.4	<0.001

Values are presented as mean (standard deviation).  $\dot{V}O_{2peak}$ : peak oxygen consumption above the resting level, PO: power output, HR: heart rate, RER: respiratory exchange ratio, E: energy consumption. <sup>a</sup>Significant difference with NW ( $p < 0.01$ ). <sup>b</sup>Significant difference with OW ( $p < 0.01$ ).



significant predictors of  $ME_{net}$  were also epinephrine ( $\beta = 0.49$ ,  $p = 0.01$ ), norepinephrine ( $\beta = 0.60$ ,  $p < 0.001$ ) and power output ( $\beta = 0.71$ ,  $p < 0.001$ ).

## DISCUSSION

To the best of our knowledge, this study was the first to investigate the relationship between ME and many potential underlying factors among obese (OB), overweight (OW) and normal-weight (NW) adolescent boys when cycling at different exercise intensities of an incremental cycle test. Our results confirmed that (1) excess body fat had a significant effect in decreasing ME at all studied intensity levels. Moreover, (2) exercising at similar relative intensities brought about higher metabolic and physiological responses for the OB group that also presented the lowest ME values in comparison to the NW and OW groups. Finally, results showed that (3) body weight, %LO and energy consumption were significant predictors of  $ME_{net}$  at the moderate intensity (i.e., 50% $HR_{max}$ ), while this was no longer apparent at higher intensities where catecholamine levels and power output seemed to be better predictors of efficiency. The later finding was made possible by the choice of the studied population that presented different

epinephrine and norepinephrine responses to an incremental exercise, a characteristic that was not addressed in previous studies examining factors of ME.

At rest and during all tested cycling intensity levels, absolute oxygen uptake (in L·min<sup>-1</sup>) was significantly higher for the OW and OB adolescents as compared to the NW adolescents (Nikolaidis et al., 2018), while no differences in HR values were detected between groups. This result might suggest a higher muscle oxygen extraction capacity per heart beat and/or a larger stroke volume for our obese adolescents (Salvadori et al., 1999; Lafortuna et al., 2006). The latter goes in line with previous reports on obese adult women (Lafortuna et al., 2006) and young obese adults (Salvadori et al., 1999), and could be interpreted in relation to the excess body mass and FFM. Furthermore, it has been suggested that increases in  $\dot{V}O_2$  and E during cycling in obese individuals can result from the extra work required to move the lower limbs (Anton-Kuchly et al., 1984) and the higher postural activity (Dempsey et al., 1966).

As hypothesized, obesity seems to affect metabolic and physiological responses to exercise in adolescents. Specifically, at 50% $HR_{max}$ , our results showed a lower ME for the OB group in comparison to the OW and NW groups. As previously established by Butte et al. (2007), ME is negatively affected by

energy expenditure rates at this intensity level in overweight children. These authors found that the higher energy expenditure in overweight children and adolescents were attributed in a large part to differences in body size and composition. Indeed, excess body weight, as presented in our OB adolescents, constitutes a major contributor for energy expenditure increases as more energy is consumed at a given work output (e.g., moderate aerobic level). Additionally, it was found that lipid oxidation rate (%LO) was a significant predictor of ME at 50%HRmax, in a way that those having a high %LO during the moderate aerobic stage were also more efficient. In fact, our OB and OW groups presented significantly higher respiratory exchange ratio values, which is a potential indicator of an impaired fat oxidation capability during exercise (van Baak, 1999). Accordingly, a recent study of Laaksonen et al. (2018) conducted in groups of subjects with different ME levels during cycling, showed a higher use of fatty acids for more efficient individuals during prolonged exercise at moderate exercise intensity. For these authors, the shift in relative contributions of fats and carbohydrates may explain the changes in ME. As assumed by Laaksonen et al. (2018), ME depends on the effectiveness of lipid oxidative capacity at moderate aerobic intensities. Despite that other complementary analyses are needed to confirm our assumption, the present study was the first to establish the link between %LO and ME at moderate aerobic intensity among obese adolescents.

At higher intensity levels (i.e., 75%HRmax and at  $\dot{V}O_{2peak}$ ), subjects' efficiency increased as compared to its value at the moderate aerobic level (Figure 1B). This could be explained by the increases of both workload and the amount of energy consumed (Jabbour et al., 2013). At these two intensity levels (i.e., 75%HRmax and  $\dot{V}O_{2peak}$ ), ME was negatively affected by body fat and weight status with the lowest values found for OB as compared to OW and NW adolescents. The lower ME observed for OB and OW groups may be a consequence of lower muscle performance. Accordingly, the power output developed at this stage was higher for NW as compared to OB and OW groups leading to increases in the magnitude of the numerator in the ME model, thus in the ME value. Furthermore, results revealed significantly lower epinephrine and norepinephrine responses to exercise for the OB and OW groups at all studied intensity levels. The latter supports previous findings on adolescents (Eliakim et al., 2006) showing a substantially attenuated catecholamine responses to bout of cycling exercise above the anaerobic threshold. Interestingly, the power output developed during 75%HRmax and  $\dot{V}O_{2peak}$  stages were significantly associated with epinephrine and norepinephrine concentrations, which was not the case at lower intensity. Indeed, as intensity increases, the reliance on fiber type-II to meet the imposed performance demand becomes greater (Sale, 1987), therefore the catecholamine responses are further stimulated by changes in acid-base balance and reduced oxygen availability to the working muscle (Schneider et al., 1992). The latter influences exercise performances by regulating muscular glycogenolysis (Richter et al., 1981).

Taken together, our results may offer a new insight in terms of ME's assessment, especially when exploring a high intensity exercise. Actually, the inclusion of the anaerobic

energy production in ME's calculation is still unavailable and therefore not represented. Indeed, anaerobic contribution is increasingly involved in energy supply at intensities above the lactate threshold 2 (intensity corresponding to 75%HRmax in our study). This might have limited our results, especially when comparing the three groups for which the anaerobic energy contribution at 75%HRmax seemed to be the highest for the OB group (estimation simply based on lactate concentrations) potentially leading to an underestimation of ME. Moreover, ME was determined from incremental 2-min stages, which did not take into account potential differences in gas exchange kinetics between the three groups. Therefore, further studies are needed to include the anaerobic component in the determination of ME that would be examined at different steady state intensities to determine the extent of the phenomenon. Moreover, the use of net ME in this study, as opposed to gross ME, allowed us to control for group differences in baseline (i.e., resting) energy expenditure, thus increasing the reliability of ME values. However, our method of ME calculation, similar to that considering the baseline as the energy cost of unloaded cycling (i.e., work efficiency), does not take into account variations in energy expenditure required to maintain homeostasis (Moseley and Jeukendrup, 2001). This also offers perspectives into calculation methods of cycling ME in relation to differences arising from group characteristics.

## CONCLUSION

In conclusion, the present study highlights an important issue regarding predictors of ME in adolescent boys with different body fat percentages. It appears that underlying factors of ME may diverge according to the intensity of exercise. Our assumption of different underlying factors for ME is supported, and goes beyond the simple relation to the mass of body segments and the energy cost involved in movements (Lafortuna et al., 2006, 2009; Butte et al., 2007). Indeed, at moderate aerobic intensity, the energy consumption and lipid oxidative rates may be important factors contributing to lowering ME among obese and overweight individuals. At the contrary, at higher intensities, ME may be better explained by factors such as muscle power and catecholamine responses that are attenuated in obesity. Based on this relationship, further investigations are needed to provide a more complete profile regarding energy/metabolic forms (aerobic, anaerobic) to ensure that they are well represented in the ME model. From a practical standpoint and given the importance of ME as an indicator of exercise tolerance, it seems important to include both moderate- and high-intensity exercises to programs targeting obese adolescents, where different benefits would be expected.

## AUTHOR CONTRIBUTIONS

GJ conceived and designed the study, collected the data, and drafted the manuscript. GJ and LM performed the data analysis and interpreted the data, and revised, read and approved the submitted version.

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# Estimating Physical Activity in Children Aged 8–11 Years Using Accelerometry: Contributions From Fundamental Movement Skills and Different Accelerometer Placements

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Accelerometers are widely used to assess physical activity, but it is unclear how effective accelerometers are in capturing fundamental movement skills in children. This study examined the energy expenditure during different physical activities (PA) and calibrated triaxial accelerometry, worn at the wrist, waist and ankle, during children's PA with attention to object control movement skills and cycling. Thirty children (14 girls) aged 8 to 11 years wore a GENEActiv accelerometer on their non-dominant wrist, dominant wrist, waist and ankle. Children undertook eight, 5-min bouts of activity comprising being lay supine, playing with Lego, slow walking, medium walking, medium paced running, overarm throwing and catching, instep passing a football and cycling at 35 W. VO<sub>2</sub> was assessed concurrently using indirect calorimetry. Indirect calorimetry indicated that being lay supine and playing with Lego were classified as sedentary in nature (<1.5 METs), slow paced walking, medium paced walking and throwing and instep passing were classified as light (1.51–2.99 METs) and running, cycling and instep passing were classified as moderate intensity (>3 METs). ROC curve analysis indicated that discrimination of sedentary activity was excellent for all placements although the ankle performed better than other locations. This pattern was replicated for moderate physical activity (MPA) where the ankle performed better than other locations. Data were reanalyzed removing cycling from the data set. When this analysis was undertaken discrimination of sedentary activity remained excellent for all locations. For MPA discrimination of activity was considered good for waist and ankle placement and fair for placement on either wrist. The current study is the first to quantify energy expenditure in object control fundamental movement skills via indirect calorimetry in children aged 8–11 years whilst also calibrating GENEActiv accelerometers worn at four body locations. Results suggest throwing and catching is categorized as light intensity and instep kicking a football moderate intensity, resulting in energy expenditure equivalent to slow or medium paced walking or cycling and running, respectively. Ankle worn accelerometry appears to provide the most suitable wear location to quantify MPA including ambulatory activity, object control skills and cycling, in children aged 8–11 years.

**Keywords:** motor competence, motor development, cut-points, indirect calorimetry, energy expenditure

## INTRODUCTION

Accelerometers are becoming the most widely used measure of physical activity (PA) in public health research (Vale et al., 2015) as they provide an objective assessment of energy expenditure and time spent in different intensities of PA (Crouter et al., 2018). Over the past decade there has also been increasing use of accelerometry to estimate PA in children (Rowlands et al., 2014; Crouter et al., 2018). Such methods provide objective data which is more reliable and valid in children than alternative methods such as self-report (Rowlands and Eston, 2007). Despite this, accelerometry is not without its challenges in relation to PA assessment. They are relatively expensive to use, compared to other methods of PA assessment, require more complex data handling and processing techniques to estimate PA and their accuracy in tasks requiring greater use of the upper body, cycling, or non-linear movement is not fully established (Rowlands and Eston, 2007). Given the increase in popularity of accelerometry as an assessment tool, there have also been considerable efforts made to calibrate accelerometer cut-points which is needed to more accurately estimate physical activity in pediatric populations (Ryan and Gormley, 2013; Phillips et al., 2014; Duncan et al., 2016; Roscoe et al., 2017).

Accelerometry derived cut-points are useful in determining the extent to which children meet current PA guidelines for health and, as a consequence, are widely used in prospective population based studies as a means to determine efficacy of PA interventions and to inform public health practice. Determination of cut-points that are specific to age group (e.g., children), model of accelerometer and wear location are critical in ensuring accuracy of PA assessment. Despite this, there remains a need to refine the accuracy of accelerometry to better understand the influence of PA on child health outcomes (Crouter et al., 2018). In particular, the choice of placement site can impact wear compliance and precision of the prediction equation for PA (Crouter et al., 2018). The process of, and activities used in, accelerometer calibration also has a meaningful influence on the precision of the accelerometer prediction equation where accelerometers more easily classify ambulatory based activity (Ryan and Gormley, 2013).

The waist has traditionally been the most commonly used placement site when using accelerometers to measure PA (Montoye et al., 2016). Waist worn accelerometers also tend to perform better than their wrist worn counterparts (Rowlands et al., 2014). Conversely, studies looking at participant compliance find much higher levels of compliance when accelerometers are wrist worn, especially in young children (Rowlands et al., 2014). More recently, the ankle has shown promise as an accelerometer placement site to obtain valid estimates of PA (Crouter et al., 2018). There are, however, only a limited number of studies that have used ankle placement, and to date, only one study has examined this issue in children. Previous work using adults have reported that ankle worn accelerometry performs poorly compared to waist worn accelerometry (De Vries et al., 2011), whilst others have shown the use of the ankle location was similar or better than waist or wrist worn locations

for estimating energy expenditure (Kim et al., 2014; Hibbing et al., 2018). More recently Crouter et al. (2018) reported, using a sample of 8–15-year olds, that energy expenditure estimated from an ankle worn actigraph was not significantly different from that determined by indirect calorimetry and estimates of time spent in light, moderate and vigorous physical activity as well as sedentary behavior using both methods were comparable. Given the paucity of studies examining the utility of ankle-based accelerometer estimates of PA in children, and the fact that recent work by Crouter et al. (2018) did not compare ankle-based placement to other more commonly used locations, research examining this issue is needed to better clarify the optimal accelerometer placement for children's PA assessment.

Another important influence on the process of accelerometer calibration relates to the activities which the accelerometer cut-points are derived from. The majority of studies have tended to use a procedure which includes periods of time supine, seated and in ambulatory activity executed on a treadmill (Ryan and Gormley, 2013; Phillips et al., 2014; Duncan et al., 2016; Roscoe et al., 2017). The premise for such procedures is that as locomotor activity is the predominant activity in an individual's day, the validation of accelerometers during this activity is of primary importance (Welk, 2005). However, children's PA tends to be sporadic and omnidirectional in nature (Rowlands and Eston, 2007) and thus, accelerometer cut points derived predominantly using locomotor activities may not accurately reflect the actual physical activity levels of children. In particular, fundamental movement skills such as throwing and catching are conceptualized as important and a regular feature of children's PA (Holfelder and Schott, 2014). Recent research has suggested it is important to specifically understand how the repeated performance of various types of object control skills contributes to activity intensity as there are no published MET values associated with object control skills in children (Sacko et al., 2018). This type of intermittent movement is a noted limitation in accelerometry-based assessment of PA (Trost et al., 2005), and while accelerometer-based assessment of object control skills has been examined in older adults (Hooker et al., 2011), there appears to be no studies that have examined this issue in children. Likewise, cycling, another common movement skill in children is rarely examined in pediatric accelerometry calibration studies potentially leading to erroneous estimates of habitual physical activity when using cut-points that are not derived using a cycle-based activity within its protocol. Such observations have been noted in adult based studies (Mannini et al., 2013) where the stable position of the wrist during cycling may result in activity intensity being systematically misclassified during cycling activity when using wrist worn accelerometers. Such a criticism may also be leveled at waist worn monitors. Ankle worn accelerometry may be a more practical option that may better reflect activities such as cycling. However, no studies to date have examined this issue in children.

The current study sought to address key gaps in the literature by (a) examining the energy expenditure during object control skills in children, as related to ambulatory activity and; (b) calibrating triaxial accelerometry, worn at the wrist, waist and ankle, during children's PA with particular attention to object

control fundamental movement skills and cycling alongside the more traditionally used locomotor-based calibration protocol.

## MATERIALS AND METHODS

### Participants

An opportunistic sample of 30 healthy, Caucasian, children (14 girls, 16 boys) aged between 8 and 11 years of age ( $9.4 \pm 1.4$  years) from central England took part in this study following institutional ethics approval, parental written informed consent and child assent. Mean  $\pm$  SD of height, mass and body mass index (BMI), was  $1.4 \pm 0.4$  m,  $34.6 \pm 8.6$  kg and  $17.6 \pm 2.5$  kg/m<sup>2</sup>, respectively. All children were involved in grassroots junior football as part of their recreational sports activities.

### Procedures

Participants wore a GENEActiv monitor (Brand name used with permission) on their non-dominant wrist, dominant wrist, and dominant waist, similar to other work (Routen et al., 2012) as well as an additional monitor placed on the dominant ankle. In the case of the dominant ankle, this was determined by asking the children were asked which leg they considered the leg they most used for kicking and then verifying this with their parents. Monitors were worn through the testing period. The GENEActiv has been described in detail previously (Esliger et al., 2011) but in brief, the GENEActiv is a lightweight triaxial accelerometer which provides raw acceleration data. In the work by Esliger et al. (2011) it was found to have high intra and interinstrument reliability (coefficient of variation = 1.8 and 2.4%, respectively), good criterion-referenced validity ( $r = 0.97$ ) when compared to a multi-axis shaking table and high concurrent validity with the Actigraph GT1M accelerometer. Esliger et al. (2011) also reported that, irrespective of whether the accelerometer was worn at the wrist or hip, the GENEActiv could be used to distinguish between sedentary, light, moderate, and vigorous activity behavior in adults.

The GENEActiv was chosen as it provides three-axis raw accelerometry data from monitors that can be worn on multiple body locations. The GENEActiv is also capable of capturing high frequency data (up to 100 Hz) for multiple days (up to 7 days at 100 Hz or 45 days at 10 Hz) and is thus attractive for researchers interested in assessing free-living PA. In the current study the GENEActiv was set to record at 80 Hz and 1 s epochs. Throughout the testing procedure  $VO_2$  and  $VCO_2$  were assessed using a MetaMax 3B (Cortex Biophysik GmbH, Leipzig, Germany) breath by breath gas analyzer. Participants wore a junior face mask (Hans Rudolph) and the MetaMax was calibrated with gasses of known concentration each day prior to commencing testing. All testing took place in the morning (9am–12pm). Prior to beginning the protocol, each participant was fully familiarized with the treadmill being used in the study (Woodway Inc., Wisconsin, United States).

After briefing and being fitted with the GENEActiv monitors and gas analyzer, each participant performed a series of activities reflective of different levels of PA. These were lying supine,

seated and playing with Lego, slow walking, medium walking, and a medium paced run. These were performed in order as per prior work by Phillips et al. (2014). Participants then performed bouts of overarm throwing and catching a standard size tennis ball, instep passing a football (Size 3) and cycling (Lode Corival Pediatric, Lode BV, Groningen, Netherlands). All activities were performed for 5 min with a 5-min rest in between. Using previous protocols (Puyau et al., 2002; Ryan and Gormley, 2013) as guidelines, walking and running speeds were set at 3, 4.5, and 6.5 kmph<sup>-1</sup> to represent slow, medium pace walking and running, respectively. Cadence for overarm throwing and catching and passing a football was set to ensure one complete action (e.g., a throw or football pass) was completed every 3 s. Specific instructions were given to the children in respect to each motor skill followed by a demonstration of each activity. For throwing participants were instructed to, rotate their hips and shoulders to the point where their non-throwing arm faced 90 degrees from their starting position, to transfer weight by stepping forward with their dominant foot prior to ball release and then to follow through beyond ball release diagonally toward the non-preferred side. When catching, the children were asked to move their arms in preparation with hands in front of the body and elbows flexed. To step forward with arms extended, reaching for the ball as it arrived and to only use the hands to catch. For instep passing the children were instructed to take a step forward immediately prior to ball contact with the non-kicking foot placed alongside or slightly behind the ball and to pass with the instep of the foot only.

### Data Processing

Upon completion of the protocol, each participant's accelerometer and calorimetry data was downloaded and stored on a computer. The first and last minute of each bout were discarded leaving a 3-min period for analysis. This ensured that MET values for each bout were at the required intensity and is consistent with prior work (Phillips et al., 2014; Roscoe et al., 2017). Using the GENEActiv post processing software (Version 2.9), the raw 80 Hz signal from all three axes were summarized into a single vector magnitude (gravity subtracted) (SVM gs), congruent with prior work by other authors (Esliger et al., 2011; Phillips et al., 2014; Roscoe et al., 2017). The correction for gravity was undertaken to focus the outcome variable on dynamic rather than static accelerations, as recommended by Esliger et al. (2011), and used by prior authors (Phillips et al., 2014; Roscoe et al., 2017).

Data were saved in raw format as binary files and then data for each wear location were summed into a signal magnitude vector (gravity subtracted) expressed in 1 s epochs, as is conventional (Esliger et al., 2011; Phillips et al., 2014).

The  $VO_2$  values were then converted into METs using age-specific values (Harrell et al., 2005) and coded into one of four intensity categories (sedentary <1.5 METs), light (1.5–2.99 METs), moderate (3–5.99 METs) and Vigorous (>6 METs). However, on inspection none of the activities undertaken by the participants resulted in MET values in excess of 6. Data were then subsequently recoded into three intensity categories reflecting sedentary, light and moderate PA (MPA).

## Statistical Analysis

Prior to analysis data were checked for normality which confirmed that data were non-normal via the Shapiro-Wilk test (all  $P < 0.05$ ). As a consequence Spearman's rank correlations were employed to examine criterion validity of the GENEActiv output at each wear location and METs. Following this, separate Spearman's correlations were performed between METs at each intensity (sedentary, light, moderate) and accelerometer counts at each wear location in order to provide greater clarity of the validity of the GENEActiv output at each intensity of activity. Receiver operating characteristic (ROC) curve analysis was undertaken (Jago et al., 2007) to determine SB and MPA cut-points. The area under the curve (AUC) was calculated for each analysis as a measure of diagnostic accuracy with AUC values of;  $\geq 0.90$  considered excellent,  $0.80$ – $0.89$  good,  $0.70$ – $0.79$  fair, and  $< 0.70$  poor (Metz, 1978). ROC curve analysis was conducted as described previously (Esliger et al., 2011; Phillips et al., 2014) and cut-points that maximized sensitivity (Se) and specificity (Sp) were derived (Perkins and Schisterman, 2006). In line with prior work, AUC was determined for SB and MPA leaving accelerometer counts that fell between the sedentary and MPA cut-points were then classified as light PA, in line with prior work (Phillips et al., 2014). Cut-points for light PA were classed as those higher than SB but lower than MPA but did not require AUC, Se or Sp values to be determined as per Phillips et al. (2014). These are subsequently labeled as not applicable (NA) in **Tables 1, 2**. ROC analysis was undertaken using the Statistical Package for Social Sciences (SPSS, version 24). Cut-points reflected recommendations that the lower Se or Sp values should be  $\geq 60\%$  (Lugade et al., 2014). This prioritization approach minimizes the risk of individuals being misclassified in the target behavior and is common in accelerometer calibration (Mackintosh et al., 2012) and fitness standards research (Welk et al., 2011).

## RESULTS

Results from indirect calorimetry are presented in **Figure 1**. When the child was lay supine and playing with Lego were classified as sedentary in nature ( $< 1.5$  METs), slow paced

walking, medium placed walking and throwing and catching were classified as light ( $1.51$ – $2.99$  METs) and running, cycling and instep passing were classified as moderate intensity ( $> 3$  METs).

Spearman's rank correlations indicated significant weak relationships between METs and GENEActiv counts at the non-dominant wrist ( $r = 0.415$ ,  $P = 0.0001$ ), dominant wrist ( $r = 0.458$ ,  $P = 0.0001$ ), waist ( $r = 0.505$ ,  $P = 0.0001$ ), and a moderate relationship at the ankle ( $r = 0.752$ ,  $P = 0.0001$ ). When analysis was rerun removing cycling-based activity the strength of the relationship between METs and GENEActiv counts at each location increased. Spearman's correlation values between METs and GENEActiv counts were  $r = 0.715$  ( $P = 0.0001$ ) for the non-dominant wrist,  $r = 0.720$  ( $P = 0.0001$ ) for the dominant wrist,  $r = 0.774$  ( $P = 0.0001$ ) for the waist and  $r = 0.790$  ( $P = 0.0001$ ) for the ankle, demonstrating appropriate criterion validity between GENEActiv output and overall activity. Subsequent analysis examining the association between GENEActiv counts and METs at each intensity of activity revealed a similar pattern to that of the overall activity where significant weak to moderate relationships were found for all wear locations within sedentary activity, light activity and moderate activity (all  $P < 0.01$ , See **Table 1**) irrespective of whether cycling was included or excluded in the analysis. The strongest associations between METs in each intensity and GENEActiv output was observed for the ankle placement with the exception of light METs when cycling was included in the analysis where the waist performed marginally better than the other wear locations.

Receiver operating characteristic curve analysis for the GENEActiv monitors worn at the non-dominant wrist, dominant wrist, waist and dominant ankle were able to successfully discriminate different intensities of activity. Sensitivity, specificity, AUC and resultant cut-points for each GENEActiv monitor are presented in **Table 2**. Discrimination of sedentary activity was excellent although the ankle performed better than other locations. This pattern was replicated for MPA where the ankle location performed better than other locations. Ankle discrimination was considered good, discrimination at the waist fair but discrimination at non-dominant and dominant wrist was considered poor. Ankle worn GENEActivs had the highest sensitivity for sedentary behavior and MPA. Waist worn GENEActivs had the highest specificity for sedentary behavior whereas the highest specificity for MPA was for ankle worn GENEActivs.

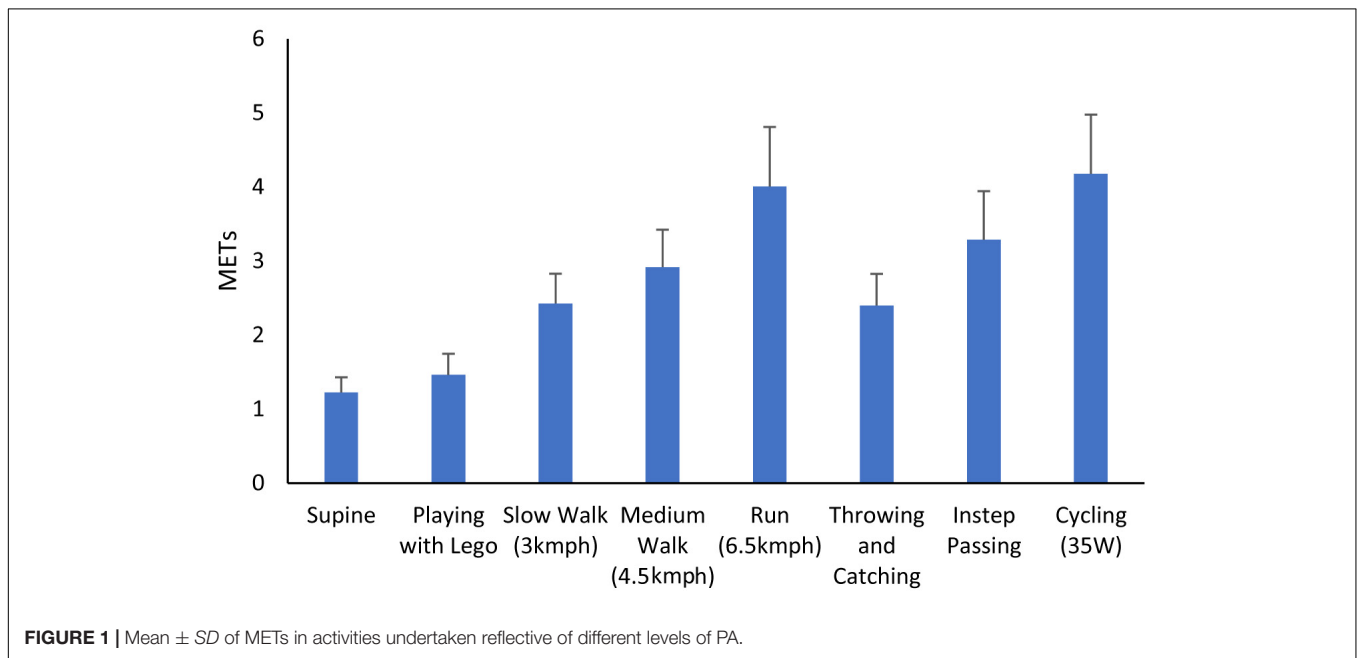
Considering the recognized issues where the stable position of the wrist during cycling resulting in activity being misclassified when using wrist worn accelerometers, data were reanalyzed with cycling activity removed from the analysis (See **Table 3**). When this subsequent analysis was undertaken, discrimination of sedentary activity remained excellent for all locations, although waist placement performed slightly better than the ankle or either wrist. For MPA activity, discrimination of activity was considered good for waist and ankle placement and fair for placement on the non-dominant and dominant wrist. There was similar sensitivity for all monitor locations for sedentary activity, but the wrist worn GENEActiv had lower specificity for sedentary activity compared to wrist and ankle locations. For MPA wrist worn

**TABLE 1** | Spearman's rank correlations between GENEActiv output and METs during sedentary, light and moderate intensity activities with and without cycling removed from analysis ( $*P < 0.01$ ).

	Non-dominant wrist	Dominant wrist	Waist	Ankle
<b>Cycling included</b>				
Sedentary	0.177*	0.154*	0.228*	0.429*
Light	0.110*	0.114*	0.120*	0.105*
Moderate	0.530*	0.542*	0.508*	0.611*
<b>Cycling excluded</b>				
Sedentary	0.268*	0.371*	0.099*	0.489*
Light	0.091*	0.061*	0.129*	0.182*
Moderate	0.413*	0.443*	0.480*	0.689*

**TABLE 2** | Sensitivity, specificity and area under the curve and resultant cut-points for each GENEActiv monitor.

Intensity	Location	AUC	95% CI	Sensitivity	Specificity	Cut-point (gs)
<b>Sedentary</b>	Non-dominant wrist	0.901	0.891–0.911	87.6	83.4	4.8
	Dominant wrist	0.912	0.903–0.922	89.1	77.7	5.3
	Waist	0.934	0.926–0.942	88	94.6	4.3
	Ankle	0.977	0.974–0.981	95.3	85.7	4.4
<b>Light</b>	Non-dominant wrist	NA	NA	NA	NA	4.9–11.99
	Dominant wrist	NA	NA	NA	NA	5.4–14.6
	Waist	NA	NA	NA	NA	4.4–8.2
	Ankle	NA	NA	NA	NA	4.5–129.1
<b>MPA</b>	Non-dominant wrist	0.669	0.650–0.688	79.5	60.6	12.0
	Dominant wrist	0.661	0.642–0.680	79.3	60.9	14.7
	Waist	0.742	0.724–0.759	81.6	64.7	8.3
	Ankle	0.869	0.858–0.880	98.8	73.8	129.2

**FIGURE 1** | Mean  $\pm$  SD of METs in activities undertaken reflective of different levels of PA.

monitors had lower sensitivity and specificity than waist and ankle worn monitors.

## DISCUSSION

This study provides novel data quantifying the energy expenditure in fundamental movement skills and calibrating the GENEActiv accelerometer in children aged 8–11 years across four different wear locations and with particular attention to object control fundamental movement skills and cycling. The quantification of energy expenditure during fundamental movement skills in children has previously not been reported and there are no directly established (e.g., via indirect calorimetry) MET values associated with object control skill performance in

children. The current study is the first to provide this insight and addresses recent calls for this information to be provided (Sacko et al., 2018).

The results of the present study suggest that participants' metabolic expenditure while performing object control skills was light (throwing and catching) to moderate (instep kicking) in nature. These data suggest that practicing object control skills in the form of instep football kicking would be classified as MPA and illustrates that repetitive performance of fundamental movement skills can contribute to achieving recommended guidelines for physical activity in children. Given the paucity of studies on this topic in children it is difficult to draw conclusions with prior work. However, recent work by Sacko et al. (2018), conducted in adults, reported execution of blocked trials of kicking, throwing and striking executed with maximal effort and at different

**TABLE 3** | Sensitivity, specificity and area under the curve and resultant cut-points for each GENEActiv monitor with cycling removed from analysis.

Intensity	Location	AUC	95% CI	Sensitivity	Specificity	Cut-point
<b>Sedentary</b>	Non-dominant wrist	0.974	0.969–0.979	98.3	82.7	8.9
	Dominant wrist	0.977	0.973–0.981	97.8	80.2	11.5
	Waist	0.993	0.969–0.978	99.2	88.1	6.4
	Ankle	0.974	0.969–978	95.9	88	4.4
<b>Light</b>	Non-dominant wrist	NA	NA	NA	NA	9.0–34.6
	Dominant wrist	NA	NA	NA	NA	11.6–29.4
	Waist	NA	NA	NA	NA	6.5–30.5
	Ankle	NA	NA	NA	NA	4.5–121.3
<b>MPA</b>	Non-dominant wrist	0.798	0.783–0.813	87.7	73.1	34.7
	Dominant wrist	0.776	0.759–0.792	85.7	71.4	29.5
	Waist	0.861	0.849–0.873	92.1	71.0	30.6
	Ankle	0.856	0.844–0.869	96	74.0	121.4

cadences, produced metabolic expenditure that was moderate to vigorous in nature. In the current study, and using a different protocol, only instep kicking entered the moderate threshold with throwing and catching being of light intensity.

The GENEActiv accelerometers at each wear location demonstrated acceptable criterion validity with METs based on both the results from Spearman's correlations, showing the relationships between GENEActiv output and MET values, and AUC data from ROC analysis which gives an indication of classification accuracy of the GENEActiv output to the criterion (METs). However, the strength of association of Spearman's correlation was lower when cycling activity was included in the protocol. This was particularly the case for accelerometers worn at the wrist and the waist. The inclusion of cycling with accelerometer calibration protocols has been a point of debate. Cycling is a lifetime physical activity which is health enhancing but results in minimal movement at the waist and wrist, compared to other more ambulatory activities (Mannini et al., 2013). This often results in misclassification of cycling activity by accelerometers worn at the wrist and waist (Welch et al., 2013). In the present study the strength of association between METs and accelerometer counts from the wrist and waist were weaker when cycling was included compared to when it was removed from the protocol. Irrespective of protocol, the strongest association and therefore best criterion validity, between accelerometer counts and METs was for the ankle wear location. This observation was consistent when total METs was examined and when separate analysis was conducted for SB, LPA, and MPA. Such a finding aligns with recent work by Crouter et al. (2018) which also highlighted the utility of ankle worn accelerometry for estimating physical activity in youth.

Receiver operating characteristic curve analysis also supports the validity of ankle worn accelerometry given that the largest AUC values were found for MPA assessment at this wear location. Where the highest AUC were observed for the ankle in SB and MPA (See **Table 2**). The results of the present study extend prior work in this area (e.g., Phillips et al., 2014; Duncan et al., 2016;

Roscoe et al., 2017) that have used calibration activities involving predominantly ambulatory activity and examined wrist and waist worn devices. These aforementioned studies provide distinct AUC data and subsequent cut-points for the waist and wrist based on activities that are, arguably, the easiest for an accelerometer to quantify. Children's movement patterns are omnidirectional and rarely comprise solely of walking/running type physical activity. In the current study we included cycling, given its role as a lifelong health enhancing physical activity, and two object control skills, throwing and catching and instep kicking. These object control skills were included given their importance in participation in physical activity (Morgan et al., 2013). For this reason, accelerometer cut-points for use in pediatric samples should be sensitive to detecting these forms of movement. Without considering these types of activities there is likely to be a drastic underestimation of energy expenditure in activities that include object control skills such as football, basketball, and racquet sports (Rowlands and Stiles, 2012).

No study to date has examined the utility of GENEActiv accelerometers worn at the ankle to classify PA in children. It is therefore difficult for the present study to draw comparisons with prior studies. However, the current study is supportive of work conducted by Crouter et al. (2018) suggesting ankle worn accelerometry (Actigraph) has potential to measure physical activity accurately in youth. Unfortunately, Crouter et al. (2018) did not compare ankle worn accelerometry to estimations derived from other locations. Other work with youth and using Actigraph accelerometers (De Vries et al., 2011) has suggested the waist location may be better than the ankle in predicting adult physical activity using artificial neural networks, whereas research using the Actical accelerometer (Heil, 2006) has reported no differences in energy expenditure estimation from devices worn at the wrist, ankle or waist. In the present study, however, ankle worn accelerometry appears to offer a more accurate means to estimate physical activity using the GENEActiv accelerometer when cycling and object control skills are also considered. The cut-points presented for the ankle, wrist and waist placements

are not, however, interchangeable as body segments will move with different amounts of acceleration for different intensity movements. One-second epoch were also used, as is conventional in calibration studies (e.g., Phillips et al., 2014; Roscoe et al., 2017) and allows for upscaling to larger epochs which may be more useful in monitoring of habitual activity over multiples days.

The data presented here are based on activities conducted in a laboratory setting. This is a needed first step to establish energy expenditure in the movement skills of interest and to calibrate the accelerometer against breath-by-breath indirect calorimetry derived energy expenditure. A useful next step for researchers is to apply the cut-points derived in the current study in free living physical activity in children providing cross-validation of the cut-points presented in this study. In practical terms using the cut-points we present that include cycling within the calibration may be better reflective of the diversity of activities that children undertake habitually.

It would be beneficial to also understand if the cut-points presented here correctly classify different object control skills as light (throwing and catching) or moderate (kicking) in nature. Cross-validation of the current cut-points is needed to answer this question. Subsequent use of machine learning approaches to activity classification may also be an interesting technique to answer this question. In comparison to prior work, only Phillips et al. (2014) present cut-points for the age of population we examined in the present study, and only examined the wrist and waist locations. The cut-points we present for SB for those locations are similar to those reported by Phillips et al. (2014). However, the cut-points for MPA are slightly lower than those reported by Phillips et al. (2014). This discrepancy is not unexpected as the work by Phillips et al. (2014) relied primarily on treadmill based activity alongside a primarily upper body and linear activity on the Nintendo Wii, whereas the protocol employed in the current study comprised more varied activities, typical of children's habitual PA. Comparing accelerometer counts worn at all four locations against estimates of energy expenditure from direct observation or, if possible, expired gas, in settings where fundamental movement skills are typically performed (e.g., children's organized sports), would be a useful future research study. In the current study, participants were children who engaged with grassroots football. In this way we sought to pragmatically recruit children who were engaged in activity that necessitated use of fundamental movement skills as part of regular recreation. However, the results presented here are therefore indicative of children who had "good" motor competence and were all within "healthy" BMI based weight status categories. Level of technical skill may contribute to total energy expenditure (Sacko et al., 2018) and it is possible that children who are not fully competent in their fundamental motor skills will expend more energy for the same movements and young sports performers who are highly technically proficient may be more economical in their movement patterns resulting in less energy expended for the same movement. To date this issue has not been investigated in the context of assessing physical activity using accelerometry. We are also conscious that this study evidences utility of accelerometers worn at different locations. Although ankle worn accelerometry produced better

classification of physical activity we did not examine any issues around compliance to ankle worn wear protocols. Compliance to wear protocols in habitual physical activity studies with children are also important. Prior work (Rowlands et al., 2014) has suggested higher wear compliance for wrist worn, compared to waist worn accelerometry with children. Other research (Tudor-Locke et al., 2015) has suggested acceptable compliance rates using ankle worn accelerometry over 24 h. Future research examining this issue using the GENEActiv accelerometer would be useful in translating the results of the current study into wider use for multi day assessment of physical activity. In practical terms, understanding how children respond to ankle worn accelerometry when worn over multiple days would be useful in establishing whether this is a viable alternative to the more commonly used wrist and waist worn protocols for the assessment of habitual PA. It is important to note that the protocol employed in the present study did not result in children undertaking energy expenditure of a vigorous intensity. Therefore, the cut-points established represent the threshold for MPA only. While the MPA threshold is essential for classifying whether children meet current physical activity guidelines, understanding differentiation of moderate and vigorous physical activity would be a useful next step. Related to this point, the activities employed in the current study to represent sedentary behavior comprised being lay supine and seated playing with lego. Inclusion of standing as a discrete sedentary behavior would also have been useful given that standing is sedentary behavior recognized as distinct from sitting or lying (Barone Gibbs et al., 2015). The time commitment and physical demand needed by children to undertake the current protocol did not, however, permit us to include additional activities or treadmill speeds. The safety aspect of asking children to run at faster speeds than those used in the present study also needs to be considered by future researchers, as in the current study requesting participants to run at any faster speed than was used was not feasible.

This study extends the literature in the area of physical activity assessment by quantifying energy expenditure in object control fundamental movement skills via indirect calorimetry in children aged 8–11 years and also calibrating the GENEActiv accelerometer during PA including object control skills and cycling and when worn at different body locations. The results of the current study suggest throwing and catching is categorized as of light intensity and instep kicking a football moderate intensity, resulting in energy expenditure equivalent to slow or medium paced walking or cycling and running, respectively. GENEActiv accelerometers demonstrated acceptable criterion validity although, when cycling was considered, validity of wrist and waist worn accelerometers was lower. Ankle worn accelerometry appears to provide the most suitable wear location to quantify MPA including ambulatory activity, object control skills and cycling, in children aged 8–11 years.

## ETHICS STATEMENT

The research presented in this manuscript was approved by the institutional ethics committees of Coventry University and

the University of Derby and adhered in full to the Declaration of Helsinki in the treatment and use of human participants in research studies.

## AUTHOR CONTRIBUTIONS

MD conceived the study, collected data, performed data processing and analysis, and drafted and edited the manuscript. CR and MF collected data, performed data processing, and edited

the manuscript. JT conceived the study, drafted and edited the manuscript. EE conceived the study, collected data, and drafted and edited the manuscript. MD, CR, MF, JT, and EE approved the final version.

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# Longitudinal Changes of Functional Capacities Among Adolescent Female Basketball Players

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**Background:** The interpretation of young athletes' performance during pubertal years is important to support coaches' decisions, as performance may be erroneously interpreted due to the misalignment between chronological age (CA), biological age (BA) and sport age (SA).

**Aim:** Using a Bayesian multilevel approach, the variation in longitudinal changes in performance was examined considering the influence of CA, BA (age at menarche), SA, body size, and exposure to training among female basketball players.

**Method:** The study had a mixed-longitudinal design. Thirty eight female basketball players (aged  $13.38 \pm 1.25$  years at baseline) were measured three times per season. CA, BA and SA were obtained. Anthropometric and functional measures: countermovement jump, Line drill (LD), Yo-Yo (Yo-Yo IR1). Based on the sum of the z-scores, an index of overall performance was estimated. The effects of training on longitudinal changes in performance were modeled.

**Results:** A decrease in the rate of improvements was apparent at about 14 years of age. When aligned for BA, the slowing of the rate of improvements is apparent about 2 years after menarche for LD. For countermovement jump longitudinal changes, when performance was aligned for BA improvements became linear. For Yo-Yo IR1 and performance index, both indicators showed a linear trend of improvement when aligned for CA and BA, separately. Older players showed higher rates of improvement for Yo-Yo IR1 and performance index from pre-season to end-season. When considering performance changes aligned for BA it was apparent an improvement of performance as players became biologically mature.

**Conclusions and Implications:** The alignment of CA with BA and SA provides important information for coaches. Human growth follows a genetically determined pattern, despite variation in both tempo and timing. When the effects of maturation reach their end, all the girls went through the same process. Hence, there is no need to artificially manipulate youth competitions in order to accelerate gains that sooner or later reach their peak and tend to flat their improvement curve.

**Keywords:** youth sports, menarche, athletes, Bayesian multilevel modeling, adolescence

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## INTRODUCTION

The interpretation of young athletes' performance development during pubertal years is of importance to support coaches' short- and long-term decisions. Particularly in the context of talent development where, despite ethical issues, early identification and selection is the *modus operandi* of high performance sport, talent selection or de-selection decisions have different levels of risks and consequences for young players (Baker et al., 2018).

Most notably, performance assessment may be confounded due to the misalignment between chronological age, biological age and sport age (accumulated training and competitive experience in sport). Therefore, the mechanisms that predict future successful players and those dropping out from organized sports are multifactorial and highly complex, especially in sports like basketball, where structured training systems start before or during puberty (Deprez et al., 2015; Soares et al., 2016; Gonçalves et al., 2018).

Basketball is a team sport that requires movement patterns that involve short, intense and repeated episodes of activity requiring frequent rapid changes in direction (McInnes et al., 1995; Ben Abdelkrim et al., 2007, 2010; Staunton et al., 2018; Stojanovic et al., 2018). Although basketball movement patterns mainly involve intermittent activities which are aerobic in nature, maximal intensity short-term activities (e.g., sprinting, jumping, cutting) are decisive for the performance in the game (Ben Abdelkrim et al., 2007; Stojanovic et al., 2018). Hence, when interpreting young basketball player's performance, coaches and/or researchers should consider both maximal short-term output and basketball related intermittent endurance. On the other hand, body size, particularly stature, is relevant for basketball performance (Drinkwater et al., 2008), and is highly valued by coaches when attempting to select and/or predict future outcomes (Pearson et al., 2006). However, young players vary substantially in growth and maturity status, as well as complex environmental factors, often complicates interpretation of performance in young athletes (Abbott et al., 2005; Pearson et al., 2006).

There is an emphasis in youth sports, including youth basketball, on talent identification, development and selection (Gonçalves et al., 2012). Achievement of athletic expertise at high level when adults is limited to a very narrow group of players.

In youth basketball, coaches' decisions regarding the career path of adolescent players are influenced by players size and functional performance level (Drinkwater et al., 2008; Carvalho et al., 2018). Although differences in adolescent players' physique and performance are transient, are likely exacerbated by the interactions between pubertal growth rate, chronological age and accumulated sport-specific experience (Carvalho et al., 2018; Gonçalves et al., 2018; Leonardi et al., 2018). Thus, the appropriate interpretation of the young basketball players' performance is crucial for both coach and athlete (Leonardi et al., 2018).

Available data considering functional capacities in young basketball players is mainly based on male players (Montgomery et al., 2008; Carvalho et al., 2011a,b,c; Sisic et al., 2016; Torres-Unda et al., 2016). Although there is an increasing number of

female young athletes involved in intensive training programs and high level competitions, available knowledge concerning the functional capacities of young female basketball players remains scarce (Mcmanus and Armstrong, 2011). Interpretations of young female athletes' functional capacity may be complicated by sexual dimorphism (Mcmanus and Armstrong, 2011), given the large variation between-girls in the timing and tempo of biological maturation, as well as primary sex differences (Sherar et al., 2004). Hence, interpretations based on male athlete samples may be inadequate and examining functional capacity in young female athletes engaged in sport-specific training merits attention.

Understanding changes and development of performance during pubertal development is an increasingly studied topic (Nevill et al., 1998; Thomis et al., 2000; Beunen et al., 2002; De Ste Croix et al., 2002; Martin et al., 2004; Drinkwater et al., 2005; Bidaurrazaga-Letona et al., 2014; Carvalho et al., 2017). Amongst young athletes exposed to organized training and competition programs, researchers usually need a long time planning, extensive resources for data collection in the field, rather than the laboratory. Attrition from injuries or loss of interest complicates data analysis. Furthermore, the need to consider chronological age, biological age and "the age in the sport" (i.e., the amount of accumulated training and competition experience in the sport) represent a level complexity that may be difficult to appropriately fit and interpret using traditional statistical models (i.e., based on repeated measures analysis of variance) (Gueorguieva and Krystal, 2004; Kristensen and Hansen, 2004). Multilevel modeling provides a flexible and powerful approach to fit complex hierarchical structured data, such as repeated measures (Gelman and Hill, 2007; Goldstein, 2011). Furthermore, Bayesian methods are especially attractive in this context, as they perform well with small sample sizes (Van De Schoot et al., 2015), perform well with complex models such as multilevel modeling (McElreath, 2015), and allow incorporation of available prior information about the parameters in evaluating the data consequently improving out-of-sample predictions (Heino et al., 2018).

Considering a Bayesian multilevel approach, we examined the influence of chronological age, biological age (age at menarche), age in the sport, body size and composition, and exposure to training and competitive basketball season on the longitudinal changes in functional performance during the pubertal years among female basketball players.

## METHODS

### Study Design and Participants

This study was based on a mixed-longitudinal design. A total of 38 adolescent female basketball players aged, on average, 13.38 (1.25) years at baseline, were measured three times per season between August 2015 and December 2017. Within the season, measurements were performed pre- (March), mid- (August) and end-season (December). The period of observation comprised two full competitive seasons (from March 2016 to December 2017), and a half season (August 2015 to December 2015). A total of 177 observations were considered for analysis, during the

observation period as follows: August 2015,  $n = 10$ ; December 2015,  $n = 9$ ; March 2016,  $n = 31$ ; August 2016,  $n = 37$ ; December 2016,  $n = 35$ ; March 2017,  $n = 24$ ; August 2017,  $n = 17$ ; December 2017,  $n = 14$ ). The players considered in the present study had at least three measurements across the period of observation. The distribution of measurements per players was as follows: three measurements,  $n = 6$ ; four measurements,  $n = 10$ ; five measurements,  $n = 13$ ; six measurements,  $n = 9$ ). All The players were engaged in formal training and competition within under 13 ( $n = 23$ ) and under 15 ( $n = 15$ ) teams from two clubs from the Campinas metropolitan region of Brazil, and competed at regional level competition supervised by the *Associação Regional de Basquetebol* (ARB). During the study, all players trained regularly ( $\sim 300$ – $360$  min/wk) over a 9-month season (March to November). The typical week was composed by three training sessions, with a duration of 120 min per session. In general, sessions were composed by a warm-up section ( $\sim 30$  min), an individual technical development section ( $\sim 30$  min), tactical development section ( $\sim 30$  min, mostly small sided games), and game session ( $\sim 30$  min). No player was suffering from injury at the time of testing or during 6 months before testing.

The study was approved by the Research Ethics Committee of the University of Campinas. Participants were informed about the nature of the study, that participation was voluntary and that they could withdraw from the study at any time. Players and their parents/legal guardians provided written informed consent.

## Measures

Chronological age was calculated to the nearest 0.1 year by subtracting birth date from date of testing. Years of training in formal basketball were attained by interview. Age at menarche was obtained from an individual interview by the coaches of the players (female coaches in all cases). To align age at menarche with chronological age (i.e., distance to menarche) we subtracted chronological age by age at menarche. Negative values indicate time before age at menarche and positive values indicate time after age at menarche.

Anthropometric measurements were performed by a single experienced observer. Stature was measured with a portable stadiometer (Seca model 206, Hanover, MD, USA) to the nearest 0.1 cm. Body mass was measured with a calibrated portable balance (Seca model 770, Hanover, MD, USA) to the nearest 0.1 kg. The triceps, subscapular, suprailiac and medial calf skinfolds were measured and summed as a measure of relative body fat distribution. Skinfold sites were measured with a Lange skinfold caliper (Cambridge Scientific Industries, Inc., Cambridge, MD). Reliability estimates for the observer are published elsewhere (Carvalho et al., 2011a,b).

Three measures of functional capacity for basketball were considered: vertical jump with countermovement (Bosco et al., 1983), a short-term maximal running protocol, the Line drill (LD) test (Semenick, 1990; Carvalho et al., 2011a) and an intermittent endurance test, the Yo-Yo Intermittent Recovery level 1 test (Yo-Yo IR1) (Bangsbo, 1994). Based on the sum of the z-scores, we estimated an index of overall performance, i.e., functional performance index (lower-limb explosive strength,

agility and anaerobic power, and intermittent endurance). Note that z-scores were reversed for the LD performance; as lower times indicate better performance.

Tests were performed in two sessions separated by at least 48 h, where the first session included the vertical jump and LD test, and the second session the Yo-Yo IR1. Before testing a standardized warm-up was taken by all athletes.

The countermovement jump test was tested on a jump mat (Multisprint System, Hidrofit, Brazil). Participants started from an upright standing position. Players were instructed to begin the jump with a downward movement, which was immediately followed by a concentric upward movement, resulting in a maximal vertical jump. During jumping, hands were held on the hips during all phases of the jumping. Three trials were allowed and the best retained for analysis. The coefficient of variation, based on replicate measures separated by 1 week in 18 players, was 6.9% (95% CI 5.1–10.5).

In the LD protocol (Semenick, 1990; Carvalho et al., 2011a), players ran 140 m as fast as possible in the form of four consecutive shuttle sprints of 5.8, 14.0, 22.2, and 28.0 m within a regulation basketball court. Players began the test one meter behind the baseline of the basketball court, where a pair of photoelectric cells (Multisprint System, Hidrofit, Brazil) was aligned with the baseline. Verbal encouragement for an all-out effort was given throughout the test. Time was recorded in seconds. Reliability estimates were reported previously (Carvalho et al., 2011a).

The Yo-Yo IR1 was performed by all players (Bangsbo, 1994). The protocol is based on repeated 2 x 20-m runs back and forth between the starting, turning, and finishing line at a progressively increased speed controlled by audio bleeps from a tape recorder (Bangsbo, 1994). The athletes have a 10-s active rest period between each bout, jogging in a distance of  $2 \times 5$ -m. Players ran until they were no longer able to maintain the required speed; the test was completed when athletes failed twice to reach the finishing line in time. Covered distance was measured in meters. Based on replicate measures on a subsample of 11 players measured twice within 1 week, the coefficient of variation was 6.0% (95% CI 4.5–9.5%), which is within the range of reproducibility reported for the Yo-Yo IR1 (Bangsbo et al., 2008).

## Statistical Analysis

### Modeling Functional Performance Aligned by Chronological Age or Age at Menarche

The first modeling step was to use a basic two-level polynomial growth model curve (Goldstein, 1986) to model functional performance indicators against chronological age and age at menarche, i.e., distance to menarche in years, separately. The model describes each player's successive measurements over time defining the player's change at each measurement point and its variation (level-1), differences in trajectories between players and its variation (level-2). To capture the possibility of non-linear longitudinal changes during pubertal years we considered time (chronological age or distance to menarche) coefficients up to the quadratic terms, at least. When modeling functional capacity indicators against chronological age we centered each player's value at the sample grand mean (13.94 years). This allows for the

model to provide predicted values with meaningful information within the range of observations, in particular the intercept term. We allowed for between-participants variation at group-level (level-2) across the intervals of observations.

Since both time indicators were centered, we used weakly informative prior distributions for population-level, normal priors (0.50), and for group-level effects, half-cauchy priors (0.2). This conveniently allows for easier achievement of model convergence, as well as ensuring that results reflect the knowledge available from the current data.

### Modeling the Influence of Body Size and Training Experience on Functional Performance Longitudinal Changes

In this step of the analysis we explored whether body size (stature, body mass and adiposity represented by the sum of four skinfolds) and years formal training experience influenced longitudinal changes in functional performance. For computational convenience and for interpretation when variables have different scales (McElreath, 2015) we used z-score transformation on both dependent variables (functional performance indicators) and independent variables (i.e., the candidate explanatory variables training experience, stature, body mass and adiposity). We added the explanatory variables to each of the basic two-level polynomial growth model modeling functional performance indicators against chronological age and distance to menarche. In the models for this step we also used weakly informative prior distributions for population-level, normal priors (0.10), and for group-level effects, half-cauchy priors (0.2).

### Modeling the Effects of Exposure to 9-Months Competitive Season on Longitudinal Changes in Functional Performance

Based on the results of the previous analytical step, we explored the effects of training exposure on longitudinal changes in functional performance with indicators aligned for years of formal training in basketball, controlling for chronological age and distance to menarche. Since measurements were made and pre-, mid- and end-season across two and a half years, we first examined the pattern of change within the 9-month competitive season. A linear trend of change was observed, thus we included a dummy variable (pre-season coded as 0; mid-season coded as 1; end-season coded as 2) to identify each moment of observation in the models. We then included the dummy variable (i.e., season) in each of the initial models predicting functional performance indicators against chronological age and distance to menarche in years. We also considered an interaction term of the time variable with the dummy variable (e.g., distance to menarche interaction with season). The inclusion of the dummy variable for season and the interaction terms allow us to examine whether there are differences in players' functional performance indicators rates of change within the 9-month season, as well as changes within the 9-month season change with chronological age, distance to menarche or years of training experience (interaction term between time of measurement and season measurement).

Similar to the precedent models, we used weakly informative prior distributions for population-level, normal priors (0.10), and for group-level effects, half-cauchy priors (0.2), allowing model convergence, as well as ensuring that results reflect the knowledge available on the current data.

### Model Checking and Computation

We used posterior predictive checks to confirm that we did not omit relevant interactions (Gelman et al., 2013; Vehtari et al., 2016). We used the widely applicable information criteria (WAIC) to compare models and to ensure we had not overfit our data (Gelman et al., 2013; McElreath, 2015; Vehtari et al., 2016).

For each model we run two chains for 2,000 iterations with a warm-up length of 1,000 iterations. The models were implemented with Bayesian methods via Markov Chain Monte Carlo (MCMC) simulation and using Hamiltonian Monte Carlo and its extension, the No-U-Turn Sampler using Stan (Stan Development Team, 2018), via "brms" package (Bürkner, 2017), available as a package in the R statistical language (R Core Team, 2015).

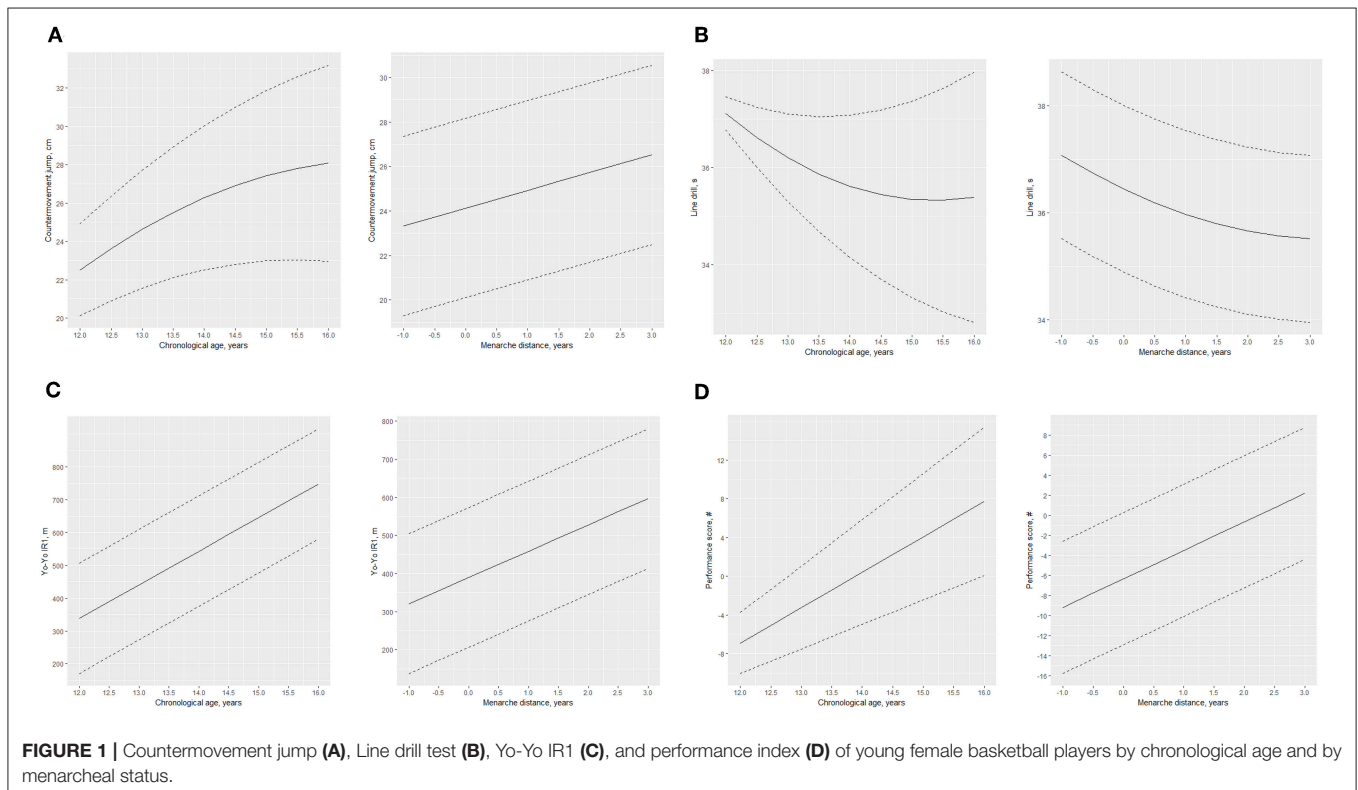
## RESULTS

The average age at menarche for the present sample of adolescent female basketball players was 11.82 (1.25) years. Five players attained menarche during the study. The posterior predictions and 90% credible intervals for indicators of functional performance aligned by chronological age and distance to age at menarche of young Brazilian female basketball players are summarized in **Table 1** and **Figure 1**. Corresponding Bayesian multilevel models from where posterior samples were derived are summarized in **Supplementary Tables 1, 2**. A non-linear trend was observed for both countermovement jump and Line drill performances when aligning performance by chronological age. A decrease in the rate of improvements in both jump and Line drill changes was apparent at about 14 years of age. When aligned for distance to menarche, the slowing of the rate of improvement was apparent about 2 years after menarche for Line drill performance. As for jump performance longitudinal changes, when performance was aligned for age at menarche improvements became linear. For Yo-Yo IR1 and functional performance index, both indicators showed a linear trend of improvement in performance when aligned for chronological age and distance to menarche. For all functional performance indicators except Yo-Yo IR1, variation between players in longitudinal changes was substantial when aligned for chronological age (see **Supplementary Table 1**). However, between-player variation was not apparent when functional performance was aligned for age at menarche (see **Supplementary Table 2**).

The relative contributions of formal experience of training, stature, body mass and adiposity on the longitudinal changes in functional performance indicators aligned for chronological age are summarized in **Table 2**, and aligned for age at menarche in **Table 3**. Adiposity had a negative influence on players' functional performance. Between-player differences in body mass did not influence longitudinal changes in functional performance,

**TABLE 1** | Posterior predictions and 90% credible intervals for longitudinal changes of functional performance aligned both by chronological age and age at menarche.

	Countermovement jump, cm	Line drill test, s	Yo-Yo IR1, m	Performance index, #
12 years	22.52 (21.56 to 23.50)	37.11 (36.72 to 37.51)	338.3 (330.6 to 347.20)	-6.90 (-7.16 to -6.65)
13 years	24.63 (23.81 to 25.46)	36.20 (35.91 to 36.51)	440.7 (418.9 to 463.3)	-3.25 (-3.96 to 2.51)
14 years	26.26 (25.08 to 27.41)	35.61 (35.13 to 36.31)	543.1 (490.6 to 596.0)	0.40 (-1.27 to 2.13)
15 years	27.41 (25.41 to 29.38)	35.34 (34.36 to 36.34)	645.5 (562.3 to 728.7)	4.05 (1.42 to 6.79)
16 years	28.09 (24.77 to 31.34)	35.39 (33.61 to 37.19)	747.9 (634.0 to 861.4)	7.70 (4.11 to 11.44)
1 year before age at menarche	23.33 (22.11 to 24.45)	37.07 (36.74 to 37.41)	320.9 (264.8 to 370.0)	-9.18 (-11.14 to -6.82)
Age at menarche	24.13 (22.35 to 25.81)	36.44 (35.73 to 37.11)	389.8 (306.5 to 466.5)	-6.34 (-9.28 to -3.28)
1 year after age at menarche	24.93 (22.59 to 27.17)	35.97 (34.72 to 37.17)	458.7 (348.2 to 563.0)	-3.50 (-7.42 to 0.26)
2 years after age at menarche	25.73 (22.83 to 28.53)	35.66 (33.71 to 37.59)	527.6 (389.9 to 659.5)	-0.66 (-5.56 to 3.8)
3 years after age at menarche	26.53 (23.07 to 29.89)	35.51 (32.70 to 38.37)	596.5 (431.6 to 756.0)	2.18 (-3.7 to 7.34)



whether performance was modeled against chronological age or age at menarche.

The results for the model exploring the effects of exposure to 9-months competitive season on longitudinal changes in functional performance are summarized in **Table 4**, when aligning for chronological age, and **Table 5**, when aligning for age at menarche.

Considering performance changes aligned for chronological age and accounting for between players' differences in training experience and adiposity, it was observed a trend for substantial improvements of all performance indicators across the 9-months season (i.e., between pre-, mid- and end-season). Also, it was apparent that older players showed higher rates of improvement for Yo-Yo IR1 and functional performance

index from pre-season to end-season. Also, when considering performance changes aligned for age at menarche (**Table 5**), there was an apparent substantial improvement of performance across the season. However, for countermovement jump, Yo-Yo IR1 and functional performance index from pre-season to end season improvements were substantially higher as players became biologically mature (greater distance to menarche).

## DISCUSSION

The present study modeled longitudinal changes in functional performance considering the influence of chronological age, biological age (age at menarche), age in the sport, body

**TABLE 2 |** Relative contributions of years of formal experience of basketball training, body size and adiposity on longitudinal changes in functional performance aligned by chronological age.

	Countermovement jump	Line drill test	Yo-Yo IR1	Performance index
<b>POPULATION-LEVEL EFFECTS (90% CREDIBLE INTERVAL)</b>				
Intercept	-3.38 (-12.37 to 5.56)	3.10 (-3.92 to 10.40)	-5.25 (-41.22 to 30.22)	-14.85 (-54.58 to -26.03)
Chronological age	0.34 (0.00 to 0.70)	-0.04 (-0.29 to 0.22)	2.74 (1.47 to 4.09)	2.77 (1.27 to 4.40)
Years of formal training experience	0.39 (0.04 to 0.72)	-0.36 (-0.60 to -0.12)	0.68 (-0.61 to 2.01)	1.65 (0.13 to 3.14)
Years of formal training experience <sup>2</sup>	-0.18 (-0.28 to -0.07)	0.10 (0.01 to 0.20)	-	-
Stature	-0.09 (-0.13 to 0.31)	-0.08 (-0.26 to 0.09)	0.14 (-0.74 to 1.03)	0.38 (-0.63 to 1.37)
Body mass	-0.18 (-0.52 to 0.16)	0.12 (-0.13 to 0.38)	0.53 (-0.94 to 2.01)	0.53 (-1.08 to 2.14)
Sum of four skinfolds	-0.24 (-0.54 to 0.08)	0.18 (-0.03 to 0.39)	-1.91 (-3.31 to -0.54)	-2.60 (-4.11 to -1.05)
<b>GROUP-LEVEL EFFECTS (90% CREDIBLE INTERVAL)</b>				
<b>Level 1 standard deviation (within player)</b>				
Within-individuals	0.71 (0.63 to 0.80)	0.72 (0.63 to 0.81)	3.65 (3.26 to 4.09)	3.80 (3.35 to 4.25)
<b>Level 2 standard deviation (between players)</b>				
Intercept	1.06 (0.80 to 1.38)	0.48 (0.11 to 0.78)	3.61 (2.56 to 4.76)	4.07 (2.79 to 5.55)
Chronological age	0.25 (0.02 to 0.57)	0.31 (0.02 to 0.70)	1.10 (0.13 to 2.27)	1.05 (0.07 to 2.41)
Years of formal training experience	0.24 (0.02 to 0.56)	0.32 (0.03 to 0.69)	1.11 (0.12 to 2.31)	1.47 (0.23 to 2.74)

variables were standardized.

**TABLE 3 |** Relative contributions of years of formal experience of basketball training, body size and adiposity on longitudinal changes in functional performance aligned by age at menarche.

	Countermovement jump	Line drill test	Yo-Yo IR1	Performance index
<b>POPULATION-LEVEL EFFECTS (90% CREDIBLE INTERVAL)</b>				
Intercept	-5.14 (-13.72 to 3.39)	2.22 (-5.32 to 9.07)	-15.15 (-48.43 to 19.29)	-21.37 (-61.89 to 18.70)
Distance to menarche	-0.10 (-0.44 to 0.27)	0.05 (-0.19 to 0.29)	2.11 (0.76 to 3.52)	2.04 (0.57 to 3.62)
Years of formal training experience	0.69 (0.46 to 0.94)	-0.41 (-0.63 to -0.18)	1.93 (0.81 to 3.10)	2.73 (0.152 to 4.01)
Years of formal training experience <sup>2</sup>	-0.19 (-0.29 to -0.08)	0.10 (0.01 to 0.21)	-	-
Stature	0.14 (-0.07 to 0.35)	-0.06 (-0.23 to 0.12)	0.32 (-0.51 to 1.14)	0.48 (-0.51 to 1.48)
Body mass	-0.09 (-0.44 to 0.25)	0.07 (-0.17 to 0.32)	0.43 (-1.03 to 1.94)	0.48 (-1.13 to 2.06)
Sum of four skinfolds	-0.32 (-0.62 to -0.01)	0.19 (-0.01 to 0.40)	-1.99 (-3.47 to -0.61)	-2.58 (-4.16 to -1.02)
<b>GROUP-LEVEL EFFECTS (90% CREDIBLE INTERVAL)</b>				
<b>Level 1 standard deviation (within player)</b>				
Within-individuals	0.73 (0.65 to 0.82)	0.72 (0.64 to 0.81)	3.67 (3.30 to 4.08)	3.87 (3.42 to 4.37)
<b>Level 2 standard deviation (between players)</b>				
Intercept	0.85 (0.36 to 1.33)	0.44 (0.07 to 0.84)	0.97 (0.06 to 2.55)	3.52 (1.19 to 5.57)
Distance to menarche	0.32 (0.04 to 0.69)	0.16 (0.01 to 0.39)	0.76 (0.08 to 1.91)	1.25 (0.09 to 2.96)
Years of formal training experience	0.28 (0.02 to 0.59)	0.39 (0.05 to 0.77)	1.21 (0.73 to 1.75)	1.52 (0.27 to 2.81)

variables were standardized.

size and body composition, and exposure to training and competitive basketball season over the pubertal years in female basketball players. There is a body of literature that addresses the growth curves and functional performance development of pubertal girls (Nevill et al., 1998; Yagüe and De La Fuente, 1998; Armstrong et al., 2000; Thomis et al., 2000; De Ste Croix et al., 2002; Geithner et al., 2004), but mostly in non-athletic populations. A challenging question of interest with young athletes is to understand the complex interactions of growth and development with the exposure to athletic and sport-specific performance. However, to our knowledge, this is the first study that aims to align

performance development with chronological age, biological age and sport experience, and to shed light on their relative effect on performance.

Chronological age and biological age are genotype variables and age in the sport is a phenotype one, meaning that the knowledge of the contribution and the interaction of each of the variables to performance in the developmental years is an important issue for researchers and practitioners. The findings in the present study showed that the genetic-determined variables tend to converge in the final stages of maturation, following a linear evolution curve, although the between-players variability does not disappear. There were substantial

**TABLE 4 |** Posterior estimates for longitudinal changes in functional performance aligned by chronological age and partitioning the influence of exposure to 10-month competitive seasons.

	Countermovement jump	Line drill test	Yo-Yo IR1	Performance index
<b>POPULATION-LEVEL EFFECTS (90% CREDIBLE INTERVAL)</b>				
Intercept	-0.01 (-0.35 to 0.34)	0.19 (-0.04 to 0.42)	-1.78 (-2.70 to -0.84)	-2.12 (-3.25 to -0.95)
Chronological age	0.11 (-0.19 to 0.41)	0.14 (-0.08 to 0.36)	1.65 (1.02 to 2.29)	2.14 (1.39 to 2.92)
Years of formal training experience	0.46 (0.16 to 0.77)	-0.42 (-0.70 to -0.16)	-	1.11 (1.42 to 2.86)
Years of formal training experience <sup>2</sup>	-0.17 (-0.26 to -0.08)	0.11 (0.01 to 0.22)	-	-
Season	0.34 (0.22 to 0.45)	-0.36 (-0.47 to -0.24)	1.70 (0.94 to 2.48)	2.14 (1.42 to 2.86)
Season × chronological age interaction	-	-	0.62 (0.14 to 1.10)	0.63 (0.16 to 1.12)
Sum of 4 skinfolds	-0.29 (-0.49 to -0.09)	0.20 (0.04 to 0.36)	-0.97 (-1.65 to -0.28)	-1.62 (-2.45 to -0.75)
<b>GROUP-LEVEL EFFECTS (90% CREDIBLE INTERVAL)</b>				
<b>Level 1 standard deviation (within player)</b>				
Within-individuals	0.65 (0.58 to 0.74)	0.64 (0.56 to 0.71)	2.93 (2.60 to 3.30)	2.96 (2.60 to 3.37)
<b>Level 2 standard deviation (between players)</b>				
Intercept	1.02 (0.78 to 1.31)	0.35 (0.05 to 0.67)	2.37 (1.58 to 3.29)	3.18 (2.18 to 4.35)
Chronological age	0.21 (0.02 to 0.51)	0.43 (0.12 to 0.73)	-	1.10 (0.16 to 2.18)
Years of formal training experience	0.25 (0.02 to 0.61)	0.60 (0.26 to 0.91)	-	-
Season	-	-	2.02 (1.32 to 2.78)	1.69 (0.90 to 2.56)

variables were standardized.

**TABLE 5 |** Posterior estimates for longitudinal changes in functional performance aligned by menarche age and partitioning the influence of exposure to 10-month competitive seasons.

	Countermovement jump	Line drill test	Yo-Yo IR1	Performance index
<b>POPULATION-LEVEL EFFECTS (90% CREDIBLE INTERVAL)</b>				
Intercept	-0.44 (-0.06 to 0.06)	0.03 (-0.39 to 0.32)	-3.82 (-5.37 to -2.32)	-3.57 (-5.57 to -1.70)
Distance to menarche	-0.33 (-0.66 to -0.01)	0.16 (-0.05 to 0.39)	1.51 (0.50 to 2.52)	0.59 (-0.19 to 1.39)
Years of formal training experience	0.69 (0.47 to 0.92)	-0.40 (-0.65 to -0.15)	0.91 (0.19 to 1.63)	1.84 (0.87 to 2.80)
Years of formal training experience <sup>2</sup>	-0.19 (0.27 to -0.10)	0.11 (0.1 to 0.22)	-	-
Season	0.23 (0.06 to 0.41)	-0.36 (-0.47 to -0.15)	-	-
Season × distance to menarche interaction	0.06 (-0.00 to 0.13)	-	0.78 (0.10 to 1.48)	0.85 (0.61 to 1.10)
Sum of four skinfolds	-0.26 (-0.46 to -0.06)	0.20 (0.06 to 0.34)	-1.10 (-1.84 to -0.36)	-1.49 (-2.33 to -0.63)
<b>GROUP-LEVEL EFFECTS (90% CREDIBLE INTERVAL)</b>				
<b>Level 1 standard deviation (within player)</b>				
Within-individuals	0.66 (0.58 to 0.74)	0.63 (0.56 to 0.72)	2.95 (2.60 to 3.34)	2.94 (2.59 to 3.35)
<b>Level 2 standard deviation (between players)</b>				
Intercept	0.86 (1.08 to 2.27)	0.33 (0.05 to 0.64)	2.46 (1.63 to 3.41)	2.83 (1.19 to 4.43)
Distance to menarche	0.22 (0.03 to 0.43)	-	-	1.21 (0.12 to 2.63)
Years of formal training experience	-	0.60 (0.32 to 0.90)	-	-
Season	-	0.11 (0.01 to 0.25)	2.11 (1.36 to 2.96)	1.89 (1.18 to 2.71)

variables were standardized.

improvements in performance during the basketball season, and these improvements continued to occur with older players. However, performance improvements tended to slow down, leveling-off when the players approach adult maturity status. Thus, variance can be explained by sport experience, notably less evident in tests where the movement of body mass over short distances is needed, such as the LD test.

This interpretation is of relevance applied to basketball player selection. The mean age at menarche was 11.82 (1.25) years, which is earlier than worldwide observations (Eveleth and

Tanner, 1991), as well as on observations based on Brazilian data (Duarte, 1993). Hence, the present sample of female basketball players was, on average, advanced in maturity status expressed by mean age at menarche. This trend is consistent with observations in adolescent male basketball players where an overrepresentation of players with advanced maturity status has been noted (Carvalho et al., 2011b, 2013, 2018; Te Wierike et al., 2015; Torres-Unda et al., 2016). Hence, youth basketball coaches likely are not considering the transient influence of maturation when interpreting young athletes' performance.



The interpretation of the random effects allows us to determine that all the evolution paths are linear even in tests that require explosive short-term strength. The importance of body mass and adiposity on functional performance are well known (Nevill et al., 2004), particularly in young populations (Barker and Armstrong, 2011). However, there was no substantial influence of body size on longitudinal changes in performance when aligning for chronological age or age at menarche in the present sample of female adolescent basketball players. Only adiposity had a negative influence on performance, which is consistent with longitudinal observations in non-athletic girls (Welsman and Armstrong, 2000; Armstrong et al., 2001). On the other hand, it should be expected that relative gains of body fat around 25 to 30% will occur by the end of puberty in the average adolescent girl (Matthews et al., 2006; Sherar et al., 2007; Mcmanus and Armstrong, 2011). Although young athletes tend to be leaner than non-athletic girl (Mcmanus and Armstrong, 2011), it appears that coaches should still need to consider pubertal body composition changes when interpreting female players performance development.

There was substantial variability between players across the 9-month competitive season exposure, however paths of performance development remained consistent across puberty. Differences between players at the beginning of the competitive seasons remained at the end of the season, although substantial variation on rates of changes across the season highlight the need for coaches to look at the players from an athlete-centered perspective. Furthermore, differences between players' rate of change in functional performance across competitive seasons during pubertal years appeared to be positively related to chronological age and biological age.

The alignment of chronological age with biological age and accumulated years of experience in the sport provide important information for youth sport organizers and coaches. Children and adolescents' growth with age follows a pattern that is genetically determined, albeit substantial between individuals' variation in both tempo and timing of growth between individuals (Malina et al., 2004). When the effects of maturation reach their end, about 15–16 years in average girls, all the players in the present sample, despite their variability, went through the same process. Hence, there is no need to artificially manipulate youth competitions in order to accelerate gains that sooner or later reach their peak and tend to flatten their improvement curve. At the same time, coaches need to be aware of the alignment of their interventions in preparation, providing the athlete with the training stimuli that match their readiness and knowing that there is no point in trying to force or accelerate those stimuli because the gains tend to slow down with the advance in chronological and biological age. These observations are of particular relevance given the recent calls promoting biobanding as a new paradigm for youth sports and training (Cumming et al., 2017; Rogol et al., 2018). The present results suggest the need to be cautious when interpreting the young athletes' performance, the need to consider athletes development over time, and to avoid decisions (competition groups, exclusion or promotion of athletes) based on snapshots using maturity status estimations that, at best, have limited validity (Malina et al., 2012; Malina and Koziel, 2014; Koziel and Malina, 2018).

We acknowledge that several limitations in the present study. The sample size is small and there was attrition between measurements. This may in part reflect the particular characteristics of context of the study (i.e., youth female basketball in Brazil), warranting caution when generalizing interpretations. Also, attrition is an important limitation in longitudinal studies during growth and training (Kemper, 2008). On the other hand, we only considered the follow-up of body dimensions and functional capacities in this study given the available time and context of assessment. Future studies may consider tracking also behavioral and in-game performance, but these pose considerable challenges when studying young players' development during pubertal growth. Nevertheless, the present data add valuable insights for the study of young female basketball players' physical and functional development. Moreover, Bayesian multilevel modeling was adopted to deal with the analytical challenges posed in a design with repeated observations within players over time, with different levels and sources of variation (within- and between-players). In contrast with traditional statistical approaches used in sports science, Bayesian multilevel modeling is a flexible and powerful approach to interpret young athletes' performance.

In summary, this study provides a description and interpretation about the development of functional performance across adolescence in female basketball players, accounting for the influence of growth, maturation and training on competitive basketball performance. It shows the need to account for chronological, biological and training experience, i.e., age in sport, and partition their influence on body size. Human growth follows a genetically determined pattern, despite substantial variation in both tempo and timing during puberty. When the effects of maturation reach their end, all the girls went through the same process. Hence, coaches, sport scientists, and others involved in the selection and development of youth basketball players should consider that there is no need to artificially manipulate youth competitions in order to accelerate gains that sooner or later reach their peak and tend to flat their improvement curve.

## ETHICS STATEMENT

This study was approved and carried out in accordance with the recommendations of the local Institutional Ethics Review Board. All participants were informed about the nature of the study, that participation was voluntary and that they could withdraw from the study at any time. Players and their parents/legal guardians provided written informed consent in accordance with the Declaration of Helsinki.

## AUTHOR CONTRIBUTIONS

HC and CG were involved in the conceptualization of the study, data analysis, and the writing of the manuscript. RP and CF were involved in the conceptualization of the study and the writing of the manuscript. TL and AS were involved in the data assessment, and the writing of the

manuscript. All authors contributed approved the final version of the manuscript.

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## SUPPLEMENTARY MATERIAL

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# Physiological, Anthropometric, and Motor Characteristics of Elite Chinese Youth Athletes From Six Different Sports

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Several talent selection programs in elite sport schools are based on motor diagnostics for the purpose of recommending or transferring promising talents to general groups of sports; game sports, combat sports or endurance sports, and to more concrete sports such as gymnastics, skiing, or tennis. However, the predictive value of such testing is unclear. This study evaluated the concurrent validity of physiological performance prerequisites, body dimensions, as well as specific motor performances. The sample consisted of  $N = 97$  youth athletes from all ninth grade classes of a Shanghai Elite Sport school belonging to six different sports including basketball ( $n = 7$ ), fencing ( $n = 23$ ), judo ( $n = 20$ ), swimming ( $n = 10$ ), table tennis ( $n = 15$ ), and volleyball ( $n = 22$ ). The performance diagnosis took place between September 2016 and March 2017, and comprised five physiological measurements of the heart rate at rest, vital capacity, systolic and diastolic blood pressure, and hemoglobin concentration in the blood, eighteen anthropometric parameters, and two motor tests on back strength and complex reaction speed. The aim of the study was to investigate whether U15 age group athletes participating in six different sports already at this age show a sport specific anthropometric, motor performance, and physiological profile which is in line with the specific requirements of each of the sports. A discriminant analysis and a Neural Network (Multilayer Perceptron) were used to test whether it is possible to discriminate between athletes of the six sports and to assign each individual of the Under-15 athletes to his own sport on the basis of a unique profile of the morphological, motor, and physiological prerequisites. All diagnostic methods exhibited medium to high validity to discriminate between the six different sports. The relevance of the eighteen body dimensions, five physiological measures, and two motor tests for talent identification was confirmed.

**Keywords:** talent, youth sport, discriminant analysis, neural network, test validity

## INTRODUCTION

Participation in elite sport training at youth age is associated with the selection of athletes with specific prerequisites and the development of the specific anthropometric, motor and physiological characteristics of a particular sport (Pion et al., 2015). For example, judo athletes at age U13 exhibit a higher sideward jumping ability compared to Karate and Taekwondo athletes (Pion et al., 2014). In fencing, Krishnan et al. (2017) could not find a superior broad jump ability in U21 fencers compared to weightlifters and wrestlers. In swimming, Bencke et al. (2002) found that male gymnasts showed better jumping abilities compared to swimmers, handball, and tennis players. Opstoel et al. (2015) also found in their study that U12 year old swimmers did not differ from ballsports players, dancers, gymnasts, martial arts and other sports participants in twelve different motor tests, although they were slowest in ball dribbling. In table tennis, Maehner (2018) found U15 players to be superior to soccer players in sideward jumping and push-ups. In volleyball, talented youth players are characterized by a higher stature as well as by a better jumping ability (Rikberg and Raudsepp, 2011). In basketball, at least female players exhibit a lower level of static and dynamic balance compared to gymnasts and soccer players, respectively (Bressel et al., 2007). On the basis of such findings, the discriminating sports specific characteristics can be recommended for talent identification purposes. But, as all studies mentioned above were executed with caucasian athletes from Europe, there is a lack of knowledge in regard to the make-up of Chinese youth athletes. To our knowledge this study is the first one comparing Chinese elite youth athletes from different sports in regard to sports specific characteristics. Thus, the purpose of this study was to investigate whether U15 age group athletes participating in six different sports under the condition of two daily training sessions in a Chinese elite sports school already at the U15 age show a sport specific anthropometric, motor performance, and physiological profile which is in line with the specific requirements of each of the particular sports, and which could serve as scientific knowledge background for sports specific talent identification purposes.

In long-term talent development programs, talent identification procedures include morphological measures and motor tests as well as physiological data. According to Pion et al. (2015), talent identification is related to homogeneous samples and aims at pinpointing the most promising young athletes to engage in long-term, elite sport training. Following this idea, several talent identification programs in elite sport schools have implemented morphological, motor, and physiological diagnostics (e. g., Hoare, 1995; Fuchslocher et al., 2011; Douglas, 2014; Kinugasa, 2014; Pion, 2015; etc.) to select or transfer young athletes into certain sport groups, such as combat sports, game sports, or endurance sports (Pion et al., 2014), and even more concrete to specific sports, like e.g., alpine skiing (Mueller et al., 2015). Talents in particular sport disciplines exhibit a specific make-up of natural abilities (nature) and well-developed performance prerequisites (nurture) (Pion et al., 2015). Therefore, the predictive validity of such talent characteristics is paramount when identifying promising youth athletes.

Although some academics warn against talent identification procedures that are conducted too early (Meylan et al., 2010), these procedures targeted at juveniles are worthwhile for the purpose of helping sport federations maintain focus on their resources regarding the most talented young athletes (Unnithan et al., 2012; Höner and Votteler, 2016).

Many sports are based on a complex, multi-dimensional performance profile (Buekers et al., 2015). Thus, the talent selection should be focused on a multifaceted variety of general physical, physiological, psychomotor, and psychological performance diagnostics (Williams and Franks, 1998; Williams and Reilly, 2000). In general, there is a lack of research investigating the discriminative value of different performance prerequisites over a range of different sport disciplines. Nevertheless, there were promising attempts to discriminate various sports by means of their profile of sports specific performance prerequisites. So, Leone et al. (2002) could distinguish 88% of athletes from four different sports (figure skating, swimming, tennis, and volleyball) by means of a discriminant analysis including anthropometric and motor characteristics. Opstoel et al. (2015) reported a correct classification of 85.2% of high active U12 athletes into their own sport (ball sports, dance, gymnastics, martial arts, raquet sports, and swimming). Also, Pion et al. (2015) could assign 96.4% of 141 adolescent Flemish athletes into nine different sports. Even more promising were the findings of Pion et al. (2014) in elite male U18 athletes, as the investigators found a 100% correct classification within the more interrelated martial arts disciplines judo, karate, and taekwondo. In contrast to the aforementioned studies, the discriminant analysis is less accurate, when a hold-out of one case ( $n = 1$ ) is used which has to be classified on the basis of the discriminant functions obtained from all other cases ( $n-1$ ). Using this cross-validation strategy in a discriminant analysis with 56 12–16 years old youth athletes from six different sports (water polo, volleyball, soccer, cross-country skiing, running sprint, and alpine skiing), Hohmann et al. (2015) reported a correct assignment of 76.8%. Using alternatively the neural network method multilayer perceptron (MLP) the authors reported a lower classification rate of 69.6%.

Thus, reliable and valid information regarding the potential of talented athletes in certain sports on the basis of morphological parameters, motor abilities and skills, and physiological diagnostics is a valuable tool in talent development programs for clubs and sport federations. The main reasons for these scientific uncertainties in talent orientation arise from the often-undifferentiated mixture of general as well as sport-specific tests in talent identification campaigns; the unsystematic timing of cross-sectional diagnostics at single points in time during the long-term athletic development process also contributes to the aforementioned uncertainty. Thus, it is not surprising that the great variety of study design parameters have led to inconsistent research results, providing an inconsistent picture with regard to the discriminative validity of talent features addressing general and sport-specific performance prerequisites. Therefore, for talent orientation, there is a need for a multifaceted test battery that allows the ability to distinguish between the specific skills/physical attributes necessary for various sports.

Although the prediction of long-term success is still debatable, the talent orientation method of recommending suitable sports to children in accordance with their individual talent make-up seems feasible (Pion et al., 2015). Thus, the general aim of this study is to discriminate elite adolescent male athletes from a Shanghai Elite Sport school from seven different sports by means of morphological, motor, and physiological tests. It was hypothesized that a generic test battery consisting of 25 diagnostic tests has enough discriminative validity to assign athletes to their own sport on the basis of their individual profile of test scores.

## MATERIALS AND METHODS

A sample of  $N = 97$  Under-15 and Under-16 youth athletes from six different sports (age:  $M = 178.2$  mon;  $SD = 6.9$ ;  $Min = 168$  mon;  $Max = 191$  mon) attending the ninth grade classes of the Shanghai sports school took part in this study (see **Supplementary Material**). All athletes take part in 1–2 daily training sessions which amount to more than 20 h total training time per week ( $M = 20.8$  h/w).

Due to the character of the Shanghai sport school as an institution that promotes peak performance athletes only, the athletes of the ten incorporated sports sections of the school had to be selected according to age and training history, so that the participants matching these criteria represent a resulting sample out of six sports only. As the track and field group consisted of athletes from six different disciplines (pole vault, long jump, high jump, hurdle sprint, running sprint, decathlon) it had to be cut out from the study. Three other disciplines (modern pentathlon, baseball, and badminton) did not comprise enough male athletes in the interesting U15 and U16 age group. The participants were recruited according to the ethical standards of the Shanghai University of Sports (SUS). Ethics approval and parental written informed consent was obtained from the participants of this study in accordance with the declaration of Helsinki. All athletes' parents were informed about this study protocol, which was outlined in an information letter. No data collection took place without parents' consent. All athletes were performing at a high level in their respective sport, representing China and/or the Shanghai province in international competitions.

### Measurements

The participants completed five physiological, eighteen morphological, and two motor tests that were administered by expert sport school staff members. All tests were conducted on the same day in both the gym and sport science laboratory on campus. The testing started at 10 a.m., and all athletes refrained from strenuous exercise one day prior to the test session.

### Morphological Characteristics

Body height (BH) and sitting height (SH) to the nearest 0.1 cm (Height Tester, Donghuateg Sports Apparatus Ltd, Beijing, China), arm span (AS), arm length (AL), leg length (LL), lower leg length (LLL), shoulder width (SW), crista width (CW) to the nearest 0.1 cm (Martin Ruler, Donghuateg Sports Apparatus

Ltd, Beijing, China), chest girth (CHG), calf girth (CAG), waist girth (WG), thigh circumference (TC), ankle circumference (AC) to the nearest 0.1 cm (Circumference ruler, Donghuateg Sports Apparatus Ltd, Beijing, China), Achilles tendon length (ATL) to the nearest 0.1 cm (Martin Ruler, Donghuateg Sports Apparatus Ltd, Beijing, China), subscapular angle (SA) to the nearest  $1.0^\circ$  (Protractor, Donghuateg Sports Apparatus Ltd, Beijing, China), abdomen skinfold thickness (AST), upper arm skinfold thickness (UAST) to the nearest 0.1 cm (Harpenden skinfold caliper, British Indicators, United Kingdom), and body weight to the nearest 0.1 kg (calibrated Seca Alpha 770) were measured according to standardized test prescriptions (Hawes and Martin, 2001; Stewart et al., 2011).

### Motor Characteristics

Maximal dynamic back strength (measured by power dead lift) and simple reaction time (ms; PsyTech Sports; Xinyi Electronic Technology Company, Shanghai, China) were tested by expert staff members from the elite sport school.

In basketball (Chaouachi et al., 2009), as well as in volleyball (Bunn et al., 2017) maximal dynamic back strength turned out to be a relevant predictor of sport performance. Also, in judo it was shown by Drid et al. (2015) that elite athletes exhibit a higher maximum dynamic strength in deadlift and squat testing than their subelite counterparts. In fencing (Turner et al., 2014) as well as in crawl sprint swimming (Morouço et al., 2011), the power of the squat movement is a relevant predictor for the lunge speed, and the swimming power, respectively. Although there was no report on the validity of back strength testing in table tennis, the high reliability of the dead lift test ( $ICC = 0.99$ ; Comfort and McMahon, 2015) allowed for the use of this measurement in all six sports of this study.

Before the dynamic back strength test, subjects performed a warm-up consisting of cycling and dynamic stretching. During the test the standardized procedures for the one repetition maximum (1RM) deadlift was followed (Hoffman, 2006). A low-intensity set of 5–10 repetitions was performed using 40–60% of the perceived 1RM. After a 1-minute rest, subjects performed a set of 2–3 repetitions at 60–80% of the perceived 1RM. Subsequently, subjects performed 3–5 maximal trials, followed by an assessment of 1RM deadlift strength.

Computerized measurements of simple reaction times show a sufficient reliability ( $ICC = 0.51$ ; Eckner et al., 2011). Although it is known that game sports athletes show shorter simple reaction times than non-athletes, there is only few evidence for the validity of a simple reaction time assessment to distinguish between different sports (Badau et al., 2018). In this study, it was assumed that at least in the games sports (basketball, volleyball, and table tennis) and in the combat sports (fencing and judo) participants might exhibit different levels of performance in the computer-based test of the single-choice reaction time, especially when compared to swimming.

In the simple reaction time assessment the test device was prepared to measure the time of a simple response to light stimulation. The subject sat in front of the test instrument, placed his right index finger on the button, and pressed the button when the red light was on. The measurement included 20

repetitions, and the average value was calculated and used for all further data analysis.

**Physiological Characteristics**

Resting heart rate (bpm; Polar H10 Heart rate sensor, Polar Electro Inc., Finland), vital capacity (ml; High precision digital electronic spirometer, Donghuateng Sports Apparatus Ltd, Beijing, China), hemoglobin mass (mg; HemoCue Hb 201; HemoCue AB, Angelholm, Sweden), and blood pressure (mmHg; HEM-1000, Blood Pressure Monitor, Omron Health Care Inc., Japan) were diagnosed by medical personnel of the Shanghai University of Sport.

The long-term training effect of a reduced heart rate at rest in elite endurance sports is well known (Wilmore and Costill, 1994), whereas up to now the adaptation of blood pressure parameters on sports performance was investigated primarily in strength sports, e.g., weightlifting (Dhamu et al. (2012). Generally, it is assumed that the training-induced decrease of the systolic blood pressure is more pronounced in weight training than in endurance sports (Hagberg, 1990).

Vital capacity only changes little with training, although water polo players exhibit higher amounts of air expelled after maximal inspiration than e.g., basketball, handball or soccer players (Durmic et al., 2015). Due to the innate character of this feature of the respiratory system, it might also be useful for the talent classification in the six sports investigated in this study.

Hemoglobin mass was also suggested for talent identification purposes (Eastwood et al., 2012) as it is not only relevant in endurance sports, but also could predict future success at least in young soccer players (Prommer et al., 2018).

**Statistical Analysis**

All data were analyzed with SPSS (Version 25.0; SPSS Inc., Chicago, IL, United States) and statistical significance was set at  $p < 0.05$ . All test data were collected from the ninth grade classes on September 30th, 2016. The discriminative validity of the eighteen morphological, two motor, and three physiological measures was determined using a classification of athletes by means of a linear discriminant analysis (DA) and a nonlinear neural network MLP. In both classification procedures, the six

sports served as the dependent grouping variable, whereas the test results were used as an independent variable set. The stepwise DA was based on the “leave-one-out” method. This means the classification of each individual was calculated using a function derived from all other cases without the single one case that was held out for final classification. Similarly, for the MLP analysis, three subsets were created for (i) training, (ii) testing of the predictive model, and (iii) the final classification of the left-out cases. Subsequently, the MLP was trained with 80% of all cases, whereas ten percent was used for testing the trained network. Finally, the classification was calculated for the hold-out of the remaining ten percent of cases. This specific type of leave-out strategy was repeated ten times so that each case should at least once belong to the left-out athletes that were finally classified. To quantify the validity of this talent identification strategy, the percentage of correct hits of the neural network classification was averaged over the ten trials and the mean value was used from there on. The classification quality of both methods was expressed by the proportion of correct hits, and was also classified as the percentage of athletes that were assigned as true positives to their own sport. An athlete was defined as false positive if he was classified as a participant of a specific sport for which he did not practice.

**RESULTS**

**Classification by Linear Discriminant Analysis and Nonlinear Neural Network**

In the DA, three cases of the fencers were sorted out due to missing data. In a first attempt, a DA with the remaining total sample of  $n = 94$  cases was calculated and a classification rate of 98.9% was obtained. In this analysis, only one table tennis player was assigned erroneously to judo. In a second attempt, a cross-validated DA was applied, where each of 94 athletes was iteratively used as a single hold-out case which has to be solely classified. On the basis of this leave-one-out procedure, 71.3% of all athletes were classified correctly and assigned as true positives to their own sport (**Table 1**). The best classification result of 85.0% correct hits was obtained in fencing, where only three

**TABLE 1** | Original and cross-validated classification of  $n = 94$  single cases of youth athletes from six different sports on the basis of the 25 performance characteristics.

		Fencing	Basketball	Volleyball	Table tennis	Judo	Swimming	Total	
Cross-validated	N	Fencing	17	0	2	1	0	0	20
		Basketball	4	2	1	0	0	0	7
		Volleyball	2	1	16	0	1	2	22
		Table tennis	2	0	1	11	1	0	15
		Judo	0	0	0	2	14	4	20
		Swimming	0	0	1	0	2	7	10
	%	Fencing	85,0	,0	10,0	5,0	,0	,0	100,0
		Basketball	57,1	28,6	14,3	,0	,0	,0	100,0
		Volleyball	9,1	4,5	72,7	,0	4,5	9,1	100,0
		Table tennis	13,3	,0	6,7	73,3	6,7	,0	100,0
		Judo	,0	,0	,0	10,0	70,0	20,0	100,0
		Swimming	,0	,0	10,0	,0	20,0	70,0	100,0

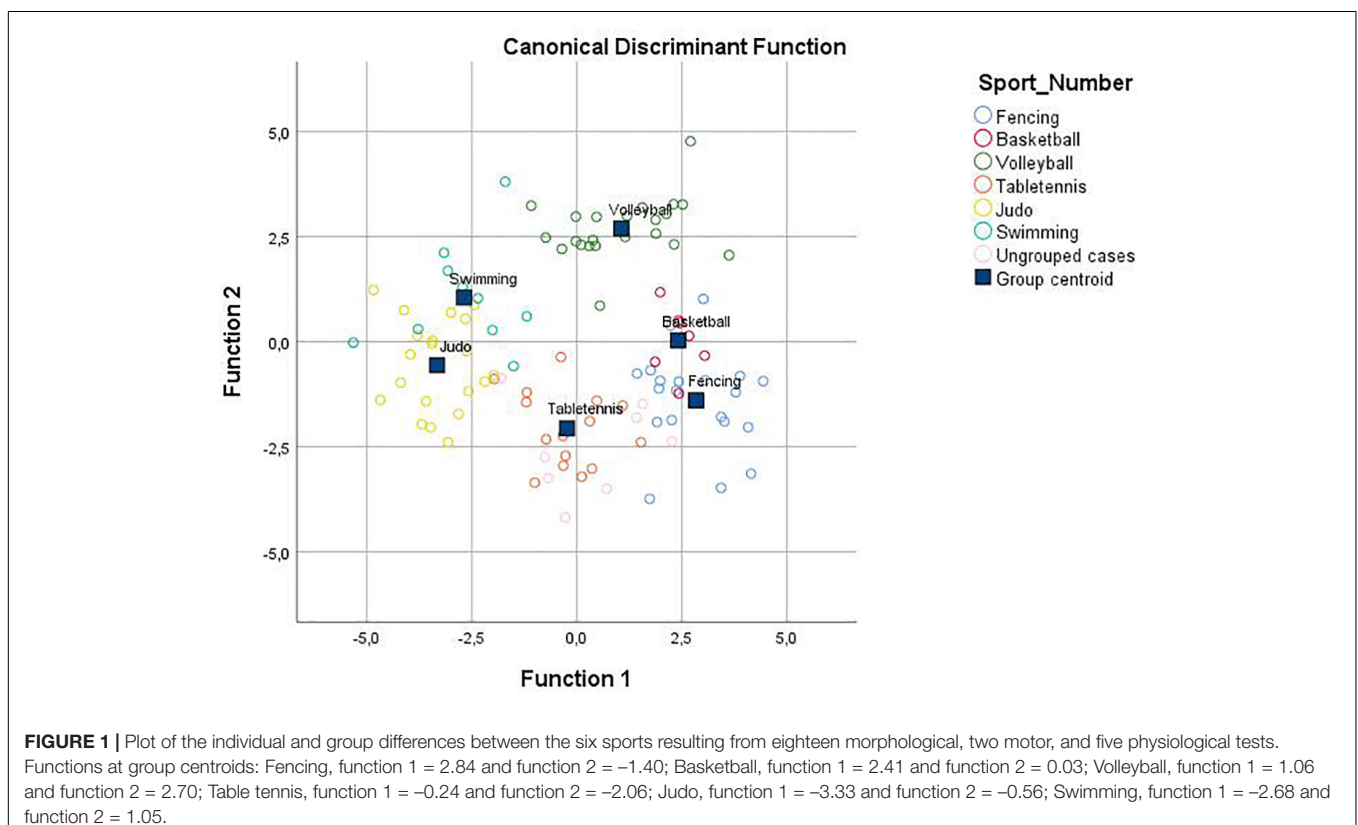
out of twenty athletes were collated as false negatives to another sport (two athletes to volleyball, one athlete to table tennis). The highest fraction of false negatives was found in basketball (28.6%), mostly due to an erroneous assignment of four (57.1%) youth basketballers to the fencing group, and one (14.3%) to the volleyball group.

Since there were six different sport groups, six linear discriminant functions were established. The first two functions accounted for 77.5% of the variance and are represented in **Figure 1** on the X- and Y-axes. The athletes from the six sport groups are distributed around their respective centroids, which are located on distinct areas of the plot. The first function (Eigenvalue: 5.92) was the most important, accounting for 50.4% of the variance and was related primarily to morphological body dimensions. The second function (Eigenvalue: 3.18) accounted for 27.1% of the variance.

In the neural network analysis, the same three cases of fencers with incomplete data were cut out. In accordance with the linear analysis described above, in a first attempt, the MLP was trained with all complete cases ( $n = 94$ ). According to the learning character of neural networks methods, the MLP also makes minimal assumptions in regard to relations within the data. Thus, the MLP is able to determine linear as well as nonlinear relationships by its iterative learning mechanism. Along the learning process of neural networks, the prediction result may partly depend on the start vector that is set in a random mode; consequently, the results of the MLP may vary slightly from repetition to repetition (Pion

et al., 2016). Therefore, all MLP analyses were repeated ten times to secure that the mean value of this series represents the overall quality of the results obtained by means of that neural network method.

The application of the nonlinear MLP in a first attempt was based on the total sample of  $n = 94$  athletes, and the ten trials led to an average classification rate of  $M = 99.3\%$ , indicating that on average 93 of 94 original cases were collated correctly in regard to their respective sport. In the row of the ten repetitions of the MLP analysis, on only three occasions were one, two, and three athletes assigned to any other sport; in the other seven repetitions, 100% correct hits were calculated. In a second attempt, a cross-validated MLP analysis was applied, where in each of the ten repetitions 80% of the 94 athletes were used as training data set to calibrate the network, ten percent of the cases were taken to test the network solution, and the remaining ten percent of cases served as a hold-out data set for the cross-validated prediction of the sport group in which these athletes performed. On the basis of this leave-ten-percent-out procedure, 71.0% of the 94 athletes were classified correctly and assigned as true positives to their own sport (**Table 2**). The best classification result of 83.4% correct hits were obtained in volleyball, where only an average four of 22 athletes were collated as false negatives to another sport (leaning toward judo and swimming). The smallest fraction of true positives was found in basketball (20.0%), mostly due to an erroneous assignment of the MLP of most youth basketballers to the fencing group and somewhat fewer to the volleyball group.





## Prioritization of Talent Characteristics for Each Sport

To analyze which particular talent features specifically distinguish between the participants of one specific sport and all other athletes, three stepwise DA and MLP analyses were calculated. The aim was to prioritize the most relevant talent characteristics of the respective single sport. In both analyses, only two groups were formed and served as the dichotomic dependent variable: one group of participants of the single sports discipline under investigation, and a second group of all other athletes from the remaining five sports. Descriptive statistics of the 25 variables

measured in the  $N = 97$  male athletes, which were included into the three stepwise DA and MLP analyses as documented in **Table 3**. Also, the mean values of the test age are reported, which do not differ systematically between the six sport groups ( $F_{5;91} = 1,90; p = 0.102$ ).

### Basketball

In the stepwise DA of the anthropometrical measures, it was shown that the youth basketball players differed from the rest of the sport groups in lower leg length ( $F = 15.41$  and  $p < 0.05$ ), shoulder width ( $F = 7.78$  and  $p < 0.05$ ), and thigh circumference

**TABLE 2** | Cross-validated classification results (mean value from ten repetitive calculations) from the nonlinear neural network analysis (multilayer perceptron).

	Basketball ( $M_{percent}$ )	Fencing ( $M_{percent}$ )	Judo ( $M_{percent}$ )	Swimming ( $M_{percent}$ )	Table tennis ( $M_{percent}$ )	Volleyball ( $M_{percent}$ )
Basketball ( $n = 7$ )	20.0					
Fencing ( $n = 23$ )		70.7				
Judo ( $n = 20$ )			80.0			
Swimming ( $n = 10$ )				37.5		
Table tennis ( $n = 15$ )					83.3	
Volleyball ( $n = 22$ )						83.4

**TABLE 3** | Morphological, motor, and physiological prerequisites of the athletes from six different sports.

	Basketball ( $n = 7$ )	Fencing ( $n = 20$ )	Judo ( $n = 20$ )	Swimming ( $n = 10$ )	Table tennis ( $n = 15$ )	Volleyball ( $n = 22$ )
Age (mon)	179.7 ± 6.7	179.1 ± 6.4	180.0 ± 6.6	172.8 ± 6.5	178.8 ± 7.5	177.0 ± 6.6
<b>Morphological characteristics</b>						
Body height (cm)	182.6 ± 5.5	178.9 ± 7.3	*177.7 ± 7.3	180.0 ± 7.1	170.7 ± 4.0	*192.4 ± 3.3
Sitting height (cm)	94.1 ± 2.4	*94.5 ± 3.9	93.6 ± 3.4	94.3 ± 3.6	90.4 ± 3.6	100.3 ± 2.8
Arm span (cm)	182.9 ± 6.8	179.7 ± 7.3	181.5 ± 7.6	184.3 ± 7.2	173.3 ± 3.9	195.7 ± 4.6
Arm length (cm)	78.1 ± 3.9	77.0 ± 3.4	77.6 ± 3.1	79.2 ± 3.9	74.5 ± 1.5	83.5 ± 2.1
Leg length (cm)	96.4 ± 3.9	93.0 ± 4.0	*92.6 ± 4.7	94.9 ± 6.3	88.3 ± 3.0	100.7 ± 2.5
Low leg length (cm)	*49.6 ± 2.7	47.6 ± 2.6	*47.7 ± 2.6	47.7 ± 2.1	§*44.3 ± 1.0	51.4 ± 1.1
Shoulder width (cm)	*37.7 ± 1.5	§*38.4 ± 1.5	40.3 ± 1.2	40.1 ± 1.8	38.5 ± 2.4	§*42.6 ± 1.3
Chest width (cm)	§28.2 ± 0.9	28.2 ± 1.7	28.0 ± 1.6	*27.0 ± 1.5	26.7 ± 0.8	*30.3 ± 1.6
Chest girth (cm)	81.6 ± 4.6	§*80.5 ± 4.3	*92.8 ± 6.4	§*90.3 ± 4.3	83.9 ± 5.4	91.0 ± 6.0
Calf girth (cm)	34.3 ± 0.9	36.5 ± 2.0	36.9 ± 2.5	*36.2 ± 1.7	35.0 ± 2.8	38.9 ± 3.5
Waist girth (cm)	67.6 ± 1.5	69.5 ± 3.2	78.2 ± 7.5	74.2 ± 5.9	72.1 ± 3.3	79.7 ± 7.3
Thigh circumference (cm)	*49.2 ± 1.4	*52.6 ± 3.3	56.7 ± 5.1	§*51.7 ± 3.3	51.8 ± 5.2	58.3 ± 4.6
Ankle circumference (cm)	21.7 ± 1.2	22.4 ± 1.2	*22.7 ± 1.7	21.9 ± 1.1	21.1 ± 1.2	§23.6 ± 1.8
Achilles tendon length (cm)	25.8 ± 2.1	*23.6 ± 1.4	23.0 ± 2.4	*25.4 ± 1.3	21.9 ± 2.9	*26.3 ± 1.2
Subscapular angle (deg)	6.4 ± 1.4	7.4 ± 1.2	§*11.4 ± 3.5	8.9 ± 3.0	6.9 ± 1.4	*9.7 ± 3.6
Abdomen skinfold thickness (mm)	7.7 ± 1.2	8.4 ± 2.4	15.2 ± 9.3	9.4 ± 4.5	9.7 ± 5.8	12.8 ± 7.3
Upper arm skinfold thickness (cm)	8.9 ± 0.9	9.3 ± 2.9	12.3 ± 4.6	8.6 ± 2.7	8.7 ± 3.7	11.0 ± 4.9
Body weight (kg)	59.4 ± 2.7	63.6 ± 7.6	73.4 ± 11.0	66.8 ± 9.5	60.5 ± 8.3	82.1 ± 10.5
<b>Motor characteristics</b>						
Dynamic back strength (kg)	§*88.7 ± 15.5	§*96.7 ± 15.3	§*123.3 ± 17.0	102.7 ± 12.6	§*94.8 ± 16.5	*114.7 ± 18.5
Simple-reaction time (ms)	217 ± 33	218 ± 24	236 ± 27	213 ± 29	233 ± 35	§230 ± 24
<b>Physiological characteristics</b>						
Resting heart rate (bpm)	67.7 ± 6.2	62.7 ± 6.2	*60.7 ± 7.3	65.1 ± 5.9	67.5 ± 4.7	65.0 ± 6.4
Vital capacity (ml)	4121 ± 295	§4290 ± 579	4420 ± 793	§*5071 ± 863	§*3823 ± 462	§*5067 ± 1114
Hemoglobin mass (mg)	130.6 ± 12.2	140, 1 ± 8.4	§*144.3 ± 7.1	138.5 ± 8.7	136.2 ± 11.0	§*128.5 ± 11.2
Blood pressure (systolic; mmHg)	115.3 ± 9.3	120.2 ± 10.3	117.2 ± 13.2	122.7 ± 7.3	119.3 ± 10.7	122.4 ± 11.7
Blood pressure (diastolic; mmHg)	66.4 ± 8.4	69.7 ± 6.8	*65.0 ± 8.4	§*63.0 ± 11.6	*72.5 ± 7.7	70.0 ± 8.2

\*Significant  $F$ -value in the stepwise DA; §Characteristics with an average of more than 80% normalized importance in the ten MLP repetitions.

( $F = 3.41$  and  $p < 0.05$ ). In contrast, the MLP identified the crista width (92.6% importance) wider in these individuals than in athletes from other sports. For motor characteristics, the DA, as well as the MLP, confirmed that dynamic back strength ( $F = 5.83$  and  $p < 0.05$ , and 90.4% resp.) was lower in the basketballers than in the other athletes. In regard to the physiological features, no parameter exhibited a higher standardized importance than 80%.

### Fencing

In the DA, five anthropometrical measures of fencers differed significantly from the total group of all other youth athletes from the remaining five sports. Sitting height ( $F = 20.45$  and  $p < 0.05$ ), shoulder width ( $F = 23.52$  and  $p < 0.05$ ), chest circumference ( $F = 38.31$  and  $p < 0.05$ ), thigh circumference ( $F = 13.79$  and  $p < 0.05$ ), and also Achilles tendon length were smaller in comparison to those of their counterparts belonging to other sports. The MLP analysis confirmed by a standardized importance of more than 80% that shoulder width (85.0%) and especially chest circumference (97.4%) in fencers are less developed than in athletes belonging to the other five sport disciplines. For motor characteristics, the DA, as well as the MLP, confirmed that dynamic back strength ( $F = 6.50$  and  $p < 0.05$ , and resp. 89.9%) is lower than in athletes from other sports. In the row of the physiological variables, the vital capacity (92.2% importance) was identified by the MLP as being less voluminous than vital capacity for other sports.

### Judo

In judo, the stepwise DA led to a comparably long list of significant anthropometrical variables that discriminated between judo athletes and the other youth athletes. Body height ( $F = 14.45$  and  $p < 0.05$ ), leg length ( $F = 15.84$  and  $p < 0.05$ ), and lower leg length ( $F = 25.03$  and  $p < 0.05$ ) were smaller than in athletes belonging to other sports. On the other hand, chest circumference ( $F = 16.46$  and  $p < 0.05$ ), ankle circumference ( $F = 6.16$  and  $p < 0.05$ ), and also subscapular angle ( $F = 4.78$  and  $p < 0.05$ ) turned out to be greater than those of their counterparts from other sports. The relevance of the subscapular angle is especially corroborated by the MLP analysis, which calculated a standardized importance of 89.7% for this body characteristic. In regard to the motor tests, the DA and MLP agreed that dynamic back strength distinguishes the most (99.5%) between judo athletes and the rest of the investigated total sample. For physiological measures, the stepwise DA identified a lower heart rate at rest, lower diastolic blood pressure, and higher hemoglobin mass as significantly different from the other sports. The high importance of hemoglobin mass (93.5%) was also confirmed by the nonlinear neural network method.

### Swimming

In swimming, the stepwise DA identified five anthropometrical features that distinguished between swimmers and all other youth athletes. On one hand, the crista width ( $F = 4.82$  and  $p < 0.05$ ) and thigh circumference ( $F = 20.62$  and  $p < 0.05$ ) were smaller, and on the other hand, the chest and calf circumference ( $F = 28.81$

and  $p < 0.05$ ;  $F = 8.51$  and  $p < 0.05$ ), as well as the Achilles tendon length ( $F = 6.42$  and  $p < 0.05$ ), were greater than in the athletes from other sports. The importance of the positive and negative differences in the chest (97.5%) and thigh circumference (90.0%) were underlined by the neural MLP analysis. Both motor characteristics did not show differences between the swimmers and the U15/U16 athletes from the other five sports. In the set of physiological parameters, the stepwise DA as well as the MLP both stressed the significant relevance of the higher vital capacity ( $F = 6.41$  and  $p < 0.05$ , and 96.3%) and the lower diastolic blood pressure ( $F = 5.15$  and  $p < 0.05$ , and 84.9%) in swimmers.

### Table Tennis

The stepwise DA in the table tennis players was in line with the result of the MLP as both methods identified shorter lower leg length ( $F = 36.74$  and  $p < 0.05$ , and 88.0% resp.) as the only one anthropometric variable that was significantly different from the body dimensions of other youth athletes. For motor characteristics, DA and MLP confirmed that dynamic back strength ( $F = 5.62$  and  $p < 0.05$ , and 94.8% resp.) in table tennis players is greater than in the athletes from other sports. Another noteworthy factor in the physiological variables is that both methods agreed that vital capacity ( $F = 11.41$  and  $p < 0.05$ , and 96.2% resp.) is smaller in table tennis athletes, whereas only the stepwise DA discovered that diastolic blood pressure was lower in these players.

### Volleyball

For the list of anthropometrical measures, the stepwise DA showed that youth volleyball players displayed greater body dimensions compared to the rest of the participants in regard to body height ( $F = 2.22$  and  $p < 0.05$ ), shoulder width ( $F = 13.86$  and  $p < 0.05$ ), crista width ( $F = 6.89$  and  $p < 0.05$ ), Achilles tendon length ( $F = 5.27$  and  $p < 0.05$ ), and subscapular angle ( $F = 3.93$  and  $p < 0.05$ ). The MLP confirmed greater shoulder width (83.9% importance) and reported that ankle circumference (84.4%) was also larger in the volleyballers. In regard to the motor diagnostics, the stepwise DA stressed significantly higher dynamic back strength ( $F = 6.19$  and  $p < 0.05$ ) in youth volleyballers, whereas the MLP stressed that youth volleyballers performed more slowly in the simple eye-hand-reaction test (84.9% standardized importance). Both linear and nonlinear methods furthermore revealed that in the set of physiological variables, vital capacity ( $F = 16.94$  and  $p < 0.05$ , and 83.4% resp.) as well as hemoglobin mass ( $F = 22.14$  and  $p < 0.05$ , and 88.0% resp.) were higher in the volleyballers compared to the remaining total group of elite youth athletes belonging to other sports.

## DISCUSSION

The main objective of this research was to discriminate between youth athletes from an elite sport school—many of whom will contribute to the next generation of elite senior athletes—belonging to the six researched sports in the Shanghai province. Therefore, a generic test battery of 25 physiological, anthropometric, and motor characteristics was administered to

97 young elite athletes. It should be highlighted that, in this study, linear and nonlinear statistical methods were parallelly used to identify the most relevant talent characteristics of each of the six sports and reversely confirm the results of each method. The main findings included the high discriminative validity of the generic test battery that allowed the original correct assignment of 98.9% of cases by means of the discriminant analysis. The quota of 98.9% correct assignments in the total group of 94 youth athletes is in line with the 96.4% reported by Pion et al. (2015) for a selection of nine different sports (badminton, basketball, gymnastics, handball, judo, soccer, table tennis, triathlon, and volleyball), and the 100% reached by Pion et al. (2014) in a classification analysis in the three martial arts, judo, karate, and taekwondo. Especially, when compared to the 88.0% classification rate of Leone et al. (2002) in a group of figure skaters, swimmers, tennis and volleyball players, our results seem to be quite high. Also Opstoel et al. (2015) reported a lower figure of 85.2% for a discriminant analysis in ball sport, dance, gymnastic, martial arts, racket sports, and swimming athletes. Our result of 71.3% obtained from the discriminant analysis by means of the leave-one-out validation strategy cannot be compared directly to the findings of the research groups mentioned above, as these researchers calculated the predictive accuracy only for the original sample including all members of the total groups. When compared to the 76.8% correct DA predictions for the left out participants reported in the study of Hohmann et al. (2015), our results are still satisfactory. The best classification results of 83.4% correct hits were obtained in volleyball, which is in line with Leone et al. (2002), who attributed this finding to the relevance of the anthropometric make-up in this particular sport. In the neural network analysis by means of the MLP tool and the use of the ten-percent-hold-out strategy, we discovered that 71.0% of classified correctly into their original sport. Although, that we expected that the nonlinear neural network method would allow for a higher classification rate than the linear discriminant analysis, the equality with the DA result is in line with the findings of Hohmann et al. (2015), who reported 69.6% correct predictions by means of the MLP analysis in German youth athletes of the same age group.

The comparison of our results of the linear and nonlinear classification of the Chinese youth elite athletes with the results from the aforementioned European studies shows that the athletic make-up of the participants from the Shanghai sport school does not seem to differ systematically from that of their European counterparts. This finding might be related to the homogenization effect of the sports-specific demands posed by the international competition system.

Furthermore, in our study there were still deficits in prediction accuracy, as the origin of about one third of the participants in regard to the six different sports could not be identified correctly. This finding might be attributed to the selection of the tests. In the past, talent identification for certain sports was based primarily on sport-specific testing, which made it difficult to compare results between different sports. Also, such sport-specific tests pose the problem that athletes from

one sport who are not familiar with the specific techniques and skills of an alternate sport cannot perform tests other than those specific to their own sport with a reliable and valid personal outcome. Therefore, it is paramount that talent identification programs consist of a multidisciplinary mixture of anthropometric, motor, psychological, or physiological testing methods of low specificity to allow for a more complete assessment of each athlete as well as between-sports comparisons (Pion et al., 2014, 2015, 2016). With this in mind, the results of this study provide substantial information about the validity of the anthropometric, motor, and physiological tests for the discrimination of young athletes from different sports; furthermore, we are provided with evidence for the applicability of the different measures for talent identification in the six sports investigated.

The combination of linear DA and artificial neural networks is a fruitful approach for resolving the problem of talent identification specifically when it is assumed that different types of talent patterns exist in the make-up of promising youngsters, which may lead to the same performance outcome in particular elite sports (Philippaerts et al., 2008; Pfeiffer and Hohmann, 2012; Pion et al., 2016; Till et al., 2016). As both methods on the basis of the original data set led to a quite similar and almost perfect quota of 98.9 (DA) and 99.3% (MLP) correct assignments to the six sport disciplines, both classification methods seem to establish an almost linear relationship between the 25 predictors and the six categories of sports. This assumption is corroborated by the results of the cross-validated attempts which also showed an almost identical overall quality of 71.3% (DA) and 71.0% (MLP) correct hits in the 94 individual cases. The only relevant difference between the results of the two analytical models was found in regard to the sport with the highest fraction of correct predictions. Whereas the DA identified 85.0% of the fencers correctly, the MLP was most precise in the volleyball group (83.4%). On the other hand, the very similar and rather poor prognostic results of both methods in the group of basketballers (28.6% for DA, 20.0% for MLP) may partly depend on the small sample size of only seven players; although, both predictions were still higher than the random quota (16.7%).

The results in regard to the importance of the single talent characteristics obtained in each particular sport are in agreement with previous research conducted on performance profiles in these respective sports.

## Physiological Characteristics

It is well-known that the heart rate at rest along with systolic and diastolic blood pressure are lower for endurance sports (Cornelissen et al., 2010). Moreover, within those sport categories, the reduction of blood pressure is more pronounced in disciplines with higher exercise intensities (Goldring et al., 2014). Thus, it is not surprising that, in our study, both the resting heart rate and resting diastolic blood pressure of judo participants contributed significantly to the discrimination of swimmers and judo athletes from those belonging to other sports.

In swimming and volleyball, vital capacity of youth athletes also turned out to be higher, which helped distinguish these

players from players belonging to other sports; fencing and table tennis groups could especially be identified by their lower values in this category.

The significant contribution of a higher hemoglobin mass to the classification of judo athletes and the discrimination of this group from all other study participants is somewhat unexpected because former research by Malczewska-Lenczowska et al. (2013) found that, when compared to endurance athletes, even elite judo athletes exhibit significantly lower values of hemoglobin mass related to body weight.

## Anthropometric Characteristics

The basic anthropometric features (body height and particular body segment lengths) have been proven relevant for youth expert athletes in basketball (Ziv and Lidor, 2009), swimming (Hohmann et al., 2018), and volleyball (Lidor et al., 2007).

The Achilles tendon length is positively related to the running economy (Hunter et al., 2011, 2015) and is also relevant for jumping power (Earp et al., 2011). Thus, it is not surprising that youth elite swimmers and volleyballers possessed longer tendons since in both sports a powerful jump-off from the start block (Rebutini et al., 2016; Amaro et al., 2017) and floor (Sattler et al., 2012; Battaglia et al., 2014), respectively, is a regular necessary action; additionally, a highly-exerted push-off against the wall in swimming contributes significantly to a swimmer's overall performance. Interestingly, swimmers and fencers exhibited a smaller thigh circumference, which helped discriminate them significantly from the total group of other athletes; this particular characteristic may be attributable to the reduced body weight of the participants belonging to both sports.

The inferior subscapular angle is influenced by the muscle volume of the upper body because it is attached to the m. teres major and covers the m. latissimus dorsi. In our study, this feature was larger in judo and volleyball athletes. In Judo, this result matches the findings of Drid et al. (2015), who pointed out that the subscapular angle is greater in elite judo competitors at the international level compared to sub-elites performing only at the national level.

## Motor Characteristics

Greater upper body muscle volume, which in both judo and volleyball corresponds with better performances in the back strength test, contributed to their differentiation from other sports. Greater strength prerequisites in judo athletes that support throwing, sweeping, and clamping actions during a fight were already reported by Franchini et al. (2011). These studies agree with our discovery that higher dynamic back muscle strength of the lumbar spine contributed significantly to the discrimination of judo and volleyball athletes from the other sport disciplines. In judo, this finding corroborates the early research of Kort and Hendriks (1992), who attributed this advantage to a more specific training regimen in elite judo cadres. In elite volleyball, back squat exercises contribute an important portion of the daily training routine with the aim of creating a

balanced trunk musculature and core stability, thus protecting tall volleyballers from the elevated risk of lower back pain (Ezechieli et al., 2013).

Research on single reaction time in sports is abundant, but due to different tasks, test protocols, and performance levels, the findings are hardly comparable. As a general result, one can confirm the assumption that elite athletes from most sport disciplines differ from non-athletes when performing generic, single, and elementary reaction tasks. This holds true in basketball (Bhabhor et al., 2013), judo (Badau et al., 2018), table tennis (Nakamoto and Mori, 2008), and volleyball (Kokubu et al., 2006), but maybe not in fencing—at least when compared to fencing novices as opposed to non-athletes from the normal population (Harmenberg et al., 1991). In elite swimmers, to our knowledge, generic single reaction time has not yet been compared to the untrained population. In conclusion, the finding of this study, which concludes longer single task reaction times significantly distinguished volleyballers from the other athletes, cannot be derived sufficiently from the existing literature and therefore needs further investigation.

Our study has several limitations. The first limitation is the relatively small sample size, especially in basketball ( $n = 7$ ) and swimming ( $n = 10$ ), which was a consequence of the superior quality of athletes in a Chinese elite sport school. All applicants undergo a strict sport-specific selection procedure at an early age, which implies that members of the investigated age group of 14–15 year-old male athletes cannot be numerous by definition. A second limitation is the focus solely on male youth athletes. Besides the even smaller numbers of female youth athletes in the Shanghai sports school, the focus on solely male athletes also resulted from the complexity of the study due to the different influence of the gender-specific athletic make-up on the sports-specific performances of male and female youth athletes in most of the sports disciplines under investigation.

## CONCLUSION

The results of this study reveal that in Under-15 and Under-16 male athletes from a Chinese elite sport school in Shanghai, the between-sports differences in a battery of generic anthropometric, motor, and physiological tests allow one to distinguish more than two out of three young athletes' talents according to their individual sport provenience, independent from the classification method (DA: 71.3%; MLP: 71.0%) used. Furthermore, the overall accuracy of the talent classification in the Chinese elite youth athletes corresponds to the level found in European studies. To allow for such kind of between-sports comparisons, it is necessary that talent identification programs consist of a multidisciplinary mixture of anthropometric, motor, psychological, or physiological testing methods of low specificity. The linear and nonlinear statistical methods that were used in parallel to identify the most relevant talent characteristics of each of the six sports by means of the leave-one-out procedure reversely confirmed the quality of the results. In regard to the relevance of the different sports-specific talent

characteristics for talent identification campaigns in the practical fields of the six sports, the applied talent classification strategies underlined the importance of superior stature measures solely in volleyball. Besides longer Achilles tendons in swimmers and volleyball players, a more pronounced chest circumference was found in swimmers and judo athletes. In regard to the motor characteristics, a high back strength turned out to be helpful to discriminate between the six sports. Notably in judo, table tennis, and volleyball players this athletic performance prerequisite was specifically important. In the series of the physiological characteristics, vital capacity was relevant in swimming and volleyball, and hemoglobin mass was elevated in judo and volleyball athletes. A lower heart rate at rest was detected in judo participants alone.

The limitation of the relatively small sample size and the focus on solely male athletes needs further investigations in the talent make-up of elite youth sport cadres. Furthermore, especially a greater variety of motor tests including also speed, endurance and flexibility tests in the youth athletes assessments were reasonable.

## ETHICS STATEMENT

This study was carried out in accordance with the recommendations of “Science Research Ethics Committee at the Shanghai University of Sport” with written informed consent from all subjects. All subjects gave written informed consent

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in accordance with the Declaration of Helsinki. The protocol was approved by the “Science Research Ethics Committee at the Shanghai University of Sport.”

## AUTHOR CONTRIBUTIONS

KZ was in charge of data collection in elite sports school. AH was in charge of data analysis. YC and BZ were in charge of testing in elite sports school. JP was in charge of revising the manuscript. BG was in charge of financial support and test organization.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fphys.2019.00405/full#supplementary-material>

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# Impaired Muscular Fat Metabolism in Juvenile Idiopathic Arthritis in Inactive Disease

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**Objectives:** The objective of this study was to evaluate muscular metabolic function in children with inactive juvenile idiopathic arthritis (JIA).

**Methods:** Fifteen children with inactive JIA and fifteen healthy controls were matched by sex, biological age, and Tanner stage. Participants completed a submaximal incremental exercise test to determine their fat and carbohydrate oxidation rates.

**Results:** Between the two groups, heart rate values and carbohydrate oxidation rates were the same, regardless of the relative intensity of exercise. Lipid oxidation rates were lower in JIA patients, regardless of the percentage of  $VO_2$  peak ( $p < 0.05$ ). Respiratory exchange ratios beyond 50% of  $VO_2$  peak were higher in patients with JIA ( $p < 0.05$ ). Respective maximal fat oxidation rates (MFO) for controls and children with JIA were  $218.7 \pm 92.2$  vs.  $157.5 \pm 65.9$   $mg \cdot min^{-1}$  ( $p = 0.03$ ) and  $4.9 \pm 1.9$  vs.  $3.4 \pm 1.2$   $mg \cdot min^{-1} \cdot kg^{-1}$  ( $p = 0.04$ ). There was no difference between the two groups in heart rate, percentage of  $VO_2$  peak, or power of exercise to achieve MFO. Controls reached their MFO at an exercise power significantly higher than did JIA subjects ( $42.8 \pm 16.8$  and  $31.9 \pm 9.8$  W,  $p = 0.004$ ).

**Conclusion:** Children with JIA show metabolic disturbance during exercise, even when the disease is considered inactive. This disturbance is seen in a lower lipid oxidation rate during submaximal exercise.

**Keywords:** pediatric, physical activity, inflammation, fat oxidation, metabolism

## INTRODUCTION

In juvenile idiopathic arthritis (JIA), the most common rheumatoid disorder in pediatrics, an elevation of proinflammatory cytokines, such as IL-1 $\beta$ , IL-6, IL-8, or TNF- $\alpha$ , has been demonstrated in both the serum and synovial fluid of patients with JIA (Gorczyca et al., 2017). The highest cytokine levels were usually observed in the active phase of the disease, but they also stayed high



in the clinical remission state (de Jager et al., 2007; Macaubas et al., 2009). However, the levels of these inflammatory markers are not used as a criterion for inactivity, which is mostly inferred from a defined set of clinical observations (Wallace et al., 2011).

Despite progress in anti-inflammatory treatments designed to target certain pro-inflammatory cytokines, including TNF- $\alpha$  (etanercept, adalimumab, infliximab), only half of patients achieve full and permanent remission (Bohr et al., 2016). Children and adolescents with chronic rheumatoid diseases are thus less physically active because of pain, joint limitations induced by their disease, and increased fatigue (Bohr et al., 2015; Armbrust et al., 2016; Bos et al., 2016). This behavior is accentuated during the active phases of the disease, but also persists in patients who are controlled and in those with inactive disease. The physical abilities of these children are thus impaired, and the literature reports atrophy and muscle weakness. They also have reduced aerobic and anaerobic abilities compared to healthy children (Roth et al., 2004; Lelieveld et al., 2007; van Brussel et al., 2007; van Pelt et al., 2012; Hulsegege et al., 2014).

The anti-inflammatory treatments used to control JIA have observable adverse effects, such as dyslipidemia, a major risk factor for the development of atherosclerosis (Yeh et al., 2014; Kraakman et al., 2016). Proinflammatory cytokines that are involved in JIA, like TNF- $\alpha$ , also play a significant role in tissue insulin resistance, lipid metabolism, and muscle health (Wu and Ballantyne, 2017). However, impaired metabolic control of lipid and glucose homeostasis predisposes an individual to cardiometabolic diseases (Fernández-Hernando et al., 2013). Children with JIA therefore form a population at increased risk of developing cardiovascular disease in adulthood.

As evaluated by calorimetry, maximal fat oxidation rate during incremental exercise is tightly correlated with mitochondrial function and might be a marker of metabolic fitness (Brun et al., 2012). An ability to oxidize lipids during exercise likely reflects a profile of metabolic fitness, which is correlated with the physiological status of muscles (Brun et al., 2012). In a context of chronic inflammation and physical deconditioning, children and adolescents with JIA should present impaired oxidation of energy substrates during physical exercise compared to healthy children. This would result in reduced lipid oxidation and prompter carbohydrate oxidation compared to children with no inflammatory disorder.

To evaluate metabolic responses, we used an indirect calorimetry test to evaluate substrate oxidation during submaximal incremental exercise in healthy sex- and age-matched peers compared to children with JIA who had not been treated with anti-TNF- $\alpha$  and who were in clinical remission according to the criteria of Wallace et al.

## MATERIALS AND METHODS

### Patients

Fifteen children aged 7–18 years with inactive JIA (according to the International League of Associations for Rheumatology criteria) and fifteen healthy controls were enrolled in the study. Inactive disease status was evaluated according to the American

College of Rheumatology's criteria (Wallace et al., 2011). Subjects in the two groups were matched for age, pubertal stage (according to the Tanner stage), and sex.

Subjects were excluded if they had a physician-diagnosed infection, had received oral corticosteroids within the previous 3 months, or had received anti-TNF- $\alpha$  blockade treatment in the past. All other treatments had been administered for at least 3 months at the time of evaluation. Physical activity level (PAL) was determined according to the International Physical Activity Questionnaire for Adolescents (IPAQ-A) (Ottevaere et al., 2011), and individuals were classified into one of three levels of physical activity (PA): low, moderate, or high.

This study was carried out in accordance with the recommendations of the Comité de Protection des Personnes (CPP) Sud-Est VI, with written informed consent given by all subjects in compliance with the Declaration of Helsinki. The protocol was approved by the Comité de Protection des Personnes (CPP) Sud-Est VI (clinical trial number NCT 02977416). All participants and their parents gave their informed consent and were free to withdraw from the study at any time. All the subjects were followed between January 2017 and March 2018 at the pediatric unit of the Clermont-Ferrand University Hospital, France.

### Experimental Procedure

Participants were asked to refrain from consuming any food or liquid other than water in the 3 h before the visit. They also avoided calorie-rich food and refrained from strenuous physical activity for at least 24 h beforehand. After sitting quietly for 20 min, the subjects performed, to the point of volitional fatigue, a graded exercise test on an electromagnetically braked cycle ergometer with continuous gas collection and heart rate monitoring. Following a 2-min warm-up consisting of unloaded pedaling, subjects at Tanner stages 1 and 2 started cycling at 10 W, and their work rate was increased by 10 W every 3 min. Subjects at Tanner stages 3 and 4 started at 20 W, and their work rate was increased by 15 W every 3 min. When heart rate was unstable, this stage was extended for up to 5 min to obtain a stable heart rate to within  $\pm 5$  beats. When the respiratory exchange ratio (RER) was greater than or equal to 1.00 – indicating the absence of fat oxidation – work rate was increased by the same increments at 1-min intervals until volitional fatigue was reached. The  $\text{VO}_2$  peak was considered to have been reached when the RER was greater than or equal to 1.05 and the subject achieved his or her age-predicted maximal heart rate ( $\text{HR}_{\text{max}}$ :  $220 - \text{age}$ ), according to the methodology validated by Riddell et al. (2008).

### Measurements

All the tests were performed on a Cyclus 2 ergometer (RBM Elektronik-Automation GmbH, Leipzig, Germany).  $\text{O}_2$  consumption ( $\text{VO}_2$ ) and  $\text{CO}_2$  production ( $\text{VCO}_2$ ) were measured breath by breath through a mask connected to an  $\text{O}_2$  and  $\text{CO}_2$  analyzer (MetaMax 3b, Cortex Biophysik, Leipzig, Germany).

Ventilatory parameters were averaged every minute during the submaximal exercise test and the subsequent 10-min recovery

period. Heart rate was monitored continuously throughout the duration of the tests (Polar RS800cx monitor, Polar, Finland).

## Data Analysis

Indirect calorimetry is the recognized standard method to quantify substrate oxidation rates at rest and during exercise (Frayn, 1983). The  $\text{VO}_2$  and  $\text{VCO}_2$  values were averaged over the last minute of each work rate, the results then being used to calculate fat oxidation over a wide range of exercise intensities for each subject (Achten et al., 2002) using Péronnet and Massicotte's equation (Péronnet and Massicotte, 1991): lipids ( $\text{mg} \cdot \text{min}^{-1}$ ) =  $1.6946 \times \text{VO}_2 - 1.7012 \times \text{VCO}_2$ ; carbohydrate (CHO) ( $\text{mg} \cdot \text{min}^{-1}$ ) =  $4.585 \text{VCO}_2 - 3.2255 \text{VO}_2$ .

For each individual, a best-fit polynomial curve was constructed for fat and CHO oxidation rate (expressed as  $\text{mg} \cdot \text{min}^{-1}$ ) vs. exercise intensity (expressed as a percentage of the  $\text{VO}_2$  peak). Each individual curve was then used to determine the peak fat oxidation rate and the exercise intensity associated with the maximal fat oxidation (MFO) rate (Achten et al., 2002).

## Statistical Considerations

Sample size was estimated according to (i) the CONSORT 2010 statement, extension to randomized pilot and feasibility trials (Eldridge et al., 2016) and (ii) Cohen's recommendations (Statistical Power Analysis for the Behavioral Sciences, 2nd ed., NJ, United States, Lawrence Erlbaum, 1988), which define effect-size bounds as follows: small (ES: 0.2), medium (ES: 0.5) and large (ES: 0.8, "grossly perceptible and therefore large"). With 15 patients per group, we have an effect-size of around 1 for a two-sided type I error at 5% and a statistical power at 80%, with respect to the primary endpoint, namely maximal lipid oxidation (MFO) rate. Statistical analyses were performed using Stata software version 13 (StataCorp., College Station, TX, United States). Tests were two-sided with the type-I error set at 5%. The continuous data were expressed as mean  $\pm$  standard deviation (SD) or as median (interquartile range) according to the statistical distribution. Assumption of normality was assessed with the Shapiro-Wilk test. Comparisons between groups (children with JIA vs. healthy controls) concerning the non-repeated quantitative parameters (age, body mass index, disease duration,  $\text{VO}_2$  peak,  $\text{VO}_2$  peak  $\cdot \text{kg}^{-1}$  of body weight, MFO) were performed using the Student *t*-test or the Mann-Whitney test when assumptions required for the *t*-test were not met. Homoscedasticity was analyzed using the Fisher-Snedecor test. To account for the between- and within-patient variability due to several measures being taken for the same subject, random-effects models for the correlated data were then run rather than the usual statistical tests, as these would have been inappropriate due to an unverified assumption of independence. Time-point evaluations, the group of subjects (children with JIA vs. healthy controls), and their interactions were considered as fixed effects. The subject was considered a random effect (slope and intercept). A Sidak *post hoc* test was applied to correct the type-I error due to multiple comparisons. The normality of the residuals from these models was studied as described above using the Shapiro-Wilk test. When appropriate, the data were log-transformed to achieve normality of the dependent endpoint.

## RESULTS

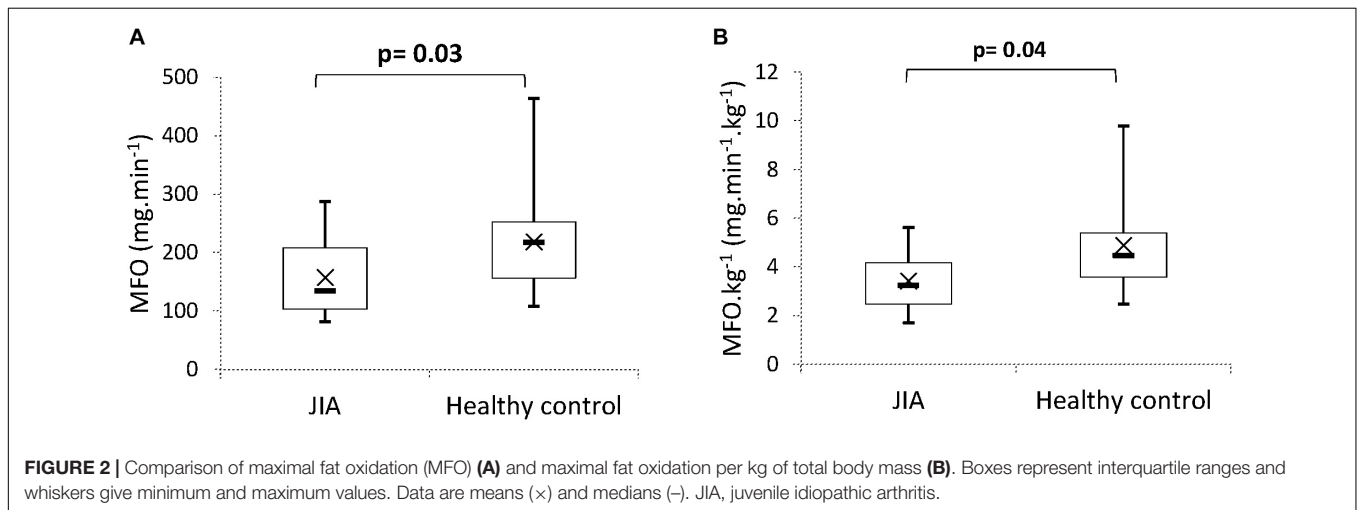
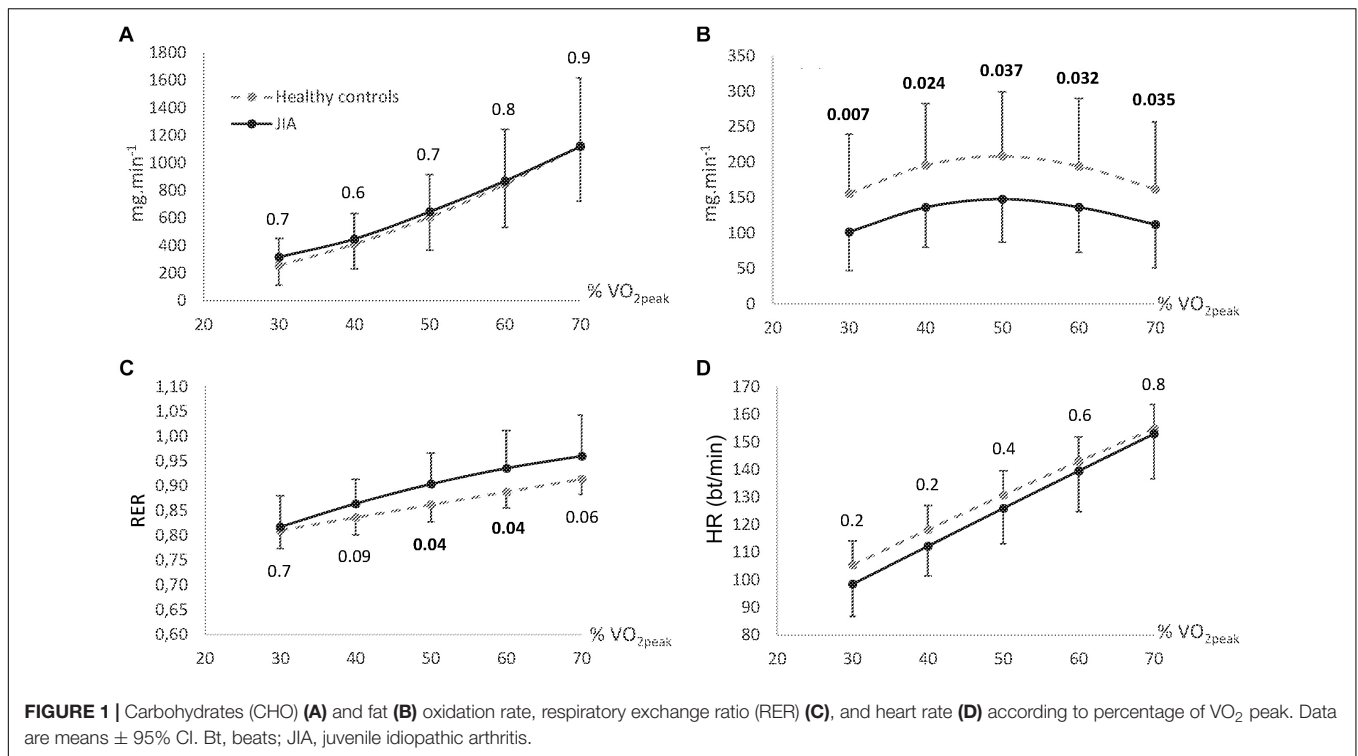
The subjects' characteristics are summarized in **Table 1**. There was no significant difference between our patients with JIA and our controls in terms of BMI,  $\text{VO}_2$  peak,  $\text{VO}_2$  peak per kilogram of body weight, rest metabolism, or physical activity level. In patients with JIA, disease duration was  $72.8 \pm 48.6$  months. Of the 15 patients, only one was treated with NSAIDs, six with methotrexate.

The oxidation rates of lipids and carbohydrates as a function of the percentage of  $\text{VO}_2$  peak are shown in **Figure 1**. For exercise intensities corresponding to the same percentages of  $\text{VO}_2$  peak, the carbohydrate oxidation rate was the same in the two groups (**Figure 1A**). However, lipid oxidation rates were statistically lower from the exercise intensities corresponding to 30% of  $\text{VO}_2$  peak up to those corresponding to 70% of  $\text{VO}_2$  peak (**Figure 1B**). Regarding the respiratory exchange ratio (RER), the intensities corresponding to 30 and 40%  $\text{VO}_2$  peak showed no difference between the two groups ( $p = 0.7$  and  $0.09$ ) (**Figure 1C**). By contrast, beyond 50% of  $\text{VO}_2$  peak, there was a statistically

**TABLE 1** | Participants' characteristics.

	JIA	Healthy controls	<i>p</i>
<i>n</i>	15	15	
Sex ( <i>n</i> ; male/female)	3/12	3/12	
Age (years) mean $\pm$ SD	13.7 $\pm$ 3.3	13.4 $\pm$ 3.5	0.85
Tanner stage ( <i>n</i> ; I-II/III-IV)	5/10	5/10	
Body mass (kg) mean $\pm$ SD	47.4 $\pm$ 13.3	47.0 $\pm$ 14.4	0.94
Height (cm) mean $\pm$ SD	155.7 $\pm$ 15.7	153.3 $\pm$ 16.4	0.70
BMI ( $\text{kg}/\text{m}^2$ ) mean $\pm$ SD	19.1 $\pm$ 2.7	19.4 $\pm$ 3.1	0.74
JIA subtype ( <i>n</i> )			
oJIA	8	–	
pJIA RF–	3	–	
ERA	2	–	
psoriatic	1	–	
Undifferentiated	1	–	
Disease duration (months) mean $\pm$ SD	72.8 $\pm$ 48.6	–	
DMARDs ( <i>n</i> )			
NSAIDs	1	–	
MTX	6	–	
IPAQ score ( <i>n</i> )			
Low level of physical activity	3	3	0.17
Moderate level of physical activity	7	6	
High level of physical activity	5	6	
$\text{VO}_2$ peak ( $\text{ml}/\text{min}$ ) mean $\pm$ SD	1486.0 $\pm$ 523.2	1695.5 $\pm$ 622.3	0.45
$\text{VO}_2$ peak/body mass ( $\text{ml}/\text{kg}/\text{min}$ ) mean $\pm$ SD	32.1 $\pm$ 7.9	36.7 $\pm$ 8.5	0.13
Rest metabolism ( $\text{kcal}/\text{day}$ ) mean $\pm$ SD	1560.3 $\pm$ 367.2	1885.5 $\pm$ 566.7	0.11

JIA, juvenile idiopathic arthritis; oJIA, oligoarticular JIA; pJIA RF–, rheumatoid factor-negative (RF–) polyarticular JIA; ERA, enthesitis-related arthritis; psoriatic, psoriatic JIA; DMARDs, disease-modifying antirheumatic drugs; MTX, methotrexate; NSAIDs, non-steroidal anti-inflammatory drugs; IPAQ, International Physical Activity Questionnaire.



significant increase in the value of the RER in patients with JIA. Finally, the heart rate values were the same for the two groups, regardless of the intensity of exercise (Figure 1D).

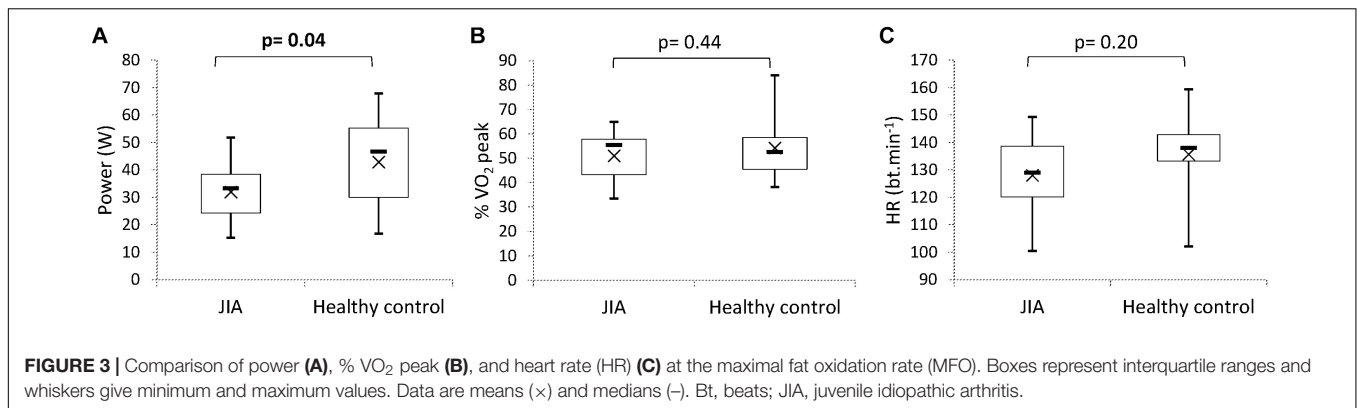
The maximal lipid oxidation rate (MFO) was significantly different between the two groups. For controls and children with JIA, the respective MFO was: 218.7 ± 92.2 vs. 157.5 ± 65.9 mg · min<sup>-1</sup> (*p* = 0.03) and 4.9 ± 1.9 vs. 3.4 ± 1.2 mg · min<sup>-1</sup> · kg<sup>-1</sup> (*p* = 0.04) (Figure 2).

Heart rate, percentage of VO<sub>2</sub> peak, and power of exercise to achieve MFO are shown in Figure 3. Between controls and patients with JIA, there was no difference in heart rate (respectively, 135.7 ± 15.2 and 128.1 ± 13.4 beats · min<sup>-1</sup>, *p* = 0.2, Cohen's *d* = 0.5) or percentage of VO<sub>2</sub> peak

(respectively, 54.3 ± 12.2 and 51.1 ± 9.4%, *p* = 0.4, Cohen's *d* = 0.2) to reach MFO. However, controls achieved a significantly higher MFO at exercise power than subjects with JIA (42.8 ± 16.8 and 31.9 ± 9.8 W, respectively, *p* = 0.004).

## DISCUSSION

Even when the disease was considered inactive, children with JIA showed a metabolic disturbance during submaximal exercise, with lower lipid oxidation rates than controls, regardless of exercise intensity, while there was no difference in carbohydrate oxidation rates. These observations imply a lower contribution



from lipids to meeting energy demand, reflected by high RER values at low relative exercise intensities, which will result in greater fatigability in these children. The lipid oxidation system seems limited and insufficient to provide the required energy, and the carbohydrate proportion then becomes predominant at relatively low exercise intensities (40–50% VO<sub>2</sub> peak). Consequently, we can expect patients with JIA to be more easily fatigable than healthy children and be unable to maintain high-intensity exercise. One of the limiting factors in lipid oxidation is the rapid turnover of enzymes involved in  $\beta$ -oxidation (Jeppesen and Kiens, 2012). The impaired energy metabolism in these children may be the consequence of mitochondrial dysfunction. Although this phenomenon has been studied only in systemic JIA, two teams have demonstrated a modification of mitochondrial gene expression that suggests that systemic JIA is not only an immunological disease, but also a metabolic disease involving mitochondrial disorders (Ishikawa et al., 2009; Omoyinmi et al., 2016). Whether this mitochondrial involvement is specific to systemic JIA or a non-specific systemic inflammation needs to be addressed. If it is a non-reversible physiological state involving dysregulation of signaling pathways or molecular functional changes (i.e., transporters, enzymes, etc.), this would imply that the metabolic impairment is constitutional in JIA. The question arises of whether the impaired energy metabolism is consecutive to chronic inflammation and deconditioning or part of the pathophysiology of this autoimmune disease. Since we matched the physical activity levels of our patients with JIA to those of controls, this impairment is likely due to the disease. A longitudinal follow-up of the oxidative profile of substrates from the diagnosis and comparison with other inflammatory diseases could help answer this question.

Half of our patients were still on medication (one being treated by non-steroidal anti-inflammatory drugs [NSAIDs] and six by methotrexate), and these treatments have a potential impact on energy metabolism. However, our results conflict with *in vitro* data that showed that methotrexate, by activation of AMPK, enhances lipid oxidation and glucose uptake in skeletal muscle (Pirkmajer et al., 2015). Nevertheless, in the profiles of the patients who were not under treatment, lipid oxidation rates remained lower than those of their paired controls [for controls and children with JIA not under treatment, the respective MFO  $\cdot$  kg<sup>-1</sup> was  $5.5 \pm 2.0$  vs.  $3.5 \pm 1.1$  mg  $\cdot$  min<sup>-1</sup> ( $p = 0.02$ ), **Supplementary Table 1**]. Furthermore, compared to the controls, children with JIA reached lower exercise powers for the same exercise intensities relative to VO<sub>2</sub> peak. This may be a reflection of lower muscle energy efficiency, lack of motor unit recruitment or autonomic nervous system dysfunction (Kuis et al., 1996). These results may also be related to insulin resistance induced by low-grade chronic inflammation and in particular higher levels of TNF- $\alpha$ . Skeletal muscle is an insulin-sensitive organ that plays a crucial role in maintaining systemic glucose homeostasis (Carnagarin et al., 2015). Inflammation and insulin resistance are closely related, and inflammatory cytokines such as TNF- $\alpha$ , IL-6, IL-1, and IL-8 may inhibit insulin signaling via multiple mechanisms (Wellen and Hotamisligil, 2005). However, in young JIA patients, there is no documentation of glucose intolerance or an increased HOMA insulin resistance index (Homeostasis Model Assessment of insulin resistance), a strong predictor for reduced glucose tolerance (Sinha et al., 2002). Nonetheless, when JIA is associated with obesity, it has been reported that the HOMA index increases (da Silva et al., 2010; Głowińska-Olszewska et al., 2013). Despite a high rate of inflammation, it can be assumed that adipocyte lipolysis is not different between our two groups and that it allows maintenance of muscular insulin sensitivity, and so does not adversely affect carbohydrate metabolism (Girousse et al., 2013). In line with our results, there is no difference in the carbohydrate oxidation rate, so there seems to be no impairment of glucose metabolism in the subjects of our study.

Finally, it is possible that this metabolic involvement results from an infra-clinical inflammation, which would mean that the criteria for defining an inactive disease are not optimal. As defined by Wallace et al. (2011), the criteria for an inactive disease are: no joints with active arthritis; no fever, rash, serositis, splenomegaly, or generalized lymphadenopathy attributable to JIA; no active uveitis; C-reactive protein (CRP) or erythrocyte sedimentation rate (ESR) level within normal limits; duration of morning stiffness less than 15 min; and a physician's global assessment of disease activity score of best possible on the scale used. Only one biological criterion is thus used to define the activity of the disease; other biological biomarkers (such as inflammatory cytokine levels), might thus be relevant in the absence of clinical signs.

Another possibility is that these children have subclinical inflammation and that the criteria used for inactivity of the disease do not reflect any real remission state. The exploration of these children's energy metabolism during exercise could therefore be an indicator of subclinical activity of the disease.

However, impaired lipid metabolism may be the whole body's adaptation to a state of less activity and low energy expenditure resulting from the low level of physical activity of children with JIA (Bohr et al., 2015; Bos et al., 2016). Lipid oxidation capacity is related to physical condition, itself related to levels of physical activity. In this case, the system could be remobilized by a physical activity program. Chronic physical activity is associated with an increase in the proportion of oxidized lipids during exercise and an improvement in the mitochondrial enzymatic capacities involved in  $\beta$ -oxidation (Tarnopolsky et al., 2007). Ongoing physical activity is also associated with an increase in insulin sensitivity because physical activity improves glucose transport and increases the expression or activity of entities involved in insulin-signaling pathways, such as protein B kinase (Akt) or AMPK (Benatti et al., 2018). Accordingly, prescription of regular physical activity could help correct this energy metabolism disturbance.

The limits of this work arise from the small size of the study population and the heterogeneity of this disorder in terms of subtypes and treatments used. More accurate data on body composition (assessed by dual-energy X-ray absorptiometry), physical activity levels (evaluated by actimetry), and inflammation (especially TNF- $\alpha$  and IL-6 levels) would need to be collected. In addition, data on glycemic and lipidemic blood markers could have told us whether any of our patients had dyslipidemia or an impairment of metabolic flexibility.

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## CONCLUSION

Our results show that even when the disease is considered inactive, muscular metabolism is disturbed, suggesting a functional impairment at the muscle level in children with JIA. This impairment could have a long-term impact on the health of these children, especially for their cardiovascular system.

## AUTHOR CONTRIBUTIONS

ER wrote the manuscript. PD and ER drafted the study design. ER and PB conducted the data acquisition and analysis. SE and EM performed the clinical assessments and data acquisition. BP performed the statistical analysis. PD and EM supervised the project. All authors contributed to the manuscript revision and read and approved the submitted version.

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# The Way to Increase the Motor and Sport Competence Among Children: The Contextualized Sport Alphabetization Model

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There is a concern to implement games that will be able to increase the students' *motor and sport competence* during the sport contents in Physical Education. Some games encompassed in Models-Based Practice (MsBP) are more beneficial for physical and physiological development than others. The main purpose of this study is to compare the degree of physical and physiological performance in several futsal games that have been implemented through two MsBP: the *Teaching Games for Understanding* (TGfU) and the *Contextualized Sport Alphabetization Model* (CSAM). The second objective is to analyze the relationship between physical and physiological variables. A quasi-experimental and cross-sectional study with pre- and post-test evaluations had been carried out. The sample was composed of 112 Primary Education students from First to Sixth grade ( $9.35 \pm 1.76$  years). Polar Team Pro® technology was implemented to compare and analyze the physical and physiological variables. Data was analyze comparing both models with a two-step cluster model. Afterward, Student's *t*-test was executed to compare the progression of both models. Besides, two-level multilevel model (MANOVA-ANOVA, followed by MANCOVA- ANCOVA) were also executed by means of applying a 4 versus 4 Small-Sided and Conditioned Game (SSCG). Finally, Pearson correlation between physical and physiological variables was calculated. Results showed that physical and physiological performance was higher in CSAM groups. In this regard, throughout the intervention of both models, results showed significant differences in physical and physiological variables at SSCGs implemented in the CSAM over the games implemented during the TGfU. Additionally, multilevel and MANCOVA post-test analyses shows significant differences in the physical and physiological performance during the post-test 4 vs. 4 SSCG at the CSAM students, in contrast to the TGfU students ( $p < 0.001$ ). These results demonstrate that both physical (e.g., distance covered) and physiological performance (e.g., Edwards' TRIMP) are significantly higher during CSAM in contrast to TGfU. Moreover, relationship between physical and physiological variables help teachers to adapt sessions to the features of the context.

**Keywords:** Small-Sided and Conditioned Games (SSCGs), physiological performance and education, child physical development, futsal, sport literacy, Models-Based Practice (MsBP)

## INTRODUCTION

There is a concern about the acquisition of fundamental motor skills that enables children to participate in physical activities satisfactorily (Barela, 2013). Indeed, the majority of curriculums around the world establishes that Physical Education (PE) is the most important subject that facilitate the development of the *motor competence* at the early childhood (from 6 to 12 years old). According to Holfelder and Schott (2014), this competence is defined as the capacity of executing a coordinated wide range of gross and fine motor skills. In fact, *motor competence* is closely related to *perceived motor competence*, which is the self-physical concept that inference in the level of physical activity (Utesch et al., 2018).

Particularly, PE has an important curricular part focused on the introductory stage of sports alphabetization learning or sports literacy (Kirk et al., 2006). Apart from the *motor competence*, the sports literacy contents also contribute to the acquisition of a specific competence, which enables students to solve a wide range of tactical/technical problems during their sports practice, called *sports competence* (Kolovelonis and Goudas, 2018). Nevertheless, pedagogical strategies are needed in both PE and extracurricular context to design suitable lesson plans or sessions to consolidate the tactical/technical elements, which included decision-making and skill-execution, respectively (Metzler, 2017). Therefore, rooted in the ideas of implementing Pedagogical Models (Haerens et al., 2011), whichever highlight the interdependence of the most important aspects of sport pedagogy (i.e., context, content, and teaching/learning process), Casey and MacPhail (2018) proposed the term Models-Based Practice (MsBP). This new concept aims to guarantee a contextualized and meaningful sport learning based on real practice.

On top of that, in spite of the fact that MsBP shared common features, such as the acquisition of *motor and sport competences*, they are divided according to their specific contents and didactic strategies that reinforce important aspects of the sport literacy. Hence, MsBP based on the teaching tactical/technical intelligence of the game, such as the Teaching Games for Understanding (TGfU; Morales-Belando et al., 2018), are included in the Games-Centred Approach (GCA; Harvey and Jarrett, 2014). According to Pill (2016), this framework is mainly composed by some modifications of Small-Sided and Conditioned Games (SSCGs) to emphasize a particular tactical and motor skill learning inside the game, as well as a to promote critical thinking by using discovery style through questions promoted by the teachers/coaches.

Specifically, TGfU aims to develop competent students who will be able to apply their tactical/technical knowledge properly according to each specify moment of the game, increasing gradually their autonomy (Harvey et al., 2018). The structure of this model are mainly divided into six parts (Metzler, 2017). First, the session starts with the implementation of a Modify Game. After the practice, pupils are asked about important tactical problems that they have just experienced during the game, as well as the best way to solve them. Thirdly, technical skill exercises are implemented in order to practice some technical skills related to the implemented game. After the exercises, students are asked

about the most important aspects to focus on the technical ability (technical awareness). Afterward, the Modify Game is implemented again emphasizing the tactical and technical aspects learned and practiced throughout the session. Finally, a general reflection of the most important outcomes is carried out to conclude the session.

However, Kirk (2017) showed some limitations in the implementation of the TGfU in the educational context: (I) the important tension between prescription and adaptation, (II) the complex teacher preparation of a flexible and adaptable lesson plans attending each students' needs and (III) the restriction of active participation during the questions moments. For this reason, Kirk (2017) proposed the design of a new model, which overcome the limitations observed meanwhile it adapt to the teacher and student necessities: the *Contextualized Sport Alphabetization Model* (CSAM). This new model aims to develop intelligent players who demonstrate cognitive (tactical), physical (technical) and social skills that enable them to gradually evolve toward more complex game formats.

On account of the limitations that have been found in the TGfU, Kirk (2017) highlighted that this new model should integrate some critical elements (inherent to the model), whichever are: (I) the pedagogy strategy based on the student-centered approach as well as the adaptation to the context; (II) the implementation of SSCGs adapted to the characteristics of the students, teacher and context; and (III) the holistic assessment which included contextual, small-group/team as well as individual criteria to ensure an integrate sports learning. Since each student has a different sport background, this new model is designed to have a flexible structure. On the one hand, the less skilled students learn, practice and consolidate the most basic tactical/technical intelligence. On the other hand, the students with an important sport background learn, practice and consolidate advanced contents using SSCGs.

Otherwise, it is observed a lack of investigation in the physiological responses during the implementations of MsBP at PE classes (Edwards et al., 2018). Above all, physiological scientific literature tends to focus on some physiological responses in high level performance SSCGs context such as the cardiorespiratory system and the exercise economy (Halouani et al., 2014), or the heart rate (HR) responses (Clemente et al., 2017). Furthermore, a wide range of research is focus in youth soccer athletes in an extracurricular context (Gäbler et al., 2018). For that reason, the lack of investigation about physical and physiological performance could be a limitation when MsBP are evaluated to be implemented in the educational context as a recommended way to increase the *motor and sport competence* (Goodyear et al., 2016).

Essentially, McLaren et al. (2017) observed that the training load (TL) has the potential to guarantee significant and adapted training sessions in team sports. Moreover, Paulson et al. (2015) also observed that the TLs help coaches or physical experts to avoid injury risks. The TLs are divided into two dimensions: (I) the external TL dimension, related to the physical demand stimulated by the athlete (i.e., distance, speed, or power); and (II) the internal TL dimension, related to the physiological and biochemical responses (i.e., metabolic, neurological, and



cardiovascular systems responses; Vanrenterghem et al., 2017). Impellizzeri et al. (2005) spotlight that the external TL is the main factor that determines the internal TL. Besides, the TLs are a great indicator for understanding the dose-response relationship between the training and the athletes' adaptation (Akubat et al., 2014). With regards to this, the internal TL is an important measurement to prevent both under- and over-training, as well as to achieve the desired athletes' performances during match and training sessions (Akubat et al., 2018).

In fact, there are a myriad of invasive and non-invasive methods to evaluate the external TLs, such as the distance covered, the body load or the number of accelerations (Buchheit and Simpson, 2017); as well as the internal TLs, such as the low-frequency fatigue, the HR frequency, the lactate levels or the session-Rate of Perceived Exertion (Heishman et al., 2018). Therefore, it exists many integrated measures to quantify the TLs (Sanders et al., 2017). One of the most popular methods, proposed by Banister et al. (1975), is the training impulse (TRIMP). This method integrates the training load duration, the mean HR of the whole training session and the intensity of the exercise. Lately, Edwards (1993) proposed a modification of the formula using five arbitrary HR zones. All in all, evidence support the use of the quantification of the training loads using HR (Hoff et al., 2002; Castagna et al., 2011).

According to Jaspers et al. (2018), the implementation of monitoring technology enables coaches, physical trainers, teachers and researchers to obtain objective and reliable results using non-invasive methods. Indeed, it is also observed that the real-time data, as well as the clarity of obtaining some of the aforementioned measurements in a "friendly" way, enables coaches and teachers to design more enriching sessions, optimizing the athletes' performance (Malone et al., 2017). Although the way of monitoring and collecting the TLs depend on the manufacturer, it is essential to use validated devices such as Polar™ or Wimu™ (Molina-Carmona et al., 2018) in order to obtain reliable results which could be used to customize the sessions and to improve the performance confidently. In addition, recent studies have analyzed physiological variables using monitoring technology in invasion games. In the soccer context, Rojas-Inda (2018) assessed the internal and external workload among young soccer athletes during SSCGs. Besides, Scott and Lovell (2017) analyzed the soccer training using external (GPS) and internal loads (HR and ratings of perceived exertion) among female soccer players.

However, as it has been exposed above, some limitations have been observed in the use of monitoring technologies in the educational context (i.e., expensive and complex devices). Even though the mentioned disadvantages, a new research line is focusing on quantifying the physical activity using accelerometers devices during the implementation of MsBP in Primary Education (Rocamora et al., 2019). However, to our knowledge, there is no studies focus on analyzing TLs during the models implementation in educational context.

For all the above, two objectives have been proposed in the present study. The main objective of this investigation is to

determine and compare the degree of physical performance, including external TLs (i.e., distance covered, speed and number of sprints), as well as the physiological factors, including internal TLs (i.e., HR variables, integrated measurement Edwards' TRIMP and calories), presented in several futsal sessions during the implementation of two MsBP: the TGfU and the CSAM. The second objective is to evaluate the relationship between external and internal TLs. The first hypothesis of this research indicated that when the game is properly adapted to the necessities of the students, an increase of physical and physiological aspects would be produced in contrast to general games for all the class. The second hypothesis stated that the performance global indicator Edwards' TRIMP are positively correlated to the external TL distance covered.

## MATERIALS AND METHODS

### Study Design and Variables Under Study

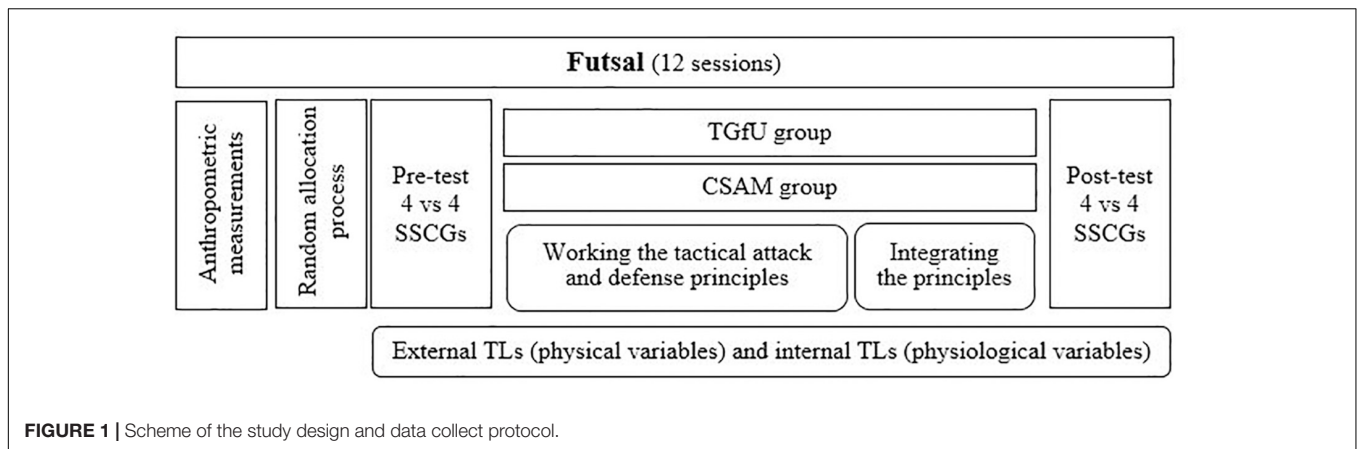
In order to achieve the objective of the current study, a quasi-experimental and cross-sectional study has been carried out in the educational context. In addition, pre-test and post-test evaluations were also implemented in order to determine the evolution of the physiological variables during the application of both models (Hernández-Sampieri, 2016).

**Figure 1** summarizes the study design. Even though the content applied in each grade was the same (i.e., tactical attack and defense principles), the lesson plans were designed according to the main features and pedagogical strategies of the TGfU and CSAM. The participant allocation in each group, based on the models, was randomized. After the anthropometric measurements, the implementation of both models was applied throughout 12 sessions of 135 min per week monitoring the physical and physiological variables. In addition, 4 vs. 4 SSCGs called "mini-futsal" was carried out at the beginning and the end of the units as pre-tests and post-tests.

The independent variables included the type of models (i.e., TGfU and CSAM) and the academic grade. In this sense, Spanish education curriculum established six mandatory grades. The first grade correspond to under-seven (U-7) years old students, second grade to under-eight (U-8), third grade to under-nine (U-9), fourth grade to under-ten (U-10), fifth grade to under-eleven (U-11), and sixth grade corresponds to under-twelve (U-12) years old students. On the other hand, the dependent variables included the physical performance variables (external TLs; e.g., total distance covered) and the physiological response variables (internal TLs; e.g., Edwards' TRIMP). In addition, anthropometric measurements, including height, weight, and waist circumference was carried out in order to contextualize the sample.

### Sample, Random Allocation, and Ethical Requirements

The sample under study was composed by 112 Primary Education (6–12 years old) male and female students from First to Sixth grade (mean age:  $9.35 \pm 1.76$ ) from a State School in Cuenca, Spain. The distribution of the participants



by courses was: 17.9% from First grade ( $n = 20$ ; mean age:  $6.95 \pm 0.38$ ), 17.0% from Second grade ( $n = 19$ ; mean age  $7.79 \pm 0.29$ ), 17.0% from Third grade ( $n = 19$ ; mean age:  $9.05 \pm 0.41$ ), 18.8% from Fourth grade ( $n = 21$ ; mean age  $9.91 \pm 0.42$ ), 13.4% from Fifth grade ( $n = 15$ ; mean age:  $11.11 \pm 0.57$ ) and finally, 16.1% was from Sixth grade ( $n = 18$ ; mean age:  $11.84 \pm 0.27$ ). As is it observed in **Table 1**, each grade was divided randomly into two groups (i.e., TGfU and CSAM group), following the CONSORT 2010 statement (Schulz et al., 2010). In each grade, every student was assigned a random identification number. The numbers were tabulated into a statistical spreadsheet. One external research from the Faculty of Education execute the random assignment command in order to obtain randomized TGfU and CSAM groups.

Throughout the investigation, the ethical standards of the Declaration of Helsinki (World Medical Association, 2013) were exhaustively followed. In addition, all the experimental procedures and the ethical considerations were approved by the Faculty of Education of Cuenca from the University of Castilla-La Mancha (UCLM). First of all, written consent had been elaborated according to the requirements of Faden et al. (1986). Secondly, after a meeting with all the members of the school (management team, teachers, and parent delegates), the head of studies, the principal and the PE teacher give their approval to carry out the investigation in the school. Finally, the participants' parents or participants' legal

guardians signed a written consent. Besides, all the participants voluntarily participated during the study. The privacy and the confidentiality of the participants' personal information were exhaustively protected.

## Instruments and Materials

In order to facilitate the interpretation of the results, the implemented instruments and materials in this study have been divided into three categories.

The first category is concerning the anthropometric measurements. An exhaustive protocol was designated in order to minimize the external influence (e.g., skin temperature or hydration status) in the body composition measurement (Pinto, 2012). Primarily, height and weight values were measured twice with a 5-min interval between measurements. Height was measured using to the nearest millimeter using the calibrated stadiometer SECA™ Model 213 (SECA, Corp., Hamburg, Germany). Weight, fat mass and lean mass was measured to the nearest 100 g using the bioelectrical impedance analysis system TANITA DC - 430 MA™ (TANITA, Corp., Tokyo, Japan; Elia, 2013). Based on the protocol, students must not have perform any kind of intense physical activity in the last 24 h, they also must not consume soft drinks (e.g., Coca-Cola™ or Fanta™) or sugared drinks (e.g., orange juice) 30 min before, and they also have to urinate at least 30 min before the bioimpedance measurement. Both objective measurements were used to calculate the Quetelet Index or the Body Mass

**TABLE 1 |** Distribution of the sample into the two models by academic grade.

Distribution	TGfU group			CSAM group		
	Percentage (%)	Frequency	Mean age	Percentage (%)	Frequency	Mean age
First grade	18.18	$n = 10$	$6.96 \pm 0.46$	17.54	$n = 10$	$6.94 \pm 0.30$
Second grade	16.36	$n = 9$	$7.77 \pm 0.33$	17.54	$n = 10$	$7.81 \pm 0.26$
Third grade	16.36	$n = 9$	$9.07 \pm 0.46$	17.54	$n = 10$	$9.04 \pm 0.39$
Fourth grade	18.18	$n = 10$	$9.73 \pm 0.50$	19.30	$n = 11$	$10.08 \pm 0.24$
Fifth grade	12.73	$n = 7$	$11.15 \pm 0.42$	14.04	$n = 8$	$11.08 \pm 0.70$
Sixth grade	18.18	$n = 10$	$11.77 \pm 0.23$	14.04	$n = 8$	$11.90 \pm 0.30$
Total	100	$n = 55$	$9.35 \pm 1.75$	100	$n = 57$	$9.35 \pm 1.78$

Index (BMI) as weight (in kilograms) divided by the square of the height (in meters). Then, the waist circumference was measured with a flexible tape in centimeters at the natural waist (which is the midpoint between the last rib and the iliac crest).

The second category is related to the monitoring devices, Polar Team Pro™ hardware and software (Polar Electro, Corp., Finland), used to collect, in an objective way, all the physical and physiological values during the implementation of the MsBP. The main important part of this technology is (I) the Polar Team Pro™ Sensor, which incorporates an integrated GPS (10 Hz), a sensor for the HR frequency, and three microelectrical mechanical components system (i.e., accelerometer, gyroscope, and digital compass; 200 Hz). This sensor has to be used with the Polar Team Pro™ soft strap. The total weight of which was 60 g. In addition, sensors are chargeable using a lithium polymer rechargeable battery station called (II) Polar Team Pro Dock™. Lastly, (III) the Polar Team Pro App™, compatible with iPad™, was used enabling the collection of the real-time data of each student. Before starting the investigation, the profiles of each student participated in the research had been created in the Polar App™, including information about the height, weight, VO<sub>2</sub> max (Patterson et al., 2018) and birthdate. During the implementation of the MsBP, each student wore a sensor around the chest. In fact, it was possible to extract the following physical and physiological variables with this system: the total distance covered; the distance per minutes; the maximum speed threshold (2.8 m/s<sup>2</sup>); the average speed; the number of sprints; the maximum, minimum, and average HR; the time in each of the five HR zones (i.e., very light: 50–60%, light: 60–70%, moderate: 70–80%, hard: 80–90%, maximum: 90–100%); and the calories burned. Goodie et al. (2000) validated the use of Polar™ technology in research contexts. In addition, Polar Team Pro™ was used in this investigation due to it is a device that can be easily adaptable to childhood (the size XS and S soft straps were used) and manage to collect all the data in a precise way in contrast to other devices made for adult population (Belton and MacDonncha, 2010).

The third category is regarding the method used to extract, the estimation of the VO<sub>2</sub> max, as well as the Edwards' TRIMP values. Due to the complexity of obtaining the exact VO<sub>2</sub> max in the educational context, the recommendation of implementing the multistage 20-m shuttle run test (Léger et al., 1988) was taken into account. In this test, students are required to run back and forth on a 20 m track. They have to touch the 20 m line before the sound signal of a prerecorded track was emitted. However, the frequency of the sound signals increases in such a way that running speed is increased by 0.5 km per hour each minute. The starting speed is set at 8.5 km per hour. Each student's test finishes when student is not able to follow the set pace. In the educational context, an extra session was previously implemented in order to explain this test (Ruiz et al., 2011). In addition, at the beginning of the test, a researcher also performed the test as a model together with the children to guide them. According to Lang et al. (2018) and Patterson et al. (2018) the VO<sub>2</sub> max could be estimated from the number

of stages (periods) of the test and the age of the students with the formula:

$$\text{VO}_2 \text{ max} = 31.025 + 3.238 \cdot \text{stage} - 3.248 \cdot \text{stage} + 0.1536 \cdot \text{stage} \cdot \text{age}$$

On the other hand, in order to obtain an internal TLs global indicator of the HR, Edwards' TRIMP was calculated using the following formula:

$$\text{Edwards' TRIMP} = (\text{minutes in maximum HR zone} \cdot 5) + (\text{minutes in hard HR zone} \cdot 4) + (\text{minutes in moderate HR zone} \cdot 3) + (\text{minutes in light HR zone} \cdot 2) + (\text{minutes in very light HR zone} \cdot 1).$$

## Procedure Protocol

### Antropometric Measurements

The previous week of starting the implementation of both models, the height, weight, and waist circumference were measured following the bioimpedance body composition protocol (see section "Instruments and Materials"; Pinto, 2012) in the school gymnasium, in order to contextualize the sample, and to configure the Polar Team Pro™ sensors. In addition, the 20-m shuttle run test (see section "Instruments and Materials") was implemented in each grade.

### Random Allocation and Pre-test SSCGs

In the first session of the model, the students in each grade were divided into the TGfU group and the CSAM group (see **Table 1**) following the randomization procedure (see section "Sample, Random Allocation, and Ethical Requirements"). In this first session, a pre-test 4 vs. 4 futsal SSCG was implemented in order to assess the initial level of the physical and physiological aspects. This SSCG is called "mini-futsal," and it is played on a pitch with dimensions of 30 m × 40 m (Tavares, 2015). The objective of the game is to score a point in the goal of the other team. However, in order to reinforce the active participation of every student, each player of the team should hold or controlled the ball at least once before shooting the ball. Each SSCGs lasts 5 min in one-isolated period. There are no goalkeepers.

The implementation of both MsBP were developed in 12 sessions of 45 min each at the same time during the classes of PE. The content implemented in both models and in every grade were futsal, an extensively sport in Spanish students. Both models were implemented at the same time in the same class in order to avoid biased data. In fact, the school sport-center was divided into two parts. In each part, one model was implemented.

### The TGfU Group

The TGfU groups followed the structure of session proposed and adapted by Metzler (2017): (I) implementation of a Modify Game, (II) common tactical awareness of the important aspect of the previous game through guided questions; (III) common technical execution and reinforcement of some technical skills; (IV) technical awareness through guided questions; (V) implementation of the Modify Game, stimulating the technical and tactical aspects developed before; and (VI) common and final reflection on the session.

In general terms, three sessions were dedicated to learn and practice each of the three tactical attack principle [(I) keep the possession of the ball, (II) progress to the rival goal, and (III) achieve the goal] and defense principle [(I) recover the possession of the ball, (II) avoid the progression of the rival team, and (III) defense the goal]. In addition, three sessions were dedicated to integrate all of the tactical principles with the best technical skill solutions. Throughout the implementation of these sessions, the students in each Modify Games were randomly changed.

The school PE teacher, an expert in the implementation of this model (more than 60 h of theoretical and practical training), designed the lesson plans together with two authors of the present research (SGV and MJSD) the TGfU lesson plans. He also was trained to implement the lesson plans in every grade, using the aforementioned structure (Metzler, 2017), by the researchers of the current paper. During the implementation, he was supervised by an external researcher (SGV).

### The CSAM Group

On the other hand, sessions of the CSAM were adapted to the necessities to the students. Even though the CSAM do not have a “non-negotiated” structure, the sessions included (I) a beginning common reflection, (II) implementations of several SSCGs oriented to a one of the basic tactical principles of attack and defense, (III) progression of the game and reinforcement of some tactical aspect of skill of the game, and (IV) final reflection and self-evaluation. Each student in this model had to bring tracking sheet. This sheet (different in each grade) is organized in several levels (i.e., basic, intermediate, and advance) in order to facilitate the adaptation of each student’s necessities and the establishment of the individual and collective objectives.

In this model, the group was divided into several teams or sub-groups. In contrast to the TGfU, the students in each team was maintained during the first classes. The session starting with 5-min common reflection in order to establish the objectives of the session in each team (i.e., to practice the dynamic passes in a SSCGs which reinforce the idea of not seeing the ball – from a third grade tracking sheet). Thereby, each team was proposed to implement different SSCGs to fulfill the objectives that had been proposed.

Instead of implementing common tactical awareness outside the game context, as TGfU did, the reflection of the most important tactical and technical aspects was carried out through “freezing the game.” At the end of the class, a 5-min reflection of the session was implemented, in order to check if the individuals and team objectives has been achieved, and to analyzed the best outcomes of the session.

One author of the present paper (MJSD), who is also a PE teacher, designed together with the rest of the researchers (SGV, JCPV, and ORCJ), the lesson plans for this group. All authors were experts in the implementation, assessment and investigation of MsBP with more than 30 years of practice. In addition, an external researcher (SGV) also supervised the implementation of this model.

### Data Monitoring and Post-test SSCGs

During each session, the Polar Team Pro<sup>TM</sup> technology collected the real-time physical and physiological data of each student. In addition, one external researcher (SGV) was present in every session in order to supervise the implementation of each model and the recording of the data.

At the end of the implementation of both models, the 4 vs. 4 SSCG called “mini-futsal” was carried out again to compare differences between physical and physiological variables during a contextualize game, as well as to assess the improvements of each variable in each model. The students of each team were the same as those of the pre-test 4 vs. 4 SSCGs.

### Data Analysis

First of all, in order to evaluate the normal distribution of the data, a Kolmogorov–Smirnov (K–S) test was carried out due to the size of the sample ( $n = 112$ ). The ordinary distribution is established when the mentioned test have a  $p$ -value superior to 0.050.

Secondly, in order to identify and assess the group of cases that share similar characteristics, the two-step cluster analysis was calculated. In this sense, this analysis could be useful to determine the quality of the allocation of the both intervention groups, identifying groups of participants that would not have been considered at the beginning of the intervention. The final clusters were compared using  $X^2$  test and cross-tabulations to determine if significant dependences existed ( $p$ -value inferior to 0.050). In addition,  $k$  Kappa was also calculated to evaluate the level of agreement between the clusters and the intervention groups (Landis and Kock, 1977). Once the clusters and intervention groups were evaluated, a descriptive analysis was implemented in order to compare the anthropometric variables between grades and groups (i.e., TGfU and CSAM group). These results has been reported as a mean  $\pm$  standard deviation (SD) following by the  $p$ -value, which is significant if it is inferior to 0.050.

Thirdly, Student’s  $t$ -test was executed to evaluate the external and internal TLs variables monitoring during the 12 sessions (i.e., distance covered,  $m \cdot \min^{-1}$ , maximum speed, average speed, number of sprints, maximum HR, minimum HR, average HR, Edwards’ TRIMP and calories burned) between the TGfU and the CSAM. In addition, the effect size was also calculated between both groups. The significant results were establish when  $p$ -value was inferior of 0.050. In addition, the effect size was also calculated between both groups.

Then, a multi-level model analysis was carried out to determine the existence of relationship between the dependent variables and the groups (i.e., TGfU and CSAM groups), including the academic grade. In this sense,  $\rho$  Intraclass Correlation Coefficient (ICC) was calculated.

Subsequently, MANCOVA and ANCOVA were carried out to compare the evolution through the time of the aforementioned variables derived from the same 4 vs. 4 SSCGs implemented after the investigation in every group. The significant results were established when  $p$ -value was inferior to 0.050. In addition, the effect size was also calculated between both groups.

Finally, on the one hand, Pearson correlation was implemented in order to evaluate the relationship between the internal TLs (i.e., total distance covered, meters per minutes  $\cdot \text{m} \cdot \text{min}^{-1}$ , maximum speed, average speed, and number of sprints) and the external TLs collected (i.e., maximum HR, minimum HR, average HR, Edwards' TRIMP, calories burned and  $\text{VO}_2$  max). Alongside the Pearson's  $r$  coefficient and  $p$ -value, it is provided the confidence intervals (CIs; Thompson, 2007) for each correlations coefficient. In addition, according to Hopkins et al. (2009), the correlation coefficient were classified in trivial ( $r$  from 0 to 0.09), small ( $r$  between 0.10 and 0.29), moderate ( $r$  between 0.30 and 0.49), large ( $r$  between 0.50 and 0.69), very large ( $r$  between 0.70 and 0.89), nearly perfect ( $r$  between 0.90 and 0.99), and perfect (when  $r$  is 1). On the other hand, partial correlations were executed to control the confounding influence of the third intervening variables (e.g., gender, course, and intervention groups). In both procedures, significance was set at  $p$ -value inferior to 0.050.

All the above mentioned statistical procedures were calculated using the Statistical Package for the Social Sciences (SPSS<sup>TM</sup>), version 24. The Cohen's  $d$  effect size value is considered small from 0 to 0.20, medium from 0.21 to 0.50, large from 0.51 to 0.80, and very large from more than 1.30 (Sullivan and Feinn, 2012).

## RESULTS

Since the sample was superior to 30 subjects, the normal distribution was assumed due to the Central Limit Theorem (CLT) (Akritas and Papadatos, 2004).

### Two-Step Cluster Procedure and Anthropometric Descriptive Analysis

The physical and physiological dependent variables were explored using the two-step cluster procedure. Results identify two clusters with a good cluster quality of 0.50 (Mooi and Sarstedt, 2011). **Table 2** shows the analysis of the dependent variables according to the two clusters. First of all, the first cluster was comprised of 51.40% of the sample with significant physical and physiological responses due to the methodology implemented. The largest relationship group in this cluster was First grade at 19.60%. The second cluster was comprised of 48.60% of the sample. This second cluster captured students with fewer means of the physical and physiological variables. A total of 20.8% of this group came from Fourth grade. The  $X^2$  analysis showed statistical relationship between both clusters and the intervention groups ( $X^2 = 97.360$ ;  $p < 0.001$ ), categorized as large effect ( $V = 0.945$ ). In addition,  $k$  Kappa showed almost perfect agreement between both clusters and the intervention groups ( $k = 0.945$ ;  $p < 0.001$ ).

Secondly, **Table 3** shows the average of the anthropometric values in each grade, divided by the intervention groups (i.e., TGfU and CSAM). In addition, Student's  $t$ -test was carried out in order to determine significant differences between both groups. The total average did not show significant statistical differences at height ( $p = 0.653$ ), weight ( $p = 0.588$ ), BMI ( $p = 0.272$ ), and waist circumference ( $p = 0.389$ ) among groups.

### During Intervention Analysis: Comparison of Both Models

In order to determine significant differences in the external and internal TLs during the 12 sessions between both models, Student's  $t$ -test was carried out. **Table 4** shows significant statistical differences between TGfU and CSAM groups. Indeed, throughout the lesson plans, better results were observed when CSAM are implemented, in contrast to TGfU, at total distance covered ( $p < 0.001$ ),  $\text{m} \cdot \text{min}^{-1}$  ( $p < 0.001$ ), maximum speed ( $p < 0.001$ ), average speed ( $p < 0.001$ ), and number of sprints ( $p < 0.001$ ). In addition, this trend was also observed at internal TLs values, which included maximum HR ( $p < 0.001$ ), minimum HR ( $p < 0.001$ ), average HR ( $p < 0.001$ ), Edwards' TRIMP ( $p < 0.001$ ), and calories burned ( $p < 0.001$ ). In this sense, Cohen's  $d$  effect size was significantly large in all the TLs variables (**Supplementary Material**).

### Multilevel Model Analysis and Intraclass Correlation Coefficient

The two-level model was used to predict the physical and physiological outcomes at the 4 vs. 4 SSCGs pre-test and post-test variables using the cluster proposed in Section "Two-Step Cluster Procedure and Anthropometric Descriptive Analysis," the groups intervention and the academic grades. **Table 5** shows that the regression coefficient for pre-test variables indicated a positive and significant relationship between the corresponding pre-test variables and the post-test dependent variables. The proportions of variation in physical and physiological variables during the 4 vs. 4 SSCGs that lies between intervention groups and grades varied from 1 to 48.4%.

### Analysis of the Pre-test and Post-test 4 vs. 4 SSCG

In relation to the homogeneity of physical and physiological variables derived from the 4 vs. 4 SSCGs carried out at the beginning of the implementation, the MANOVA result did not show statistical differences in external TLs and internal TLs [Wilks' Lambda  $\Lambda = 0.927$ ,  $F(11,100) = 0.715$ ,  $p > 0.050$ ; very large effect size,  $\eta^2 = 0.73$ ] between

**TABLE 2** | Analysis of the significant differences between both clusters.

Clusters	1	2
% of cases	51.4%	48.6%
Distance covered*	1421.68	771.85
Average HR*	179.62	146.91
Number of sprints*	13.81	5.96
Edwards' TRIMP*	21	11.45
Maximum HR*	204.76	187.99
Minimum HR*	112.25	95.99
Calories burned*	142.00	86.30
$\text{m} \cdot \text{min}^{-1}$ *	52.63	30.80
Average speed*	3.96	2.27

\*Significant results ( $p$ -values inferior than 0.050).

**TABLE 3** | Anthropometrics variables divided by grade and intervention groups.

Grade (years)	Group	Anthropometric variables			
		Height (cm)	Weight (kg)	BMI (kg/m <sup>2</sup> )	Waist circumference (cm)
First (U-7)	TGfU	115.90 ± 5.27	22.09 ± 4.24	16.31 ± 1.92	54.20 ± 6.35
	CSAM	120.70 ± 3.86	23.81 ± 2.35	16.35 ± 1.56	57.40 ± 4.37
Second (U-8)	TGfU	126.80 ± 7.62	29.12 ± 8.07	17.93 ± 3.91	55.90 ± 2.84
	CSAM	127.30 ± 9.86	26.52 ± 6.06	16.18 ± 1.45	65.22 ± 10.07
Third (U-9)	TGfU	131.44 ± 7.55	29.54 ± 7.79	16.95 ± 3.40	61.44 ± 9.64
	CSAM	133.70 ± 32.44	32.44 ± 8.32	17.96 ± 3.47	66.20 ± 12.30
Fourth (U-10)	TGfU	137.20 ± 7.92	34.50 ± 9.82	18.05 ± 3.52	66.90 ± 10.47
	CSAM	136.90 ± 6.45	33.69 ± 6.47	17.85 ± 2.41	64.82 ± 7.34
Fifth (U-12)	TGfU	140.20 ± 33.08	33.08 ± 6.69	16.71 ± 2.07	60.14 ± 4.22
	CSAM	144.25 ± 10.44	41.92 ± 13.82	19.68 ± 3.79	71.50 ± 10.70
Sixth (U-12)	TGfU	149.80 ± 6.61	51.65 ± 14.80	22.79 ± 5.43	79.00 ± 16.80
	CSAM	148.25 ± 8.46	38.46 ± 9.27	17.30 ± 2.61	63.38 ± 7.42
Total average	TGfU	133.38 ± 5.27	33.49 ± 13.05	18.23 ± 4.14	64.74 ± 12.90
	CSAM	134.43 ± 11.69	32.30 ± 9.89	17.49 ± 2.76	65.93 ± 9.32

U-x, under-age; cm, centimeter; kg, kilogram; m, meter.

**TABLE 4** | Analysis and comparison of the external and internal TLs throughout the sessions.

	TGfU group		CSAM group		p	d
	Mean	SD	Mean	SD		
Distance covered	783.22	154.57	1422.33	212.16	<0.001	3.43
m·min <sup>-1</sup>	32.22	13.20	52.28	16.58	<0.001	1.33
Maximum speed	19.35	2.85	23.48	3.63	<0.001	1.26
Average speed	2.35	0.75	3.42	1.05	<0.001	1.20
Number of sprints	5.96	1.10	13.88	3.17	<0.001	3.33
Maximum HR	188.40	9.77	204.34	7.44	<0.001	1.83
Minimum HR	97.15	10.69	111.75	7.47	<0.001	1.58
Average HR	147.48	9.09	179.55	9.16	<0.001	3.51
Edwards' TRIMP	11.42	3.37	21.30	2.11	<0.001	3.51
Calories burned	85.67	22.97	142.69	40.33	<0.001	1.73

SD, standard deviation; p, p-value; d, Cohen's d value.

models. Indeed, **Table 6** shows that pre-test ANOVA results did not show statistical differences between TGfU and CSAM ( $p > 0.050$ ).

Subsequently, MANCOVA results showed statistical differences in external and internal TLs between TGfU and CSAM among each grade during the 4 vs. 4 SSCGs carried out after the implementation of them [Wilks' Lambda  $\Lambda = 0.900$ ,  $F(10,90) = 90.715$ ,  $p < 0.001$ ; nearly perfect effect size,  $\eta^2 = 0.91$ ]. In fact, **Table 7** shows that post-test ANCOVA results were significantly different at CSAM in contrast to TGfU, observing a significant p-value in each external and internal TLs ( $p < 0.001$ ) (**Supplementary Material**).

## Relationship Between External and Internal TLs

In order to analyze the TLs as a whole, a significant relationship should be established between external and internal TLs in educational context. The correlation between the external TLs

and the internal TLs derived from the record in every session shows significant statistical differences from small to moderate among both MsBP. Specifically, the TGfU model shows that total distance covered are significantly related to maximum HR ( $r = 0.26$ ,  $p = 0.050$ , CI: 0.00 to 0.49; small), to average HR ( $r = 0.28$ ,  $p = 0.036$ , CI: 0.02 to 0.51; small), and to the calories burned ( $r = 0.27$ ,  $p = 0.042$ , CI: 0.01 to 0.50; small). Moreover, it is also observed a significant relationship between m·min<sup>-1</sup> and maximum HR ( $r = 0.31$ ,  $p = 0.018$ , CI: 0.58 to 0.53; moderate), m·min<sup>-1</sup> and average HR ( $r = 0.34$ ,  $p = 0.009$ , CI: 0.82 to 0.53; moderate), as well as m·min<sup>-1</sup> and minimum HR ( $r = 0.26$ ,  $p = 0.048$ , CI: 0.00 to 0.49; small). Maximum speed is also significantly related to maximum HR ( $r = 0.27$ ,  $p = 0.046$ , CI: 0.00 to 0.50; small). Finally it is also observed a moderated relationship between average speed with maximum HR ( $r = 0.39$ ,  $p = 0.003$ , CI: 0.14 to 0.59; moderated), and average HR ( $r = 0.44$ ,  $p = 0.001$ , CI: 0.23 to 0.60; moderate).

In addition, the CSAM shows that total distance covered are significantly related to maximum HR ( $r = 0.42$ ,  $p = 0.001$ , CI: 0.18

**TABLE 5** | Regression estimation of the pre-test and post-test variables using the two-level model.

Variables	Groups means differences		Regression coefficient	SE	p	$\rho$ ICC
	TGfU	CSAM				
Distance covered	74.46	125.58	305.104	16.860	<0.001	0.001
m·min <sup>-1</sup>	1.77	9.96	17.980	1.172	<0.001	0.011
Maximum speed	1.86	7.96	15.316	3.585	<0.001	0.021
Average speed	0.24	1.01	0.884	0.118	<0.001	0.484
Number of sprints	3.77	9.04	6.165	0.426	<0.001	0.160
Maximum HR	25.29	52.18	120.949	20.719	<0.001	0.001
Minimum HR	18.11	31.98	54.21	10.577	<0.001	0.010
Average HR	21.56	37.35	82.772	18.153	<0.001	0.004
Edwards' TRIMP	-0.09	10.31	14.072	1.088	<0.001	0.012
Calories burned	11.02	24.56	25.040	4.108	<0.001	0.026
VO <sub>2</sub> max	5.92	19.62	56.419	5.260	<0.001	0.014

ICC, Intraclass Correlation Coefficient.

**TABLE 6** | Descriptive and inferential pre-test analysis of the external and internal TLs variables.

	Pre-test				ANOVA pre-test		
	TGfU group		CSAM group		F(1,110)	p	d
	Mean	SD	Mean	SD			
Distance covered	197.96	54.22	197.89	58.90	0.0	0.99	-0.001
m·min <sup>-1</sup>	17.96	3.44	15.25	3.36	1.2	0.26	-0.797
Maximum speed	16.35	1.19	16.19	1.02	0.5	0.44	-0.144
Average speed	1.99	3.07	1.54	0.58	1.1	0.28	-0.203
Number of sprints	0.85	0.80	0.75	0.78	0.4	0.50	-0.126
Maximum HR	100.51	9.97	100.40	9.80	0.0	0.95	-0.011
Minimum HR	70.98	11.99	70.49	12.11	0.0	0.83	-0.040
Average HR	84.29	9.69	83.93	9.51	0.0	0.84	-0.037
Edwards' TRIMP	11.67	3.73	11.19	3.55	0.4	0.48	-0.131
Calories burned	33.69	10.80	32.53	9.95	0.3	0.55	-0.111

m, meter; min, minute; SD, standard deviation; p, p-value; d, Cohen's d value.

**TABLE 7** | Descriptive and inferential post-test analysis of the external and internal TLs variables.

	Post-test				ANCOVA post-test		
	TGfU group		CSAM group		F(1,110)	p	d
	Mean	SD	Mean	SD			
Distance covered	272.42	53.82	323.47	53.45	202.8	<0.001	0.951
m·min <sup>-1</sup>	19.73	3.47	25.21	3.20	102.2	<0.001	0.419
Maximum speed	18.21	2.21	24.15	2.37	281.6	<0.001	2.592
Average speed	2.23	3.01	2.55	0.36	367.2	<0.001	0.149
Number of sprints	4.62	2.92	9.79	3.81	110.0	<0.001	1.523
Maximum HR	125.80	16.18	152.58	12.31	142.6	<0.001	1.862
Minimum HR	89.09	12.76	102.47	11.10	202.8	<0.001	1.118
Average HR	105.85	19.78	121.28	13.69	169.5	<0.001	0.907
Edwards' TRIMP	11.58	3.53	21.50	2.21	366.3	<0.001	3.368
Calories burned	44.71	14.21	57.09	13.81	146.0	<0.001	0.883

m, meter; min, minute; SD, standard deviation; p, p-value; d, Cohen's d value.

to 0.61; moderate), to average HR ( $r = 0.46$ ,  $p < 0.001$ , CI: 0.22 to 0.64; moderate) and to the calories burned ( $r = 0.63$ ,  $p < 0.001$ , CI: 0.44 to 0.76; large). It is also observed a moderate relationship between total distance covered and Edwards' TRIMP ( $r = 0.39$ ,  $p = 0.003$ , CI: 0.14 to 0.59; moderate). This model also shows a significant relationship with number of sprints and maximum HR ( $r = 0.31$ ,  $p = 0.017$ , CI: 0.05 to 0.52; moderate). Besides, the number of sprints are largely related to the average HR ( $r = 0.65$ ,  $p < 0.001$ , CI: 0.46 to 0.77; large), and moderated related to the  $\text{VO}_2$  max ( $r = 0.39$ ,  $p = 0.003$ , CI: 0.14 to 0.59; moderate).

In relation to the partial correlation, taking account the confounding influence of the gender, course and intervention groups, the magnitude of the aforementioned correlations was reduced. In this sense, on the one hand, it is observed a small correlation effect between the  $\text{m}\cdot\text{m}^{-1}$  and the calories burned ( $r = 0.27$ ,  $p = 0.005$ ), as well as between the number of sprints and the minimum HR ( $r = 0.20$ ,  $p = 0.032$ ). On the other hand, it is also detected a moderate correlation effect among the total distance covered with the maximum HR ( $r = 0.31$ ,  $p < 0.001$ ), the average HR ( $r = 0.38$ ,  $p < 0.001$ ) and the calories burned ( $r = 0.46$ ,  $p < 0.001$ ). Moreover, it is also observed a moderate relationship among the number of sprints with the maximum HR ( $r = 0.30$ ,  $p < 0.001$ ), the average HR ( $r = 0.48$ ,  $p < 0.001$ ), and the calories burned ( $r = 0.39$ ,  $p < 0.001$ ).

## DISCUSSION

There is a lack of research in the assessment of TLs in the educational context, and specifically in MsBP. The main objective of this investigation was to assess and compare the degree of physical performance and the physiological factors derived from the implementation of TGfU and CSAM, in order to determine the motor and sport competence in both models. Furthermore, the second objective of the present study is to analyze the relationship between external and internal TLs. In addition, it is confirmed the main hypothesis of this research. When the sessions and the SSCGs are properly adapted to the students' necessities and abilities, engaging students to achieve individual and group objectives (use during CSAM), it is observed a significant increase of both physical and physiological variables in contrast to the use of general games and tactical awareness implemented for all the class (use of the TGfU). Secondly, it is confirm the hypothesis of the positive correlation between external and internal TLs.

First of all, two-step cluster analysis clearly identified two groups, similar from the intervention groups. Indeed, Kappa coefficient showed almost perfect agreement between these categories. In this sense, first clusters is closely related to CSAM group, where distance covered and HR variables are significant high in contrast to the second cluster, which is closely related to TGfU group.

Significant differences have not been observed regarding anthropometric measurements that have not been based on

the intervention groups. However, in line with Silva-Arantes (2018), the anthropometric measurements should be a basic index to identify sedentary lifestyle (i.e., overweight) and to orientate more effective intervention in PE related to healthy habits. In this sense, one of the most popular interventions to control the overweight and obesity is the BMI (Khambalia et al., 2012) and the waist circumference (Fredriksen et al., 2018). In the current paper, ordinary BMI and waist circumference have been observed in both models. Similar results were observed by Brown et al. (2018), obtaining that BMI improved when the intervention is oriented to lifestyle habits. Since Nuttall (2015) observed that BMI has some limitations due to it does not differentiate between fat mass and lean mass, Elia (2013) has recommended the use of bioelectrical impedance devices to identify the kind of body mass. However, in the present study it is show normal weight, closely related to the BMI results.

Regarding the comparison of the external and internal TLs variables that are derived from the recording of all the sessions, significant differences are observed in CSAM group in contrast to the TGfU group ( $p < 0.001$ ). Used Modify Games in TGfU are those ones that that have been adapted to the class needs. However, SSCGs formats in CSAM are games which follow a progression of the difficulty and have been adapted to the aims that have been proposed by each student in an individualized way (e.g., to work the demarcations) and the teams (e.g., to practice the possession of the ball using attacking inferiority). Similar results have been observed in comparable studies (Clemente et al., 2017; González-Villora et al., 2017), where the small formats of the game significantly increase the maximum HR, as well as the HR frequency in each zone.

In this line, Martín-Martínez et al. (2015) highlights that the SSCGs implementation supposes an increase of the physical and physiological response, due to it, a more significant participation of the players has been observed. The current paper has consequently verified that the active participation of the students in the implementation of SSCGs during the CSAM has increased the physical performance, such as the total distance covered ( $p < 0.001$ ), and the physiological response, such as the average HR ( $p < 0.001$ ). According to that, the concern of Kirk (2017) has been empirically demonstrated improvements are produced in the CSAM due to the SSCGs implementation during the season have been adapted to the students needs, in contrast to the Modify Games that have been organized in several games to practice the tactical/technical attack and defense principles.

On the contrary, Nathan (2017) found that TGfU is a positive model that achieve better results to enhance intensity and HR frequency in coaching environments, in contrast to strategies that are based on technical skill-drills. Similar results were found in educational context (Li et al., 2018), skill-drills are a decontextualized sport practice belonging to traditional PE approach. Even though the TGfU was created to contextualized the sport practice at introductory stage of sport learning, the result in this study also confirm that in some cases TGfU is confused as a "prescription tool" and not a pedagogical strategy which needs context adaptations (Kirk, 2017). In this sense, models should be focus on the heterogeneity of the students



and the way to satisfy their necessities at the same time as the curricular sport contents and the tactical/technical intelligence are consolidated satisfactorily.

In addition, the common tactical and technical awareness parts in TGFU (Gray and Sproule, 2011) could seem to have a negative impact on the physical and physiological responses. The current paper has demonstrated that “freezing the game” (used in CSAM) could be a better strategy to engage tactical/technical thinking (Práxedes et al., 2016), as well as to increase the active time of physical and physiological performance instead of using a tactical/technical awareness outside the game (used in TGFU). This strategy is based on short periods of reflection times, when important aspect should be considered. In this sense, tactical/technical progression of the CSAM enables a contextualize and active reflection.

In relation with the values obtaining in pre- and post-test 4 vs. 4 SSCG multilevel and variance analyses, it is observed what Cordova et al. (2012) has previously postulated, has been observed, this being: physical performance, including total distance covered,  $m \cdot m^{-1}$ , maximum speed threshold, average speed and number of sprints; as well as physiological responses, including HR values, Edwards’ TRIMP and the burnt calories have increased at the end of both models ( $p < 0.001$ ). In this sense, according to Rojas-Inda (2018), it is important to collect TLs with simply methods that could be applied in educational context, such as the distance covered or the HR values to help teacher to make decision about the design of the session or the specific games.

Considering the research of Dellal et al. (2011) in an extracurricular context, and Atl et al. (2013) in Secondary Education, it is also confirmed that the implementation of SSCGs is a beneficial strategy to increase the physical response and the physiological responses during CSAM. Furthermore, the evolution of the SSCGs, according to the needs and targets of the students in CSAM, produces significant improvements in contrast to keep the same number of players at Modify Games (TGFU). In this respect, similar results are also observed in elite soccer by Olthof et al. (2018). Recently, García-Angulo et al. (2019) highlighted that using non-linear pedagogy (such as the models used in this investigation) during the implementation of SSCGs can produced significant increase of vigorous physical activity. Indeed, CSAM students perceived SSGs as the best way to practice their tactical/technical intelligence focusing on achieving common objectives as a team inside the game, and helping other students to achieve their personal objectives proposed at the beginning of each sessions.

Regarding the relationship between the TLs, positive correlations obtaining in the present in some of the external TLs variables (i.e., total distance covered,  $m \cdot m^{-1}$ , maximum speed, average speed and number of sprints) with some of internal TLs variables (i.e., maximum, minimum, and average HR; calories burned; and  $VO_2$  max) are observed. On the one hand, this relationship confirm the idea that external TLs are the factors whichever determine the internal TLs. On the other, this fact confirm the main idea of Akubat et al. (2014), who showed that the integration of both TLs during the implementation of the

MsBP is more useful than registering only external TLs. In this respect, these results support the idea of Kirk (2017), which highlighted that pedagogical strategies should be implemented according to the real necessities and abilities of the students. In fact, monitoring devices help to obtain real-time data from all the students in order to adapt the SSCGs to the students’ characteristics and sport literacy objectives (Malone et al., 2017; Rocamora et al., 2019).

In addition, results showed in the meta-analysis by McLaren et al. (2017) highlighting that the relationships of external and internal TL could be different depending of the mode of training. This fact is also confirmed in the present study in the educational context. The relationship between external and internal TLs is different among TGFU students from CSAM students. Bartlett et al. (2017) confirmed that the total distance covered have the strongest association with internal TL in team-sports. Indeed, the correlation observed during the introductory stage of team-sport learning context in PE, seems to follow the same trend: the distance covered is significantly related with the HR values. For that reason, these results seem to reaffirm the idea observed in extracurricular sport context (Scanlan et al., 2014): external TLs, such as the total distance covered or the maximum speed threshold, seem to influence the internal response of the players.

Particularly, correlation between distance covered and Edwards’ TRIMP could be only observed in the implementation of CSAM ( $r = 0.39$ ,  $p = 0.003$ ). Even though the lack of findings in educational context, similar results was found by Casamichana et al. (2013) in soccer. In this study, it is observed a large correlation between distance covered and Edwards’ TRIMP. Finally, Haddad et al. (2017) and Fitzpatrick et al. (2018) highlight that the relationship between TLs help to examine the dose-response relationship, which is important to effective training programs. In this sense, Casamichana et al. (2013) observed that HR based methods using validated real-time devices (Malone et al., 2017) will help to objective track the internal TLs.

Nevertheless, the results should be taken cautiously due to new investigations in this area as well as models are needed. Even though the sample was composed by 112 students from every grade of Primary Education, more sample will be needed to consolidate the results observed in the present study attending to each educational context. Indeed, future research should be taken into account the comparison of dependent variables using a control group. In addition, this research investigated the physical and physiological variables, which are a group of values very important to assess new pedagogical strategies at PE or at team sport context.

However, it should be interesting to evaluate the tactical/technical knowledge as well as psychosocial factors in order to obtain a holistic evidence of the MsBP. In fact, new research could be oriented to evaluate external and internal TLs in educational context, as well as to correlate the Session-Ratings Perceived Exertions TLs with other external TLs such as the accelerometer load of the number of sustained

impacts. Therefore, future research will be focus on the teacher perception when designs and prepares this innovative model, as well as the level of training and preparation so that the model could be carried out.

## Practical Applications

Teaching/learning process at an introductory stage in sports alphabetization learning (PE and extracurricular context) is a very complex issue. For this reason, MsBP imply a great resource to orientate the pedagogical process (Casey and MacPhail, 2018). In fact, as it is observed in **Figure 2**, teachers should be taken into account some important aspects during the implementation of the models selected: (I) the kind of sport, which determine the type of specific content; (II) all the curricular elements (e.g., objectives, competences, or contents); and (III) the features of the context, which include (a) the grade or the years old of the group, (b) the previous knowledge of the content selected, (c) the needs and motivation of every students, (d) the specific materials, and (e) the area of play available. Indeed, these elements will determine the *motor and sport competence* acquisition during each session, which are divided into (I) tactical (decision-making) knowledge, (II) technical (skills) abilities, (III) physical performance or external TLs, (IV) physiological response or internal TLs, and (V) positive psychosocial values.

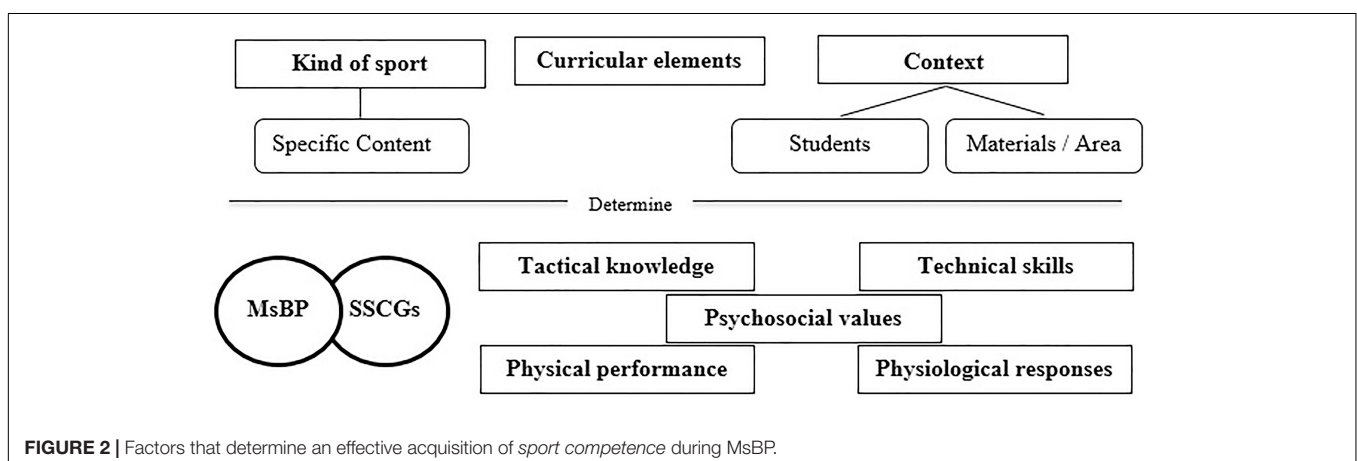
In this sense, according to Kirk (2017), the implementation of MsBP does not mean that they are “blueprints” which can be applied in every context. Particularly, the systematic review by Stolz and Pill (2014) proved that TGfU has got many versions and iterations around the world. This fact causes confusion in the educational context, where tension between prescription and adaptations is present (Kirk, 2017).

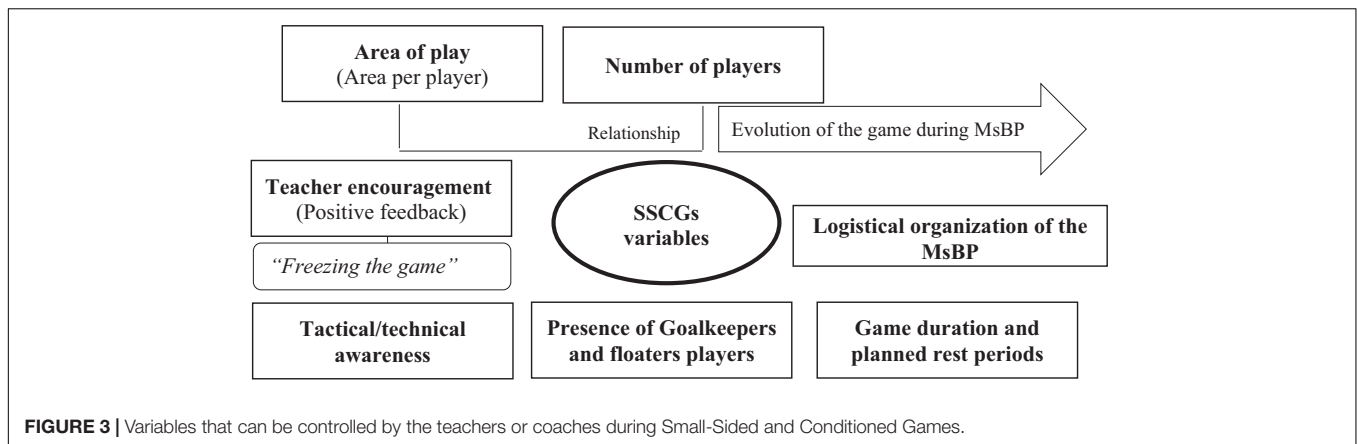
For this reason, it is important to reinforce the idea of attending to the features of each context, as well as the needs, abilities and motivations of the students and teachers. Indeed, the present study shows how CSAM could be a great resource for teachers to guide and adapt the content and the SSCGs according to the characteristics of each student, in contrast to ‘obligate’ them to adapt to the game. In this sense, teacher should know the start

point of each student, as well as the time of the sport practice outside the PE classes. For that reason, tracking sheet (used in CSAM) help students to know where they are, and where they want to be in the future regarding tactical/technical intelligence. Indeed, it is important for students to perceive a positive evolution of their abilities. In this regard, close relationship between teacher and student is necessary. Furthermore, González-Villora et al. (2018) showed that the combination of different features of the MsBP or the hybridization of them are an innovative trend to increase the *motor and sport competence* by amalgamating the basic features of each MBP.

One of the most important elements in the MsBP studied in the current paper is the implementation of SSCGs. According to Hill-Haas et al. (2011), these kinds of games should be the central axis of the PE sessions due to they offer many practical advantages. Clemente (2016) highlighted that SSCGs allow to replicate the physical performance and the physiological responses of the real match play, facilitating the evolution of tactical awareness and technical skills into a contextualized, and adapted MsBP and SSCGs atmosphere. Besides, it is observed that the implementation of SSCGs increases the students’ compliance and motivation (Hill-Haas et al., 2011). In this sense, **Figure 3** shows that there are many variables that can be controlled by the teachers or coaches to ensure holistic development of the *motor and sport competence*, which are also complemented by some logistic variables of the MsBP (e.g., grade of the students or type of sport).

In terms of measuring the physical and physiological values, Pind and Mäestu (2017) recommend to monitor TLs using real-time devices (such as validated pulsometers and/or accelerometers), as well as other strategies to understand the internal TLs responses (such as using the Edwards’ TRIMP). Even though, these kinds of devices could not be available in the educational context, teachers should take into account indirect strategies or methods to measure the HR of their students (e.g., the PSE). These measurements enable to guide or help teachers and coaches to organize and design PE sessions adequately and confidently ensuring a harmonic student development of the *motor and sport competence*.





## CONCLUSION

Within an educational context, the implementation of MsBP should not only be determined by empirical pedagogical improvements and tactical/technical progresses, but also by the physical and physiological variables which also contribute to the *motor and sport competences*. For this reason, there is a need to obtain physical and physiological variables of each MsBP. Thereby, it has been highly appreciated that the implementation of CSAM enables to overcome some of the limitations of the TGfU: the nature of the contextualized constraints in this new model will help to achieve better physical and physiological results.

It is confirmed the idea that MsBP should be adapted to the circumstances of the context (including the students' needs and motivation). Besides, the MsBP encompassed in the GCA should be organized according to an effective evolution of SSCGs. This kind of games is an effective strategy to increase physical and physiological performance when they are adapted according to the necessities and objectives of the students.

Monitoring real-time data are also ideal methods to quantify the physical performance (external TLs) and physiological responses (internal TLs) in educational context. For these reasons, there is a need to measure both external and internal TLs. Indeed, the relationship between the TLs has led teachers to 'listen to' the needs and abilities of each student in order to design and organize efficient MsBP sessions. Although improvements in physical and physiological variables at the end of both MsBP implementations have been observed, the CSAM groups have obtained better results in the physical and physiological variables in contrast to the TGfU groups.

In conclusion, *motor and sport competence* are closely related to the physical and physiological variables among others (i.e., pedagogical strategies, tactical/technical awareness, as well as psychosocial and prosocial values). However, the contribution of these elements to the above mentioned competence are not an intrinsic factor inside the MsBP or the SSCGs. There is a true need to 'listen to' the necessities and abilities of the student, as well as to design sessions according to these necessities and the context, where everybody can find his/her place inside the game, meanwhile the *motor and sport competence* effectively are being increased.

## ETHICS STATEMENT

The investigation "The way to increase the motor and sport competence among children: the Contextualized Sport Alphabetization Model" was designed based on the Ethical standards of the Declaration of Helsinki at the 64th World Medical Association (2013). The authors/researchers of this investigation have followed rigorously this Ethical Declaration due to the research involved human subjects (112 students from a Spanish State Primary School  $-9.35 \pm 1.76$  years old-). As it is shown in the *Frontiers in Physiology* manuscript, the study fulfilled with all the ethical standards, especially those related to the Privacy and Confidentiality (Principle 24); and Informed Consent (from Principles 25 to 32), adapted to Social Sciences and educational context (Faden et al., 1986). The informed consent before implementing the investigation was based rigorously on the manual "A history and theory of Informed Consent" (Faden et al., 1986). According to the current Spanish educational laws in force (the 9th of December Organic Law 8/2013, for the improvement of the educational quality – LOMCE-, as well as the 14th of July Decree 54/2014, which sets the curriculum for Primary Education in Castilla-La Mancha), the original informed consent signed by the head of studies, the principal, the Physical Education teacher, as well as each parent or legal guardian of the pupils should be kept in the school for future references. Furthermore, due to the audio-visual recording during the Small-Sided Games [as well as for others kind of activities during the academic year], the School had to request parental permission with an extra consent (Article 13.1 of the Organic Law of the Data Protection -LOPD-) at the beginning of the academic year. This consent has to be guarded in the school for future inspections.

## AUTHOR CONTRIBUTIONS

SG-V and MJS-D were involved in the conception and designed of the research and performed the experiments. SG-V, MJS-D, JP-V, and OC-J analyzed the data, edited and revised the manuscript, prepared the figures, drafted the manuscript, interpreted results of the experiments, and approved final version of the manuscript.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fphys.2019.00569/full#supplementary-material>

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# Fundamental Motor Skills Mediate the Relationship Between Physical Fitness and Soccer-Specific Motor Skills in Young Soccer Players

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Fundamental motor skills (FMS) are the basic elements of more complex sport-specific skills and should be mastered at the end of early childhood; however, the relationship between FMS and sport-specific skills has not yet been verified in prepubertal soccer players. Therefore, the aim of this study was to determine the role of FMS in the process of acquiring soccer-specific motor skills (measured using speed dribbling) with regard to physical fitness and biological maturation. Forty male soccer players ( $11.5 \pm 0.3$  years of age) at the highest performance level participated in the study. The test of Gross Motor Development – second edition and Unifittest 6–60 were used to assess FMS and physical fitness, respectively. The role of FMS in a complex theoretical model with the relationships between physical fitness, biological maturation and speed dribbling was analyzed by multiple regression path analyses (MRPA). Moderate to strong correlations were found between FMS, physical fitness, and speed dribbling ( $r = 0.56$ – $0.66$ ). Biological maturation did not appear to be a significant predictor of physical fitness or speed dribbling. The MRPA model using FMS as mediator variable between physical fitness and speed dribbling showed a significant indirect effect (standard estimation =  $-0.31$ ,  $p = 0.001$ ;  $R^2 = 0.25$ ). However, the direct correlation between physical fitness and speed dribbling was non-significant. Our results showed that FMS significantly strengthened the influence of physical fitness on the performance of speed dribbling, a soccer-specific motor skill, and thus play an important role in the process of acquiring sport-specific motor skills in prepubertal soccer players. When considering the long-term training process, especially during childhood and before puberty, a wide range of FMS activities should be applied for better and possibly faster acquisition of soccer-specific motor skills.

**Keywords:** pre-pubescence, soccer, skills, talent development, performance, motor control

## INTRODUCTION

One of the main goals of professional soccer clubs and their youth academies is to develop young, talented players into successful professional players (Huijgen et al., 2013). Many clubs and national associations (e.g., Germany, Belgium, Portugal, Netherlands) have created programs for talent identification and development (TID) to provide the best training environment and

conditions for young players with noticeable potential; enrollment in these programs often starts during early adolescence (Deutscher Fußball Bund, 2009; Huijgen et al., 2014; Leyhr et al., 2018). A player's success in a soccer match depends on complex multidimensional performance that is influenced by technical, tactical, physical, anthropometric, and mental factors (Reilly et al., 2000; Forsman et al., 2016). To ensure the highest possible efficiency in the TID process, performance tests of different soccer domains outside the context of the soccer match and notational analysis (observation and quantitative/qualitative analysis of the technical and tactical actions performed during a match) are often used in addition to coaches' expert and highly subjective assessment methods (Aquino et al., 2017).

During the last two decades, technical-tactical skills and physical fitness in particular have been frequently explored and identified as key determinants of young players' game performance, serving as discriminants between elite, subelite and non-elite youth soccer players (Meylan et al., 2010; Unnithan et al., 2012; Höner et al., 2017; Serrano et al., 2017; Leyhr et al., 2018). In particular, technical skills such as dribbling the ball, passing, shooting and ball mastery are considered critical game rudiments (Rampinini et al., 2009) and have been recognized as important motor factors within TID programs (Vaeyens et al., 2006; Deutscher Fußball Bund, 2009). Previous research suggests that technical skills develop most rapidly during the prepubertal and pubertal (10–15 years) phases (Vaeyens et al., 2006; Valente-dos-Santos et al., 2012; Leyhr et al., 2018). Specifically, the test of speed dribbling is the best discriminator of performance levels among soccer players (Vaeyens et al., 2006; Huijgen et al., 2009). Elite young players display significantly better performance in strength, speed, agility and aerobic/anaerobic endurance (Vaeyens et al., 2006; Murtagh et al., 2018; Rommers et al., 2018) than subelite young players. However, any connections between these findings and current game performance in youth players should be made with caution because differences in physical fitness and tactical skills are often caused by differences in the speed of biological maturation (Vaeyens et al., 2008; Costa et al., 2010; Meylan et al., 2010; Unnithan et al., 2012; Cumming et al., 2018). Early maturing soccer players generally show higher levels of explosive performance, sprinting, agility and aerobic endurance (Figueiredo et al., 2009; Valente-dos-Santos et al., 2012; Rommers et al., 2018). The relationship between biological maturation and the performance of technical skills is contradictory to the results of some studies confirming the influence of biological maturation status on the performance of technical skills tests (Philippaerts et al., 2006; Rommers et al., 2018) and other studies finding a lack of influence of biological maturation on the performance of technical skills (Figueiredo et al., 2009; Vandendriessche et al., 2012).

Recently, several studies have emphasized the importance of motor coordination, i.e., non-specific motor coordination, in the process of TID in youth soccer (Vandendriessche et al., 2012; Deprez et al., 2014; Deprez D. et al., 2015; Deprez D.N. et al., 2015; Rommers et al., 2018). Furthermore, these studies showed that motor coordination is a significant long-term predictor of specific aerobic fitness and explosive leg power in young soccer

players (Deprez et al., 2014; Deprez D. et al., 2015) and does not depend on biological maturation (Vandendriessche et al., 2012; Rommers et al., 2018). However, the direct relationship between motor coordination and specific technical skills (e.g., speed dribbling) was not explored in prepubescent soccer players. In another study, Deprez D.N. et al. (2015) measured motor coordination performance among club players (playing in the two highest youth soccer leagues) and dropout players (those who dropped to lower soccer leagues) over the 8-year period from age 8 years to age 16 years and found that the club players performed significantly better than the dropout players on all motor coordination tasks and on aerobic endurance and speed. The authors suggested that motor coordination performance is essential for discriminating between players in a high-level training program and dropout players from the age of 9 years until late puberty. Although the direct relationship between motor coordination and specific technical skills was not investigated in this study, one could hypothesize that the dropped players had overall worse specific technical skills and worse motor coordination than club players.

In many studies focused on motor development, the term “motor coordination” has been used to denote motor competence, motor proficiency or fundamental motor skills (FMS) to describe goal-directed human movement (Robinson et al., 2015). For the purpose of our study, we decided to use the term FMS to describe the level of general motor competence. In general, according to several key motor development theoretical models, FMS are frequently defined as the “elements” of more advanced complex movements required to participate in sports, games, or other context-specific physical activity (Clark and Metcalf, 2002; Gallahue et al., 2012). However, no clear research evidence indicates whether this theory is valid in prepubertal soccer players. Once FMS are mastered, the learning of sport-specific skills can occur more quickly and be more effective (Gallahue et al., 2012). FMS are traditionally divided into object control/ball/manipulative skills (e.g., throwing, catching, dribbling), locomotor skills (e.g., running, jumping, galloping), and balance/stability skills (e.g., non-locomotor skills such as body rolling, one-foot balance, stretching, twisting) (Gallahue et al., 2012). Although children have the developmental potential to master most FMS by the age of 6 years (Gallahue et al., 2012), recent research highlights that children and adolescent youth do not perform FMS to their expected developmental capabilities (O'Brien et al., 2016). O'Brien et al. (2016) further demonstrated that while levels of FMS vary by country, performance levels remain consistently low, with the majority of children and adolescents failing to surpass 50% mastery in most skills.

To our knowledge, little attention has been paid to the importance of FMS in the process of acquiring technical skills (e.g., dribbling, receiving, or passing a ball) in prepubescent soccer players. Moreover, current research describes the direct relationships between technical skills and other physical, motor control, or morphological factors but have not described how those factors interact with or mediate specific soccer skills. Although there is clear evidence concerning the relationships between FMS, physical fitness and biological maturation, there



is a lack of information about the influence of FMS on the performance of soccer technical skills in prepubescent-aged players. We hypothesized that FMS strengthen the influence of physical fitness and biological maturation on technical skills (e.g., speed dribbling the ball). Therefore, the aim of this study was to investigate the role of FMS in the relationships between physical fitness, biological maturation and technical skills in prepubescent soccer players.

## MATERIALS AND METHODS

### Methodological Approach

Cross-sectional measurement was performed during the competitive part of the soccer season. The participants were familiarized with the experimental protocol 1 week prior to the experiment and did not perform any exhausting activity 72 h before the experiment. After the participants' body mass (BM) was estimated, they performed the battery of FMS, speed dribbling and physical fitness tests within 1 day (two training sessions). The FMS and sit-ups (part of Unifittest 6–60) tests were performed indoors on a teraflex surface during the morning training session between 9 and 11 am. The rest of physical fitness tests were then conducted during afternoon training session on the outdoor ground with artificial grass between 3 and 5 pm.

### Participants

The research sample consisted of forty U12 soccer players (mean  $\pm$  SD; age  $11.5 \pm 0.3$  years; height  $145 \pm 7.0$  cm; body mass  $37.2 \pm 4.1$  kg). The players were members of teams from two clubs in the Prague district of the Czechia that participated in the highest Czech youth league level. These two clubs were randomly selected from a basic sample (a total of fourteen clubs in the Prague district) and then were asked to participate in the study. The weekly cycle consisted of four training sessions (7–8 h) focused primarily on technical-tactical skills during exercises and games and one competitive match. The inclusion criteria were a minimum of 6.4 years of experience with organized soccer and full attendance in ongoing habitual training cycles. Exclusion criteria were any medical problems that compromised participation or performance in the study, such as soft tissue injury, delayed muscle soreness, recent illness or recent recovery from injury. The research was approved by the Ethics Committee of the Faculty of Physical Education and Sport, Charles University, and all participants and their parents signed an informed consent form.

### Fundamental Motor Skills

The Bruininks-Oseretsky Test – 2nd edition (BOT-2; short version) was used to assess fundamental fine and gross motor skills (Bruininks, 2005). The BOT-2 has demonstrated high inter-rater reliability ( $r \geq 0.90$ ), test-retest reliability ( $r \geq 0.80$ ) and construct validity (Deitz et al., 2007). The short version contains sixteen items divided into eight dimensions (see Table 1). Raw scores from BOT-2 were transformed into standard scores according to age by ASSIST software (MN, United States). Standard scores were then used for the final analysis.

**TABLE 1** | List of dimensions and items of the BOT-2 motor test.

Fine motor precision	Balance
Drawing lines through crooked paths	Walking forward on a line
Folding paper	Standing on one leg on a balance beam – eyes open
Fine motor integration	Running speed and agility
Copying a square	One-legged stationary hop
Copying a circle	Upper limb coordination
Copying a star	Dropping and catching a ball – both hands
Copying a pencil	Dribbling a ball – alternating hands
Manual dexterity	Strength
Transferring a penny	Full push-ups
Bilateral coordination	Sit-ups – 30 s
Jumping in place – same side synchronized	
Tapping feet and fingers – same side synchronized	

### Physical Fitness Tests

Three physical fitness parameters were measured (shuttle run 4 m  $\times$  10 m, standing broad jump, and 20-m progressive shuttle run). These three tests are included in the Unifittest 6–60 test battery, which is standardized for the Czech context (Mekota and Kovar, 1995; Chytrácková, 2002) with a satisfactory level of reliability and validity (Mekota and Kovar, 1995). *Shuttle run* 4 m  $\times$  10 m, which assesses coordination and speed, was performed twice by each player, with 3–4 min of rest between the two trials. In a start position, the player stood on the starting line without moving into the space between photocells. The player sprinted to the opposite marker (10 m), turned and returned to the starting line directly adjacent to the photocell gate. This was performed twice to cover a 40-m distance. The time of the faster trial was recorded. An infrared timing gate (Alge Timing GmbH, Lustenau, Austria) placed at approximately hip height was used for the start and finish points. *Standing broad jump*, an indicator of explosive power in the lower limbs, was performed three times by each player, with 2 min of rest between trials. The player stood behind a line marked on the ground. A two-foot takeoff and landing area was used, and players were instructed to jump as far as possible while swinging their arms and bending their knees to provide forward momentum. The longest jump was recorded and used for the analysis. *Progressive shuttle run* 20 m is a measure of maximal aerobic fitness. The player continuously ran between two lines 20 m apart, keeping pace with recorded beeps, which accelerated each minute. The test was stopped when the player failed to reach the line (within two meters) after two consecutive warnings. Finally, from each test item, a standard score was obtained. The composite score of all tests on a scale from 0 to 20 was calculated as a marker of physical fitness.

### Predicted Maturity Offset

Maturity offset was estimated according to Mirwald et al. (2002) equations. Although these equations have been widely used in the sport environment (e.g., Wickel and Eisenmann, 2007;

Gastin et al., 2013; Gil et al., 2014; Meyers et al., 2015), studies have pointed to the limits of this predictive method in both sexes (Malina and Kozieł, 2014a,b; Malina et al., 2016). In the current study, we considered the finding of Kozieł and Malina (2018), who stated that maturity offset predicted from the Mirwald equations matched the observed peak high velocity (PHV) in 12-year-old boys, to be important.

$$Y\text{-PHV} = -9.326 + (\text{length of lower limbs} * \text{sitting height}) - (0.001663 * [\text{decimal age} * \text{length of lower limbs}]) + (0.007216 * [\text{decimal age} * \text{sitting height}]) + 0.02292 * [\text{weight/height}]$$

Body weight was assessed with an accuracy of 0.1 kg using medical calibrated weight (CAS DBI-C, Lesak and Zemánek s.r.o., Czechia). A portable anthropometer (A 226, Trystom, spol. s.r.o., Czechia) with a balancing point to determine the right vertical position of the anthropometer was used for the measurement of height and sitting height (0.1 cm).

## Speed Dribbling Test

The short dribbling test (SDT) (Bangsbo and Mohr, 2013; **Figure 1**) is a test of dribbling the ball at a high speed with changes in direction around a defined track. Players are required to dribble as fast as possible around the cones without touching them. The test is finished by stopping the ball in a square (defined with blue cones) at the end of the track. Each player underwent one training and one competitive trial. If the player touched any cone during dribbling, the trial failed, and the player was allowed an additional trial. Time was measured by a telemetric photocell system (Alge Timing GmbH, Lustenau, Austria), and the best time was recorded. Similar agility dribbling tests have shown acceptable reliability, with an intraclass correlation coefficient between 0.78 and 0.89 and coefficient of variation of 2.4 and 3.9 (Russell et al., 2010; Ali, 2011; Dardouri et al., 2014).

## Data Analysis

Multiple regression path analysis (MRPA) was used to test the hypothesized links, with successive multiple regression equations calculated to estimate path coefficients. Mardia's, Henze-Zirkler's and Royston's multivariate normality tests were performed using

the R package MVN, version 4.0.2, in R 3.4.1, with a cut-off of  $p$  greater than 0.05 for accepting the normality of multivariate data. The exogenous independent variables were physical fitness and maturity offset. The interacting endogenous variable was FMS. All of these variables were analyzed first using linear regressions and then using multiple regressions. Subsequently, the final path analysis model was selected. In the path model, there were specified direct paths from the exogenous variable to the endogenous variable and from the exogenous and endogenous variables to the SDT. Finally, for variables that had statistically significant predictive power ( $p < 0.05$ ) for FMS or for SDT performance, specific indirect effects via FMS were investigated. MRPA and Pearson's correlations were performed using M-plus software version 6.0 (Muthen and Muthen, 2010). All data can be found in the "Supplementary Table S1" Supplementary File.

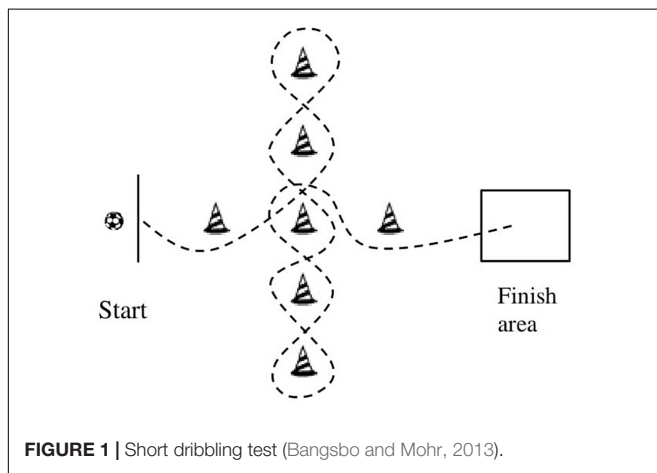
## RESULTS

The means and standard deviations of the basic descriptive statistics and correlation coefficients of FMS, physical fitness, maturity offset and speed dribbling are shown in **Tables 2, 3**, respectively. Significant moderate associations were observed between speed dribbling and FMS, speed dribbling and physical fitness, and FMS and physical fitness. However, there was no association between speed dribbling and maturity offset.

In the first step, we analyzed the predictive power of FMS, physical fitness and maturity offset (independent variables) on speed dribbling performance (dependent variable). The linear regression results (**Table 4**) showed that FMS and physical fitness are significant predictors of speed dribbling performance. Nevertheless, only the effect of FMS ( $R^2 = 0.36$ ;  $t = 2.97$ ;  $p = 0.003$ ) was significant, while the effect of physical fitness was not ( $R^2 = 0.18$ ;  $t = 1.64$ ;  $p = 0.100$ ).

Since clear evidence of the relationship between biological age and physical fitness in pubescent soccer players has been reported in previous research (biologically advanced players achieve better performance in physical fitness), we verified whether FMS and maturity offset are significant predictors of the level of physical fitness. The multiple regression model showed that the effects of FMS and maturity offset explain 22% of physical fitness performance variability ( $R^2 = 0.22$ ). Furthermore, from **Table 5**, it is clear that FMS are significantly better predictors for physical fitness than maturity offset in prepubescent players.

Considering these findings, we decided to use the analysis model where FMS plays the role of mediator between physical fitness (as the independent variable) and speed dribbling (as the dependent variable representing specific soccer skills). In the 1st path analysis model, we specified both direct and indirect paths between physical fitness and speed dribbling. The 1st path analysis model (**Figure 2**) showed that the direct effect of physical fitness on speed dribbling was non-significant (standard estimation =  $-0.17$ ,  $p = 0.247$ ), and the indirect effect through FMS was significant (standard estimation =  $-0.26$ ,  $p = 0.005$ ). To obtain better results, we decided to formulate the 2nd path analysis model without a direct effect (**Figure 3**). The 2nd model was approved as significant and acceptable with empirical data



**TABLE 2** | Basic descriptive statistics ( $n = 40$ ).

	Mean	SD	Median	Interquartile range	95% confidence interval
Age (years)	11.50	0.30	11.63	0.42	±0.09
Height (cm)	145.00	7.00	149.45	6.53	±1.98
Body mass (kg)	37.20	4.10	37.15	7.85	±2.05
Index BMI (kg/m <sup>2</sup> )	17.52	1.89	17.06	2.08	±0.59
FMS (ss)	57.33	8.88	58.50	17.25	±2.75
Physical fitness (ss)	21.05	3.34	21.00	4.50	±1.03
Maturity offset (years)	-2.88	0.30	-2.90	0.34	±0.09
Speed dribbling (s)	13.68	1.53	13.73	2.46	±0.47

FMS, fundamental motor skills; SD, standard deviation; ss, standard score.

**TABLE 3** | Correlation matrix of the study variables.

	Maturity offset	Physical fitness	FMS
<b>Maturity offset</b>	1		
<b>Physical fitness</b>	-0.21**	1	
<b>FMS</b>	-0.29**	0.50**	1
<b>Speed dribbling</b>	-0.03	-0.42**	-0.60**

FMS, fundamental motor skills; \*\*indicates statistical significance of  $p < 0.01$ .

**TABLE 4** | Linear regressions of physical fitness, FMS and maturity offset on speed dribbling.

Independent variable	B	SE B	$\beta$	t	p-Value
<b>Physical fitness</b>	-0.93	0.31	-0.43	3.28	0.001**
<b>FMS</b>	-3.5	0.74	-0.60	5.95	< 0.001**
<b>Maturity offset</b>	-0.01	0.03	-0.03	0.19	0.85

FMS, fundamental motor skills; \*\*indicates statistical significance of  $p < 0.01$ ; B, unstandardized beta; SE B, standard error for the unstandardized beta;  $\beta$ , the standardized beta; t, t-test statistic.

**TABLE 5** | Multiple regression of FMS and maturity offset on physical fitness.

Independent variable	B	SE B	$\beta$	t	p-Value
<b>FMS</b>	-0.82	0.06	0.48	3.25	0.003**
<b>Maturity offset</b>	0.18	1.64	0.08	0.50	0.62
<b>Adjusted R<sup>2</sup></b>	0.22				

FMS, fundamental motor skills; \*\*indicates statistical significance of  $p < 0.01$ ; B, unstandardized beta; SE B, standard error for the unstandardized beta;  $\beta$ , the standardized beta; t, t-test statistic.

explaining more than 25% of the model. Generally, FMS were a significant mediator between physical fitness and speed dribbling (standard estimation = -0.31,  $p = 0.001$ ).

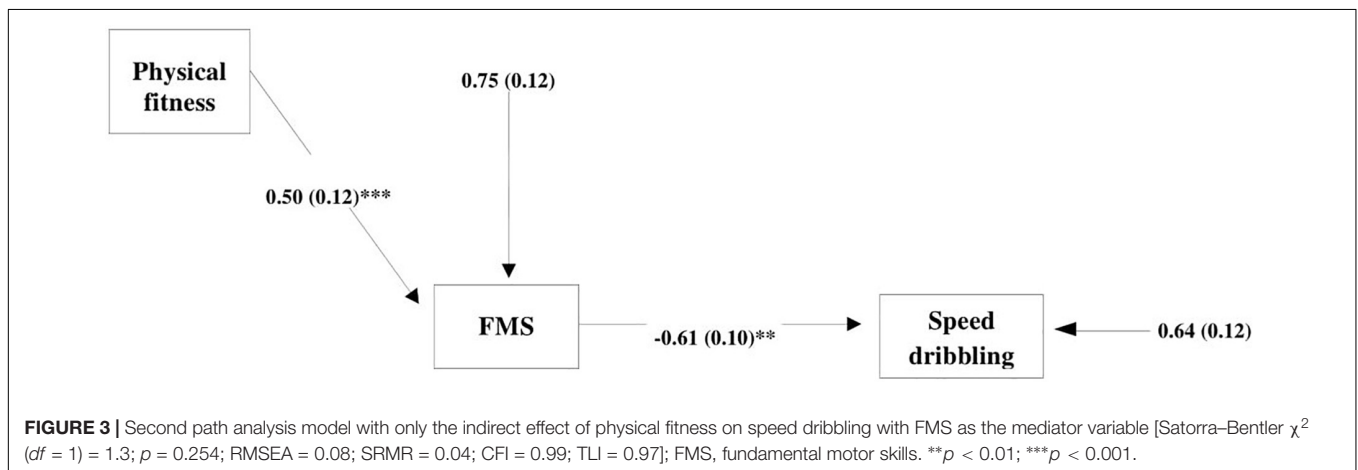
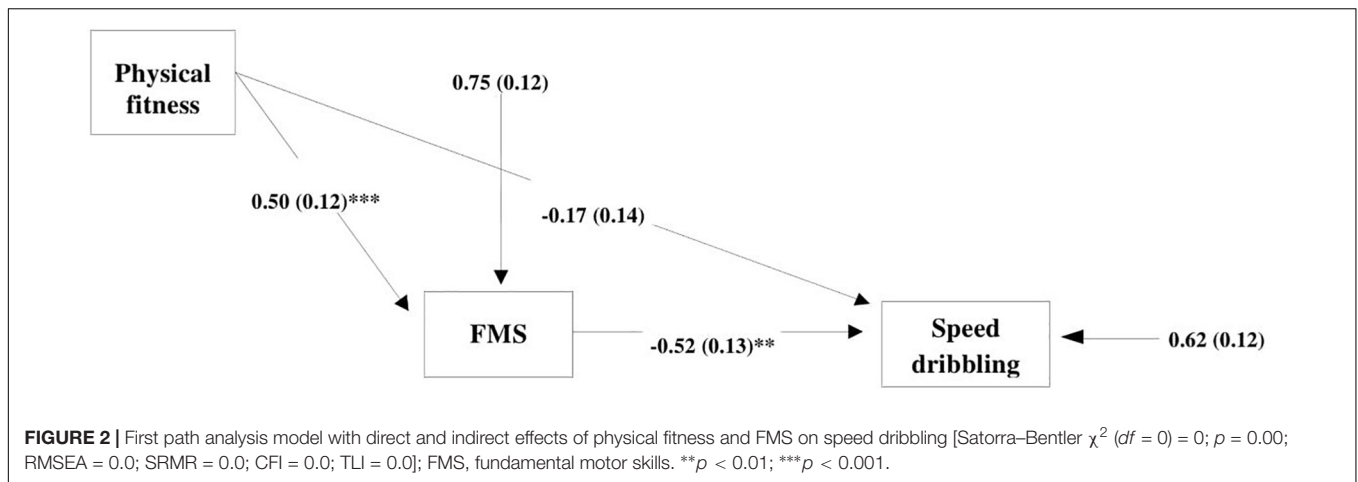
## DISCUSSION

The present study examined the possible role of FMS in the relationships between physical fitness and biological maturation and speed dribbling as a soccer-specific soccer skill in young soccer players. We found that FMS were a significant mediator of the relationship between physical fitness and speed dribbling.

Notably, biological maturation did not prove to be a significant contributor to speed dribbling performance through FMS. Despite moderate correlations between physical fitness and speed dribbling, the path model did not reveal a direct influence of physical fitness or biological maturation on speed dribbling. These findings suggest the need for a certain level of FMS (fine and gross motor skills) to acquire soccer-specific motor skills. Generally, both quantitative (physical fitness) and qualitative (FMS) motor aspects were found to be significant contributors to the performance of soccer-specific motor skills, represented by speed dribbling. Our path model revealed that FMS, physical fitness and other related factors have a prior effect on specific skill, whereas biological maturation might explain only 8.7% of motor coordination (Freitas et al., 2016).

Our results suggest that FMS mastery significantly increases the influence of physical fitness on the performance of soccer-specific skills in young players. These findings are in accordance with Clark and Metcalf's (2002) statement that FMS are basic elements for later skillfulness in a range of sport and game domains. Our results cannot be compared with similar data measured on soccer players. However, similar conclusions have been found in research involving combat sports (Bozanic and Beslija, 2010), where high correlations were found between specific karate skills and FMS ( $r = 0.74$ ) in 5- to 7-year-old members of karate clubs. This study suggested that children with higher FMS also have better karate techniques, while others have difficulties acquiring these techniques. Unfortunately, recent research has documented very poor or insufficient FMS performance in preschool and school-aged children (Okely et al., 2004; Erwin and Castelli, 2008; Hardy et al., 2010; Kokstejn et al., 2017a,b) combined with generally unresolved inactivity in children (Faigenbaum et al., 2018), which may result in impaired acquisition of more complex and difficult sport skills or delays in mastering the required skills. Similarly, the players in our study showed only an average level of FMS even though they were considered to be capable of high performance. Since a higher level of FMS and soccer-specific skills were found in players selected for Belgian professional clubs (Deprez D.N. et al., 2015) than in "dropout" players, FMS and soccer-specific skills seem to be crucial to the identification of gifted players and their likelihood of remaining in high-level talent development programs.

Our participants were at a specific age (U12) where physical development plateaus (in reactive strength and jumping) with a



change in the mechanical properties of the lower limb (decreased relative leg stiffness), which has been previously observed (Lloyd et al., 2011). This might explain why biological maturation in U12 children is not strongly related to physical fitness or motor control testing (Freitas et al., 2016) and why separate values of physical fitness and biological maturation are insufficient for predicting a player's ability to acquire soccer-specific skills. Our path models suggest that the best performance of soccer-specific skills will occur in players with adequate levels of both FMS and physical fitness. This finding is in agreement with the finding that FMS were a long-term predictor of explosive power in soccer players from childhood to young adulthood (Deprez D. et al., 2015). Therefore, we believe that well-developed FMS and the simultaneous development of PF are necessary in pre-PHV boys and that well-coordinated players will improve in power and performance with age due to increased tendon stiffness in late adolescence (Deprez D. et al., 2015).

The harmony between physical fitness and biological maturation has been highlighted by several authors (e.g., Meylan et al., 2010; Unnithan et al., 2012; Vandendriessche et al., 2012) to discriminate elite, subelite and non-elite soccer players during talent identification. Moreover, FMS were found to be a long-term predictor of soccer-specific aerobic performance in

elite pubertal soccer players (Deprez et al., 2014) and children (Hands, 2008). Specifically, children with low FMS performed worse on all fitness tests (50-m run, standing broad jump and endurance shuttle run), where endurance shuttle test differences increased between low- and high-FMS groups over 5 years (Hands, 2008). Therefore, we highlight the importance of FMS not only for soccer-specific motor skills but also for separate components of physical fitness, such as explosive power and aerobic endurance, during long-term motor development.

Several possible limitations associated with this study should be noted. The present study utilized a cross-sectional design; thus, the role of FMS in the relationships between physical fitness, biological maturation and soccer-specific motor skills should be interpreted with caution. A longitudinal follow-up of young soccer players, especially during the pubertal phase (aged 12–15 years), may provide a more accurate explanation of this mediation effect. Another possible limitation is related to the non-inclusion of psychological variables such as motivation or self-confidence, which certainly influence game performance and likely also affect the development of new soccer-specific motor skills. Lastly, although several authors consider speed dribbling the most valid soccer skill test, the inclusion of additional soccer-specific skills (e.g., passing, shooting, receiving,

or multifaceted tests) could elucidate the role of FMS in the acquisition of soccer-specific motor skills (Vanderford et al., 2004). Therefore, future research should focus on (1) performing longitudinal research to verify the role of FMS in acquiring soccer-specific skills during the pubertal phase, (2) testing more soccer-specific skills and psychological variables with respect to players' positions, and (3) using a process-oriented (assessment of movement quality) test for FMS assessment.

## CONCLUSION

This is the first study to evaluate the role of FMS in a complex theoretical model with the relationships between physical fitness, biological maturation and soccer-specific motor skill (measured using speed dribbling) in young soccer players. Our results showed that FMS significantly strengthened the influence of physical fitness on the performance of speed dribbling, a soccer-specific motor skill, and thus play an important role in the process of the acquisition of sport-specific motor skills in prepubertal elite soccer players. Conversely, physical fitness and biological maturation alone did not significantly influence speed dribbling performance. Generally, it appears that developing and improving a wide range of basic FMS as building blocks for more complex and more difficult soccer-specific motor skills is necessary during the long-term training process. Based on these findings, FMS could be included in TID programs for young elite soccer players, especially during childhood and before puberty. Thus, it is recommended that youth soccer coaches and practitioners carefully consider providing training on FMS (fine motor, locomotor, object control, balance), especially during childhood, with an emphasis on the quality of movements.

## DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and/or the **Supplementary Files**.

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## ETHICS STATEMENT

This study was carried out in accordance with the recommendations of “name of guidelines, name of committee” with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the “name of committee.”

## AUTHOR CONTRIBUTIONS

JK, MM, and PS involved in the conceptualization of the study design and the drafting of the manuscript. JK and MM involved in data collection. JK involved in performing an overview of the previous research. MM involved in conducting the statistical analysis. PW and EM-C helped with the data assessment and interpretation.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fphys.2019.00596/full#supplementary-material>

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# Multi-Stage Fitness Test Performance, $\dot{V}O_2$ Peak and Adiposity: Effect on Risk Factors for Cardio-Metabolic Disease in Adolescents

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The role of physical activity in determining the metabolic health of adolescents is poorly understood, particularly concerning the effect on low-grade chronic inflammation (chronic elevation of pro-inflammatory cytokines IL-1 $\beta$ , IL-6, TNF- $\alpha$  and acute phase protein CRP, which is implicated in the etiology of atherosclerosis) and anti-inflammatory mediators such as IL-10. Furthermore, there is limited information on the mediating effects of performance on the multi-stage fitness test (MSFT),  $\dot{V}O_2$  peak and adiposity on risk factors for cardio-metabolic disease in adolescents.

**Purpose:** To examine the effect of performance on the MSFT,  $\dot{V}O_2$  peak and adiposity on risk factors for cardio-metabolic diseases in adolescents.

**Methods:** Following ethical approval, 121 adolescents (11.3  $\pm$  0.8 year) completed the study. Risk factors for cardio-metabolic disease (circulating inflammatory cytokines, blood glucose and plasma insulin concentrations) was assessed using a fasted capillary blood sample. Participants were separated into quartiles based upon distance ran during the MSFT, the blood lactate response to submaximal exercise,  $\dot{V}O_2$  peak (determined during an uphill graded treadmill test), and adiposity (determined as the sum of four skinfolds). The blood lactate response to submaximal exercise and  $\dot{V}O_2$  peak were measured in a sub-group of participants. Data were analyzed using two-way between-subjects ANCOVA and multiple linear regression.

**Results:** Participants with the lowest performance on the MSFT had higher blood concentrations of IL-6 (3.25  $\pm$  0.25 pg mL<sup>-1</sup>) and IL-1 $\beta$  (4.78  $\pm$  0.54 pg mL<sup>-1</sup>) and lower concentrations of IL-10 (1.80  $\pm$  0.27 pg mL<sup>-1</sup>) when compared with all other quartiles (all  $p < 0.05$ ). Yet, when categorized into  $\dot{V}O_2$  peak quartiles, no differences existed in any of the inflammatory mediators (all  $p > 0.05$ ). Performance on the MSFT was the only predictor of IL-6 ( $\beta = -0.291$ ,  $p = 0.031$ ), IL-1 $\beta$  ( $\beta = -0.405$ ,  $p = 0.005$ ), IL-10 ( $\beta = 0.325$ ,  $p = 0.021$ ) and fasted blood glucose ( $\beta = -0.545$ ,  $p < 0.001$ ) concentrations. Adiposity was the only predictor of plasma insulin concentration



( $\beta = 0.515$ ,  $p < 0.001$ ) and blood pressure (diastolic:  $\beta = 0.259$ ,  $p = 0.042$ ; mean arterial pressure:  $\beta = 0.322$ ,  $p = 0.011$ ).

**Conclusion:** Enhanced performance on the MSFT, but not  $\dot{V}O_2$  peak, was associated with a favorable inflammatory profile in adolescents; whilst adiposity adversely affected plasma insulin, diastolic and mean arterial blood pressure. These findings demonstrate that enhancing performance on the MSFT and maintaining a healthy body composition are a potential therapeutic intervention for the attenuation of risk factors for cardio-metabolic diseases in adolescents.

**Keywords:** low-grade chronic inflammation, insulin resistance, cardio-metabolic disease, multi-stage fitness test,  $\dot{V}O_2$  peak, adiposity

## INTRODUCTION

Low-grade chronic inflammation is a key risk factor in the pathogenesis of cardio-metabolic diseases (including hypertension, hyperglycemia and early insulin resistance) and atherosclerotic plaques (Balagopal et al., 2011). The presence of low-grade chronic inflammation is currently the strongest predictor of cardiovascular events in adults, bettering traditional markers of dyslipidemia and hypertension (Petersen and Pedersen, 2005). Although cardiovascular disease typically presents during adulthood, the prevalence of low-grade chronic inflammation in adolescents (Balagopal et al., 2011) is of concern, as early and continued exposure increases the risk of early onset cardiovascular disease and type 2 diabetes (Gleeson et al., 2011).

Low-grade chronic inflammation is a chronic, 2- to 3- fold elevation in the concentrations of inflammatory mediators, including interleukin- $1\beta$  (IL- $1\beta$ ), interleukin-6 (IL-6), interleukin-1 receptor antagonist (IL-1ra), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and the acute phase protein C-reactive protein (CRP) (Petersen and Pedersen, 2005). Acute bouts of physical activity are implicated in the prevention of low-grade chronic inflammation through the anti-inflammatory response that occurs post-exercise (Gleeson et al., 2011). Recently, it has been shown that acute bouts of games-based activity transiently increased concentrations of anti-inflammatory mediators IL-10 and IL-1ra in healthy young people (Dring et al., 2019) and middle-aged men (Mendham et al., 2015). Increased concentrations of IL-10 and IL-1ra are reported to inhibit the synthesis of pro-inflammatory cytokines (IL- $1\beta$  and TNF- $\alpha$ ) and improve insulin sensitivity when assessed *in vitro* (Gleeson et al., 2011). Furthermore, regular participation in physical activity prevents excessive adiposity (Van der Heijden et al., 2012) and reduces adiposity in overweight adolescents (Rey et al., 2017) and adults (Alrushud et al., 2017). Although such findings support regular moderate intensity physical activity as a potential therapeutic intervention that protects against the development of risk factors for cardio-metabolic disease, the chronic effects of regular training resulting in enhanced physical fitness on low-grade chronic inflammation in adolescents are relatively unknown.

When assessing the effect of physical fitness on low-grade chronic inflammation a comprehensive range of inflammatory mediators (IL- $1\beta$ , IL-6, TNF- $\alpha$ , and CRP) should be measured

(Petersen and Pedersen, 2005). Yet, in adolescents and adults, research has focused on the relationship between physical fitness and a limited number of pro-inflammatory mediators (IL-6, TNF- $\alpha$ , and CRP) (Platat et al., 2006; Ischander et al., 2007; Bugge et al., 2012; Buchan et al., 2015). In adolescents, the findings of previous studies assessing the relationship between physical fitness and inflammatory mediators IL-6, TNF- $\alpha$  and CRP are inconclusive with no apparent relationship (Platat et al., 2006; Steene-Johannessen et al., 2013), or inverse associations observed (Bugge et al., 2012; Silva et al., 2014; Buchan et al., 2015). Furthermore, the relationship between physical fitness and concentrations of anti-inflammatory mediator IL-10 is unknown despite the potential of IL-10 to reduce low-grade chronic inflammation and improve insulin sensitivity (Petersen and Pedersen, 2005). Increasing adiposity reduces the expression of IL-10 in normal weight and overweight individuals (Esposito and Giugliano, 2004; Utsal et al., 2013), whereas the effect of physical fitness on IL-10 concentration has only been studied once, in healthy, normal weight adults (Jürimäe et al., 2017a) and once in pubertal girls (Jürimäe et al., 2017b). Jürimäe et al. (2017a) reported no relationship between maximal oxygen uptake and IL-10 concentration in well-trained adult rowers. These null findings might relate to the well-trained study population, in that the variability of fitness among the participants was not diverse enough for a relationship to be established. However, when comparing well-trained, female adolescent rhythmic gymnasts against untrained counterparts, there was still no difference across 12 markers of inflammation, which included anti-inflammatory mediator IL-10 (Jürimäe et al., 2017b). Whilst the inflammatory profiles of the trained gymnasts and the untrained controls were similar, there was no measurement of physical fitness or body composition in the pubertal girls; therefore, the relationship between physical fitness, inflammatory markers and IL-10 concentration remains unknown, particularly in young people.

In previous studies in adolescents and adults, the effect of long-term training on risk factors for cardio-metabolic disease has been determined by peak oxygen consumption when using graded treadmill tests (Ischander et al., 2007; Bugge et al., 2012; Silva et al., 2014) and graded cycle ergometer tests (Steene-Johannessen et al., 2013). The discrepant findings of previous research could relate to the limitations of  $\dot{V}O_2$

peak as a measure of physical fitness (Coyle et al., 1983), as  $\dot{V}O_2$  peak is considered to be relatively insensitive to changes in training status, with up to 50% of an individual's  $\dot{V}O_2$  peak being determined by genetics (Bouchard, 2012). Regular participation in moderate-to-vigorous activity moderates an individual's exercise capacity and is the mechanism that stimulates the transient inflammatory response that prevents low-grade chronic inflammation (Dring et al., 2019). When focusing on the relationship between physical fitness and risk factors for cardio-metabolic disease the measurement of fitness should therefore be sensitive to changes in an individual's ability to perform prolonged exercise (Strasser and Burtcher, 2018). The blood lactate response to submaximal exercise is more sensitive to changes in training status than maximal oxygen uptake in both adults (Edwards et al., 2003) and young people (Grant, 2001). Furthermore, the submaximal nature of the test allows the assessment of a heterogeneous population and therefore allows the comparison of individuals from sedentary, recreationally active and well-trained backgrounds. Performance on the MSFT is also a commonly used, reliable and easy to administer, field measure of physical fitness in young people (Ortega et al., 2008) and is sensitive to changes in training status (Aziz et al., 2005). Therefore, the blood lactate response to submaximal exercise and the MSFT are potentially better suited for examining the relationship between physical fitness (physical capacity to perform prolonged exercise) and risk factors for cardio-metabolic disease.

As excessive adiposity mediates an increase in low-grade chronic inflammation, several studies have assessed the relationship between different measures of body composition and levels of the pro-inflammatory mediators IL-6 and TNF- $\alpha$  (Galcheva et al., 2011; Bugge et al., 2012; Utsal et al., 2013; Lopez-Alcaraz et al., 2014). Findings are inconclusive in that adiposity has been reported to have no effect on the pro-inflammatory mediators in several studies (Steene-Johannessen et al., 2013; Lopez-Alcaraz et al., 2014). However, increased adiposity has been associated with higher IL-6 and TNF- $\alpha$  concentration in adolescents in other studies (Galcheva et al., 2011; Bugge et al., 2012; Utsal et al., 2013). Of the studies that have examined adiposity, only one has considered the potential mediating effects of physical fitness (Silva et al., 2014). In the study of Silva et al. (2014) maximal oxygen uptake test was the best predictor of metabolic risk (calculated from traditional risk factors including blood pressure and dyslipidemia). Although these findings suggest that physical fitness is important for the prevention of traditional cardio-metabolic risk factors, it remains unknown whether physical fitness or adiposity best predicts, or whether these variables additively predict, risk factors for cardio-metabolic disease in adolescents.

Therefore, the aim of the present study was to determine the effect of MSFT performance, the blood lactate response to submaximal exercise,  $\dot{V}O_2$  peak and adiposity on a comprehensive panel of pro- and anti-inflammatory cytokines in conjunction with traditional cardio-metabolic risk factors in adolescents. A secondary aim of the study was to determine whether peak oxygen uptake (also influenced by genetics), MSFT performance or blood lactate concentration during

sub-maximal exercise (better markers of the capacity to perform prolonged exercise) or adiposity better predict risk factors for cardio-metabolic disease in adolescents.

## MATERIALS AND METHODS

### Participant Characteristics

A cross-sectional sample of 140 adolescents aged 10–12 years were recruited to participate in the present study. Given that 19 participants withdrew from the study ( $n = 10$  due to illness,  $n = 5$  due to injury and  $n = 4$  due to reluctance to provide a capillary blood sample), 121 young people (61 male, 60 female, age  $11.3 \pm 0.8$  year) participated. All participants underwent anthropometric measures of body mass, height and sitting stature to predict age at peak height velocity (APHV, calculated using the method described in Moore et al., 2015), as the preferred measure of maturation. Body mass was measured using a Seca 770 digital scale which is accurate to 0.1 kg (Seca, Hamburg, Germany), and height was measured using a Leicester Height Measure which is accurate 0.1 cm (Seca, Hamburg, Germany), to allow the determination of body mass index BMI, [calculated as body mass (kg)/stature (m)<sup>2</sup>]. Participant characteristics were; height  $151.9 \pm 7.2$  cm, body mass  $43.1 \pm 9.5$  kg, BMI Percentile  $52.3 \pm 29.3$ ; years from peak height velocity  $1.9 \pm 0.7$  year (males  $-2.0 \pm 0.7$  year; females  $-1.9 \pm 0.8$  year).

### Study Design

Ethical approval was received from the Nottingham Trent University's Ethical Advisory Committee (SPOR-400). Participants were recruited from local secondary schools and sports clubs in the East Midlands, United Kingdom. Written parental consent and verbal child assent was obtained during recruitment. Health screen questionnaires were completed by the participants' parent/guardian and checked by a lead investigator to ensure there were no medical conditions that might affect participation in the study.

All trials were separated by a minimum of 7 days (further details of which are provided below). The field measurements (completed during the first trial) consisted of anthropometric measures (body mass, stature and sitting stature), skinfolds and the MSFT, in that order. The health measurements (completed during the second trial, which commenced at  $\sim 8.30$  am) consisted of resting blood pressure followed by a resting capillary blood sample (fasted from 9 pm the previous evening). Finally, a sub-sample of participants (68 participants, 30 male, age:  $11.6 \pm 0.6$ ; APHV:  $-1.9 \pm 0.7$  year) completed exercise laboratory tests including a submaximal treadmill test and a  $\dot{V}O_2$  peak test, which were separated by 20 min passive recovery. Only a sub-sample of participants from the study population volunteered to complete the final part of the study. Those that removed themselves from the exercise laboratory tests did so as they were not willing to take an additional day off school. Prior to all measurements, participants were asked to refrain from moderate-to-vigorous physical activity for 24 h. A telephone call was made to parents/guardians the evening prior to the testing sessions to ensure compliance with the study requirements.

## Field Measures

### Body Composition

Skinfold thickness was measured using a Harpenden Caliper (Baty International, Burgess, Hill, United Kingdom) at four sites (triceps, subscapular, supraspinale, front thigh). All measurements were taken twice in rotation and on the right-hand side of the body. An average of the two measurements was taken unless the difference between the two measurements was >5%. In this circumstance, a third measurement was taken and the median value used as the criterion measure. All skinfold measures were completed by trained kinanthropometrists using methods described in The International Society for the Advancement of Kinanthropometry (2001). The use of skinfolds in assessing body composition in young people is reported as an effective, valid and reliable method in young people (Yeung and Hui, 2010; Bugge et al., 2012). Specifically, the sum of the four skinfold thickness scores was the preferred assessment of body composition in the present study, as estimating body fat percentage from skinfold thickness has been associated with large random error and significant systematic error (Reilly et al., 1995).

### Multi-Stage Fitness Test (MSFT)

During the MSFT, participants completed progressive 20-m shuttle runs until the point of volitional exhaustion (Ramsbottom et al., 1988). The MSFT started at a speed of 8.5 km h<sup>-1</sup> and increased by 0.5 km h<sup>-1</sup> for each 1-min stage completed. Participants were fitted with a heart rate monitor (First Beat Technologies Ltd., Finland) prior to the start of the test and heart rate was monitored in real-time throughout its duration. Verbal encouragement was provided throughout to ensure participants worked to the point of volitional exhaustion. The distance ran during the MSFT was used as the criterion measure.

## Health Measures

### Blood Pressure

On arrival at the exercise laboratory following an overnight fast, participants were seated quietly for 5 min prior to the measurement of blood pressure. Two blood pressure measurements were taken from each participant's left arm, which was rested at chest height, using an HBP-1300-United Kingdom sphygmomanometer (Omron, Milton Keynes, United Kingdom). The average of the two blood pressure measures was used as the criterion measure. If systolic blood pressure differed by >5 mmHg, then a third blood pressure measurement was taken and the median value used as the criterion measure. Mean arterial blood pressure was determined using the following calculation (Smeltzer et al., 2010):  $\text{diastolic blood pressure} + \{0.33 * (\text{systolic blood pressure} - \text{diastolic blood pressure})\}$ .

### Capillary Blood Samples

Capillary blood samples were obtained early in the morning following an overnight fast and during the speed lactate treadmill test (baseline and following each progressive stage). Prior to the fasted capillary blood sample, participants' hands were warmed by submersion in warm water with clothing placed over their chosen arm to increase capillary blood flow. A Unistik single-use lancet (Unistik Extra, 21G gauge, 2.0 mm depth, Owen Mumford

Ltd., United Kingdom) was used and blood was collected into three 300 μl EDTA microvettes (Sarstedt Ltd., United Kingdom). A 25 μl whole blood sample was collected using a plain pre-calibrated glass pipette (Hawksley Ltd., United Kingdom) and immediately dispensed into 250 μl of ice-cooled 2.5% v/v perchloric acid to be deproteinised. The whole blood samples and the diluted perchloric acid samples were centrifuged at 1500 × g for 5 min (Eppendorf 5415C, Hamburg, Germany). Plasma was pipetted from the original whole blood samples and placed into one of three 500 μl plastic vials for subsequent analysis. All samples were immediately frozen at -20°C and transferred to a -80°C freezer at the earliest opportunity.

Blood glucose concentrations were determined in duplicate using a commercially available assay (GOD/PAP method, GL364, Randox, Ireland) and were read spectrophotometrically (intra-assay coefficient of variation (CV) = 2.3%). Plasma insulin concentrations were determined using a commercially available ELISA (Mercodia Ltd., Sweden; CV = 3.2%). Fasted blood glucose and plasma insulin concentration were used to calculate the HOMA index (fasting plasma insulin (μU mL<sup>-1</sup>) × fasting blood glucose (mmol L<sup>-1</sup>)/22.5), as a measure of insulin resistance in adolescents (Keskin et al., 2005). Pro-inflammatory (IL-1β, IL-6, TNF-α) and anti-inflammatory (IL-10) cytokine concentrations were determined using an AimPlex, flow cytometry-based multiplex immunoassay (YSL Bioprocess Development Company, Pomona, United States) and a Beckman Coulter Gallios™ flow cytometer and Kaluza™ acquisition and analysis software (Beckman Coulter, London, United Kingdom). CRP concentrations were determined using the same approach, but on a separate plate. The intra-assay CV based on eight repeat measurements for inflammatory cytokines were as follows: IL-6: 15.9%, IL-1β: 17.4%, TNF-α: 14.7%, IL-10: 13.2% and CRP: 10.4%. Blood lactate concentrations were determined in duplicate using a commercially available assay (PAP method, LC2389, Randox, Ireland) and were read spectrophotometrically (CV = 6.7%).

## Exercise Laboratory Measures

### Blood Lactate During Sub-Maximal Exercise

A sub-sample of participants completed a submaximal test on a calibrated treadmill (Technogym, Italy). Prior to participation, participants were fitted with a heart rate monitor (First Beat Technologies Ltd., Finland) and maximum heart rate during the final minute of each stage was recorded. Participants completed three to six, 4-min runs, interspersed with 1-min rest whilst a capillary blood sample was taken. The first stage of the test was completed at an individualized speed that was comfortable for the participant (between 6 and 8 km<sup>-1</sup> h<sup>-1</sup>), which increased by 1 km<sup>-1</sup> h<sup>-1</sup> for each stage completed thereafter. The blood lactate concentration at 8.5 km h<sup>-1</sup> was used as the criterion measure and was calculated by mathematically fitting a curve to the blood lactate-running speed relationship.

### VO<sub>2</sub> Peak Test

A sub-sample of participants completed a maximal oxygen uptake test on a treadmill to measure  $\dot{V}O_2$  peak (ml kg<sup>-1</sup> min<sup>-1</sup>).

The speed of the test was constant and individualized for each participant based on the speed that corresponded with 85%  $HR_{max}$  during the prior submaximal test. The gradient of the treadmill increased by 1% per minute of the test completed. Participants were required to run to the point of volitional exhaustion, which was indicated by the participant's rating of perceived exertion on a 6 – 20 Borg scale (Borg, 1998) in conjunction with live monitoring of their heart rate. Prior to the exercise laboratory tests, all participants were shown the Borg scale and given an age appropriate explanation of the information provided from this psychological evaluation of perceived exertion. Participants were instructed to point to the scale to indicate the rating relating to how intense the exercise felt when they were shown the scale. During the final minute of the test, participants breathed expired air into a Douglas Bag, which was later analyzed on a Servomex 1440 Gas Analyser (Servomex, United States) to calculate  $\dot{V}O_2$  peak ( $ml\ kg^{-1}\ min^{-1}$ ). Verbal encouragement was provided throughout the test to ensure the participant worked to the point of volitional exhaustion.

## Statistical Analysis

An *a priori* power calculation was performed using GPower 3.1.9.2 and based on IL-6 data in previous research (Ischander et al., 2007), with an alpha probability level of 0.05, 4 groups and 1 covariate; a total sample size of 107 was required.

Participants were separated into distinct fitness quartiles quantified by distance ran on the MSFT, blood lactate concentration at 8.5  $km\ h^{-1}$  and maximal oxygen uptake determined from the  $\dot{V}O_2$  peak test (Table 1). Adiposity quartiles were quantified from the sum of skinfolds. The first quartile (defined as the 25th percentile, which included participants with values  $\leq 25\%$  of all values in the present study) included participants with the lowest physical fitness and highest adiposity. The effect of physical fitness and adiposity quartile on risk factors for cardio-metabolic disease was analyzed via two-way between subjects ANCOVA with maturation (APHV) used as a covariate in SPSS (Version 24, SPSS Inc., Chicago, IL, United States). When significant interactions were observed between physical fitness/adiposity

and sex, *post hoc* comparisons were performed using a least significant difference (LSD) correction. *Post hoc* comparisons interrogated significant interactions between boys and girls within quartiles and within sex comparisons across quartiles. Where significant effects existed, effect sizes were calculated as Cohen's *d*. Multiple linear regression was used to examine the relationship (adjusted for APHV) between independent variables (distance on the MSFT,  $\dot{V}O_2$  peak and adiposity) and each cardio-metabolic risk factor (IL-6, IL-1 $\beta$ , IL-10, TNF- $\alpha$ , CRP, fasted blood glucose and plasma insulin, HOMA, systolic, diastolic and mean arterial blood pressure). Blood lactate concentration during sub-maximal exercise was not examined in the multiple linear regression, as the sample size did not meet the minimum criteria necessary for four predictor variables (Vanvoorhis and Morgan, 2007). For all analysis significance was accepted as  $P < 0.05$  and data are presented as mean  $\pm$  SEM.

## RESULTS

Quartiles for each variable (distance ran on the MSFT,  $\dot{V}O_2$  peak, blood lactate concentration at 8.5  $km\ h^{-1}$  and adiposity) were separately determined for boys and girls (Table 1). When considering the effect of sex on MSFT performance, boys ran further than their female counterparts across all quartiles (all  $p < 0.001$ ). Similarly, boys in quartiles one to three had a higher peak oxygen consumption, a lower blood lactate concentration at 8.5  $km\ h^{-1}$  and lower adiposity when compared with their female counterparts (all  $p < 0.001$ ). There was no difference between boys and girls in  $\dot{V}O_2$  peak ( $p = 0.970$ ) or adiposity ( $p = 0.086$ ; Table 1) in quartile four. BMI had no statistically significant effect on any of the outcome variables (all  $p > 0.05$ ).

## Inflammation

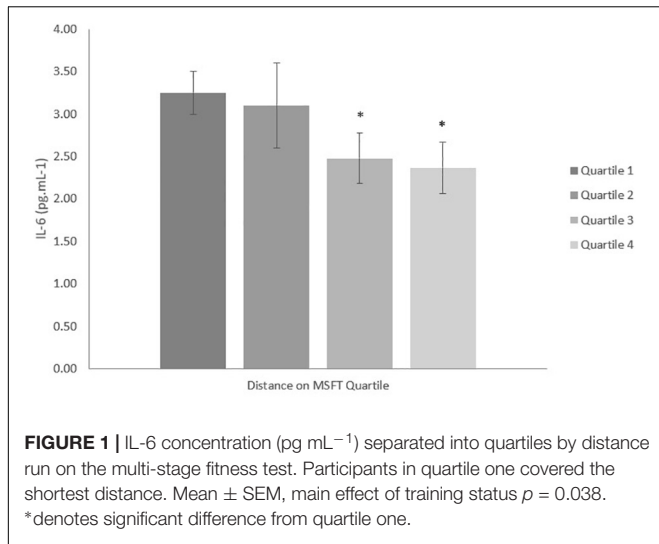
### IL-6

When separating participants into quartiles based on distance ran on the MSFT, IL-6 concentration was higher in quartile one when compared with participants in the third ( $p = 0.011$ ,  $d = 0.6$ ) and fourth quartiles [ $p = 0.009$ ,  $d = 0.7$ ; main effect:  $F_{(3,90)} = 2.9$ ,  $p = 0.038$ ; Figure 1 and Table 2]. There was no difference in IL-6 concentration when separating participants

**TABLE 1** | Performance in the multi-stage fitness test (distance run),  $\dot{V}O_2$  peak, Blood lactate at 8.5  $km\ h^{-1}$  on the speed lactate test and adiposity from sum of skinfolds separated by sex and into quartiles.

Quartile	Distance run on the MSFT (m)		$\dot{V}O_2$ Peak ( $ml\ kg^{-1}\ min^{-1}$ )		Blood lactate at 8.5 $km\ h^{-1}$ on the speed lactate test ( $mmol\ L^{-1}$ )		Adiposity from sum of skinfolds (mm)	
	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls
1	860 $\pm$ 60	480 $\pm$ 60*	40.2 $\pm$ 3.2	34.1 $\pm$ 1.5*	2.71 $\pm$ 0.17	5.20 $\pm$ 0.96*	56 $\pm$ 3	97 $\pm$ 3*
2	1300 $\pm$ 20	900 $\pm$ 40*	49.9 $\pm$ 0.6	42.9 $\pm$ 0.8*	2.30 $\pm$ 0.27	3.62 $\pm$ 0.79*	40 $\pm$ 1	54 $\pm$ 2*
3	1500 $\pm$ 20	1160 $\pm$ 20*	52.7 $\pm$ 0.4	48.9 $\pm$ 0.7*	1.95 $\pm$ 0.38	2.62 $\pm$ 0.54*	34 $\pm$ 1	39 $\pm$ 1*
4	1800 $\pm$ 40	1540 $\pm$ 60*	57.9 $\pm$ 1.2	58.0 $\pm$ 1.3	1.07 $\pm$ 0.22	1.61 $\pm$ 0.88	27 $\pm$ 1	29 $\pm$ 1

Participants in quartile one had the lowest physical fitness or highest adiposity (Mean  $\pm$  SEM). \*Denotes significant differences between boys and girls in respective quartiles.



by  $\dot{V}O_2$  peak, blood lactate concentration at 8.5 km h<sup>-1</sup> or adiposity (all *p* > 0.05), nor was there any difference between boys and girls (main effect of sex: all *p* > 0.05; interaction effect: all *p* > 0.05). The multiple regression analysis (Table 3) revealed that distance ran on the MSFT was the only statistically significant predictor of IL-6 concentration, after adjustment for APHV, with a negative relationship observed between the two variables ( $\beta = -0.291, p = 0.031$ ).

### IL-1 $\beta$

When separating participants into quartiles based on distance ran on the MSFT, IL-1 $\beta$  concentration was higher in quartile one when compared with participants in the third (*p* = 0.039, *d* = 0.6) and fourth quartiles [*p* = 0.008, *d* = 0.8; main effect:  $F_{(3,96)} = 3.1, p = 0.032$ ; Figure 2 and Table 2]. There was no difference in IL-1 $\beta$  concentration when separating participants by  $\dot{V}O_2$  peak, blood lactate concentration at 8.5 km h<sup>-1</sup> or adiposity (all *p* > 0.05). When considering the effect of sex, IL-1 $\beta$  concentration was higher in boys than girls [boys; 4.26 ± 0.44 pg·mL<sup>-1</sup>, girls; 2.94 ± 0.45 pg·mL<sup>-1</sup>; main effect of sex:  $F_{(1,96)} = 4.4, p = 0.039, d = 0.4$ ]. The effect of fitness or adiposity was not different between boys and girls (interaction: all *p* > 0.05). The multiple regression analysis (Table 3) revealed that distance ran on the MSFT was the only statistically significant predictor of IL-1 $\beta$  concentration, after adjustment for APHV, with a negative relationship observed between the two variables ( $\beta = -0.405, p = 0.005$ ).

### IL-10

When separating participants into quartiles determined by blood lactate concentration at 8.5 km h<sup>-1</sup>, IL-10 concentration was lower in quartile one when compared with participants in quartile four [*p* = 0.006, *d* = 0.9; main effect:  $F_{(3,27)} = 3.6, p = 0.035$ , Figure 3 and Table 2]. There was no difference in IL-10 concentration when separating participants by distance ran on the MSFT,  $\dot{V}O_2$  peak or adiposity (all *p* > 0.05), nor was there any difference between boys and girls (main effect of sex: all *p* > 0.05; interaction: all *p* > 0.05). The multiple regression

**TABLE 2** | Inflammatory cytokines (IL-6, IL-1 $\beta$ , IL-10, TNF- $\alpha$ ) and CRP separated into quartiles determined from distance run on the multi-stage fitness test, blood lactate concentration at 8.5 km h<sup>-1</sup> during the speed lactate test,  $\dot{V}O_2$  peak and adiposity (Mean ± SEM).

	Distance Run on the MSFT (m)				$\dot{V}O_2$ Peak (ml kg <sup>-1</sup> min <sup>-1</sup> )				Blood lactate at 8.5 km h <sup>-1</sup> during speed lactate test (mmol L <sup>-1</sup> )				Adiposity from sum of skinfolds (mm)			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
IL-6 (pg mL <sup>-1</sup> )	3.25 ± 0.25	3.10 ± 0.50	2.48 ± 0.30*	2.37 ± 0.30*	3.76 ± 0.54	3.47 ± 0.29	2.60 ± 0.37	2.60 ± 0.32	3.57 ± 0.52	3.72 ± 0.57	2.70 ± 0.50	2.88 ± 0.53	3.05 ± 0.26	3.23 ± 0.35	2.95 ± 0.29	2.47 ± 0.29
IL-1 $\beta$ (pg mL <sup>-1</sup> )	4.78 ± 0.85	4.34 ± 0.47	2.96 ± 0.29*	2.47 ± 0.29*	4.67 ± 1.70	3.14 ± 0.63	3.16 ± 0.62	3.25 ± 0.71	7.35 ± 2.60	3.15 ± 0.65	2.72 ± 1.25	2.82 ± 0.68	5.51 ± 1.06	3.36 ± 0.39	3.58 ± 0.44	2.86 ± 0.34
IL-10 (pg mL <sup>-1</sup> )	1.80 ± 0.31	2.08 ± 0.19	2.41 ± 0.41	3.80 ± 0.77	2.27 ± 0.43	2.17 ± 0.23	2.18 ± 0.37	3.82 ± 1.23	1.65 ± 0.45	2.61 ± 0.48	2.96 ± 0.61	3.62 ± 0.45*	2.18 ± 0.31	2.11 ± 0.31	2.97 ± 0.76	2.40 ± 0.38
TNF- $\alpha$ (pg mL <sup>-1</sup> )	1.93 ± 0.53	1.47 ± 0.20	1.47 ± 0.19	1.42 ± 0.24	1.71 ± 0.34	1.24 ± 0.15	1.59 ± 0.23	1.74 ± 0.32	2.32 ± 0.62	2.00 ± 0.57	1.21 ± 1.91	1.86 ± 0.42	1.89 ± 0.17	1.60 ± 0.26	1.65 ± 0.24	1.89 ± 0.54
CRP (mg L <sup>-1</sup> )	0.52 ± 0.14	0.47 ± 0.21	0.52 ± 0.31	0.35 ± 0.19	0.43 ± 0.14	0.45 ± 0.15	0.41 ± 0.16	0.30 ± 0.10	0.69 ± 0.21	0.68 ± 0.28	0.86 ± 0.39	0.45 ± 0.20	0.52 ± 0.14	0.47 ± 0.14	0.45 ± 0.16	0.38 ± 0.10

\*denotes significantly different from quartile one.

**TABLE 3** | Standardized regression summary for distance run on the MSFT,  $\dot{V}O_2$  peak, and adiposity with individual risk factors.

	Distance run on MSFT (m)			$\dot{V}O_2$ peak test ( $\text{ml kg}^{-1} \text{min}^{-1}$ )			Adiposity from sum of skinfolds (mm)		
	$R^2$ adj.	$\beta$	$p$	$R^2$ adj.	$\beta$	$p$	$R^2$ adj.	$\beta$	$p$
IL-6 ( $\text{pg mL}^{-1}$ )	0.085	-0.291	0.031*	0.035	0.060	0.800	-0.004	-0.005	0.978
IL-1 $\beta$ ( $\text{pg mL}^{-1}$ )	0.164	-0.405	0.005*	0.244	0.313	0.106	0.004	0.004	0.981
IL-10 ( $\text{pg mL}^{-1}$ )	0.108	0.325	0.021*	0.134	0.151	0.419	0.118	0.173	0.419
TNF- $\alpha$ ( $\text{pg mL}^{-1}$ )	0.098	0.167	0.397	0.054	0.107	0.489	0.120	0.178	0.420
Blood glucose ( $\text{mmol L}^{-1}$ )	0.297	-0.545	< 0.001*	-0.113	-0.145	0.390	0.152	0.190	0.246
Plasma insulin ( $\text{mU L}^{-1}$ )	-0.079	-0.097	0.563	-0.150	-0.172	0.269	0.266	0.515	< 0.001*
HOMA	-0.096	-0.127	0.488	-0.105	-0.122	0.450	0.256	0.506	< 0.001*
Systolic blood pressure (mmHg)	0.060	-0.091	0.666	-0.025	-0.102	0.855	0.31	0.142	0.825
Diastolic blood pressure (mmHg)	0.094	0.135	0.472	0.000	0.000	0.998	0.067	0.259	0.042*
Mean arterial pressure (mmHg)	0.115	0.163	0.383	-0.018	-0.023	0.892	0.088	0.332	0.011*

\*denotes significant relationship.

analysis (Table 3) revealed that distance run on the MSFT was the only statistically significant predictor of IL-10 concentration, after adjustment for APHV, with a positive relationship observed between the two variables ( $\beta = 0.325$ ,  $p = 0.021$ ).

### TNF- $\alpha$ and CRP

When separating participants into quartiles by distance covered on the MSFT,  $\dot{V}O_2$  peak, blood lactate concentration at 8.5 km  $\text{h}^{-1}$  and adiposity there was no difference in TNF- $\alpha$  or CRP concentration across quartiles (all  $p > 0.05$ , Table 2). Furthermore, there was no difference between boys and girls (main effect of sex: all  $p > 0.05$ ; interaction: all  $p > 0.05$ ). Multiple regression revealed no statistically significant predictors of TNF- $\alpha$  or CRP concentration (Table 3).

## Blood Glucose, Plasma Insulin Concentration and HOMA

### Fasting Blood Glucose

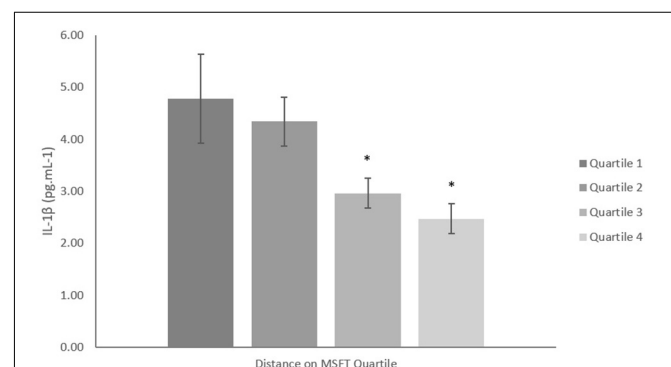
When separating participants into quartiles determined by distance run on the MSFT, blood glucose concentration was higher in quartile one when compared with quartile two ( $p = 0.025$ ,  $d = 0.5$ ), three ( $p < 0.001$ ,  $d = 1.1$ ) and four [ $p < 0.001$ ,  $d = 1$ ; main effect:  $F_{(3,110)} = 7.1$ ,  $p < 0.001$ ; Table 4]. When separating participants into  $\dot{V}O_2$  peak quartiles, blood glucose concentration was higher in quartile one when compared with participants in the fourth quartile [ $p = 0.001$ ,  $d = 1.1$ ; main effect:  $F_{(3,68)} = 3.9$ ,  $p = 0.013$ ; Table 4]. When considering the effect of sex there was no difference between boys and girls (main effect of sex: all  $p > 0.05$ ; interaction: all  $p > 0.05$ ). The multiple regression analysis (Table 3) revealed that distance run on the MSFT was the only statistically significant predictor of blood glucose concentration, after adjustment for APHV, with a negative relationship observed ( $\beta = -0.545$ ,  $p < 0.001$ ).

When separating participants into adiposity quartiles, blood glucose concentration was higher in quartile one when compared with participants in quartile four [ $p = 0.012$ ,  $d = 0.6$ ; main effect:  $F_{(3,115)} = 3.0$ ,  $p = 0.035$ ; Table 4]. Participants in quartile two also had higher blood glucose concentration when compared with quartile four (second quartile;  $p = 0.011$ ,  $d = 0.5$ ). There was

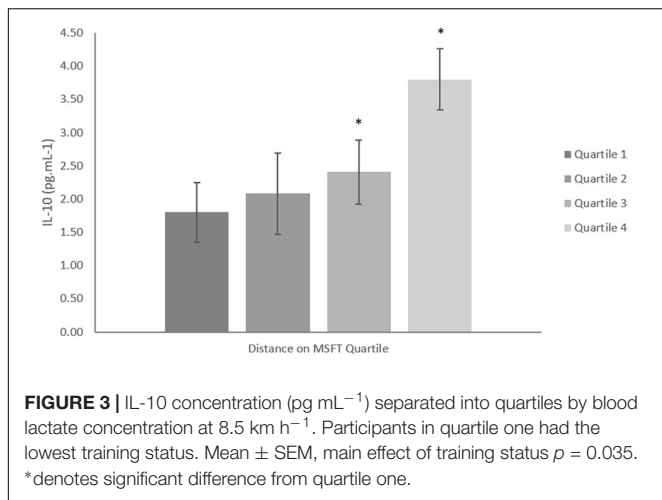
no difference in blood glucose concentration across all quartiles between boys and girls (main effect of sex:  $p = 0.637$ ). There was an effect of adiposity on sex [interaction:  $F_{(3,115)} = 3.4$ ,  $p = 0.019$ ], in that girls in the first quartile had higher blood glucose concentration ( $4.81 \pm 0.59 \text{ mmol L}^{-1}$ ) when compared with quartiles two ( $4.12 \pm 0.44 \text{ mmol L}^{-1}$ ,  $p = 0.001$ ,  $d = 1.3$ ), third ( $4.23 \pm 0.52 \text{ mmol L}^{-1}$ ,  $p = 0.004$ ,  $d = 1$ ) and four ( $4.14 \pm 0.46 \text{ mmol L}^{-1}$ ,  $p = 0.001$ ,  $d = 1.3$ ). There was no difference in blood glucose concentration across adiposity quartiles in boys (all  $p > 0.05$ ).

### Fasting Plasma Insulin

When separating participants into quartiles determined by distance run on the MSFT, plasma insulin concentration was higher in quartile one when compared with participants in quartiles three ( $p = 0.005$ ,  $d = 0.8$ ) and four [ $p < 0.001$ ,  $d = 1$ ; main effect:  $F_{(3,102)} = 5.5$ ,  $p = 0.002$ ; Table 4]. When separating participants into quartiles determined by  $\dot{V}O_2$  peak, plasma insulin concentration was higher in participants in quartile one when compared with participants in quartiles three ( $p = 0.009$ ,  $d = 0.7$ ) and four [ $p < 0.001$ ,  $d = 1$ ; main effect:  $F_{(3,62)} = 5.8$ ,



**FIGURE 2** | IL-1 $\beta$  concentration ( $\text{pg mL}^{-1}$ ) separated into quartiles by distance run on the multi-stage fitness test. Participants in quartile one covered the shortest distance. Mean  $\pm$  SEM; main effect of training status  $p = 0.032$ . \*denotes significant difference from quartile one.



$p = 0.002$ ; **Table 4**). Participants in quartile two also had higher plasma insulin concentrations when compared with quartile four ( $p = 0.009$ ,  $d = 0.7$ ). When separating participants into quartiles determined from blood lactate concentration at  $8.5 \text{ km h}^{-1}$ , plasma insulin concentration was higher in quartile one when compared with participants in quartile four [ $p = 0.012$ ,  $d = 0.9$ ; main effect:  $F_{(3,28)} = 3.8$ ,  $p = 0.043$ ; **Table 3**]. When considering the effect of sex, plasma insulin concentration was higher in girls ( $7.73 \pm 0.58 \text{ mU L}^{-1}$ ) than boys [boys:  $6.05 \pm 0.55 \text{ mU L}^{-1}$ ; main effect of sex:  $F_{(1,101)} = 4.4$ ,  $p = 0.037$ ,  $d = 0.4$ ]; yet, the effect of fitness did not differ between boys and girls (interaction: all  $p > 0.05$ ).

When separating participants into quartiles determined by adiposity, plasma insulin concentration was higher in quartile one when compared with participants in quartiles two ( $p = 0.003$ ,  $d = 0.9$ ), three ( $p = 0.044$ ,  $d = 0.6$ ) and four [ $p = 0.004$ ,  $d = 0.8$ ; main effect:  $F_{(3,105)} = 4.0$ ,  $p = 0.010$ ; **Table 4**]. When considering the effect of sex, plasma insulin concentration was higher in girls [ $7.59 \pm 0.56 \text{ mU L}^{-1}$  vs.  $5.86 \pm 0.57 \text{ mU L}^{-1}$ ; main effect of sex:  $F_{(1,105)} = 4.7$ ,  $p = 0.033$ ,  $d = 0.4$ ]. There was an effect of adiposity on sex [interaction:  $F_{(3,105)} = 3.5$ ,  $p = 0.018$ ], in that girls having the highest adiposity had higher plasma insulin concentrations than boys in the same quartile [boys:  $6.15 \pm 0.82 \text{ mU L}^{-1}$ , girls:  $11.81 \pm 1.67 \text{ mU L}^{-1}$ ,  $F_{(1,97)} = 12.9$ ,  $p < 0.001$ ,  $d = 1$ ]. Girls in quartile one had increased plasma insulin concentration when compared with girls in quartiles two ( $6.56 \pm 0.86 \text{ mU L}^{-1}$ ,  $p < 0.001$ ,  $d = 1.2$ ) three ( $6.85 \pm 0.57 \text{ mU L}^{-1}$ ,  $p = 0.002$ ,  $d = 1.1$ ) and four ( $5.11 \pm 0.57 \text{ mU L}^{-1}$ ,  $p < 0.001$ ,  $d = 1.4$ ). There was no difference in plasma insulin concentration in boys across quartiles (all  $p > 0.05$ ). The multiple regression analysis (**Table 3**) revealed that adiposity was the only statistically significant predictor of plasma insulin concentration, after adjustment for APHV, with a positive relationship observed between the two variables ( $\beta = 0.515$ ,  $p < 0.001$ ).

**HOMA**

When separating participants into quartiles determined by distance ran on the MSFT, HOMA was higher in quartile one when compared with participants in quartiles two ( $p = 0.002$ ,

**TABLE 4** | Cardio-metabolic risk factors including blood glucose, plasma insulin, HOMA, and blood pressure separated into quartiles determined from distance run on the multi-stage fitness test, blood lactate concentration at  $8.5 \text{ km h}^{-1}$  during the speed lactate test,  $\text{VO}_2$  peak, and adiposity (Mean  $\pm$  SEM).

	Distance run on the MSFT (m)				$\text{VO}_2$ Peak ( $\text{ml kg}^{-1} \text{ min}^{-1}$ )				Blood lactate at $8.5 \text{ km h}^{-1}$ during speed lactate test ( $\text{mmol L}^{-1}$ )				Adiposity from Sum of Skinfolds (mm)			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Blood Glucose ( $\text{mmol L}^{-1}$ )	4.60 $\pm$ 0.44	4.35 $\pm$ 0.48*	4.08 $\pm$ 0.44*	4.11 $\pm$ 0.53*	4.63 $\pm$ 0.58	4.32 $\pm$ 0.57	4.25 $\pm$ 0.49	4.00 $\pm$ 0.54*	4.48 $\pm$ 0.52	4.32 $\pm$ 0.65	4.36 $\pm$ 0.78	4.32 $\pm$ 0.53	4.54 $\pm$ 0.68	4.27 $\pm$ 0.44	4.19 $\pm$ 0.56	4.20 $\pm$ 0.44*
Plasma Insulin ( $\text{mU L}^{-1}$ )	8.99 $\pm$ 1.04	6.71 $\pm$ 0.76	5.60 $\pm$ 0.54*	4.86 $\pm$ 0.82*	8.49 $\pm$ 1.24	7.07 $\pm$ 0.66	5.18 $\pm$ 0.63*	4.02 $\pm$ 0.54*†	7.00 $\pm$ 0.87	5.60 $\pm$ 0.90	6.23 $\pm$ 0.67	3.51 $\pm$ 0.88*	9.08 $\pm$ 1.07	5.55 $\pm$ 0.69*	6.68 $\pm$ 0.74*	5.70 $\pm$ 0.80*
HOMA	2.00 $\pm$ 0.68	1.22 $\pm$ 0.18*	1.21 $\pm$ 0.18*	0.78 $\pm$ 0.18*	1.81 $\pm$ 0.32	1.48 $\pm$ 0.18	0.90 $\pm$ 0.09*	0.78 $\pm$ 0.13*†	1.35 $\pm$ 0.14	1.61 $\pm$ 0.17	1.01 $\pm$ 0.13	0.60 $\pm$ 0.21	1.90 $\pm$ 0.27	1.14 $\pm$ 0.14*	1.20 $\pm$ 0.15*	1.01 $\pm$ 0.12*
Systolic blood pressure (mmHg)	112 $\pm$ 2	112 $\pm$ 2	111 $\pm$ 2	111 $\pm$ 2	111 $\pm$ 2	108 $\pm$ 2	110 $\pm$ 2	110 $\pm$ 3	104 $\pm$ 2	111 $\pm$ 2	106 $\pm$ 2	113 $\pm$ 3	115 $\pm$ 2	109 $\pm$ 2	112 $\pm$ 2	110 $\pm$ 2
Diastolic blood pressure (mmHg)	69 $\pm$ 2	67 $\pm$ 1	70 $\pm$ 1	73 $\pm$ 1	70 $\pm$ 2	72 $\pm$ 2	68 $\pm$ 1	65 $\pm$ 1	74 $\pm$ 1	70 $\pm$ 2	66 $\pm$ 1	68 $\pm$ 1	73 $\pm$ 1	72 $\pm$ 2	67 $\pm$ 1*	69 $\pm$ 1*
Mean arterial pressure (mmHg)	86 $\pm$ 2	84 $\pm$ 1	82 $\pm$ 1	83 $\pm$ 1	83 $\pm$ 2	84 $\pm$ 1	82 $\pm$ 1	80 $\pm$ 1	84 $\pm$ 1	83 $\pm$ 2	79 $\pm$ 2	83 $\pm$ 1	87 $\pm$ 1	83 $\pm$ 1*	82 $\pm$ 1*	83 $\pm$ 1*

\*denotes significantly different from quartile one; †significantly different from quartile two.

$d = 0.8$ ), three ( $p = 0.002$ ,  $d = 0.8$ ) and four [ $p < 0.001$ ,  $d = 1.4$ ; main effect:  $F_{(3,101)} = 9.4$ ,  $p < 0.001$ ; **Table 4**]. When separating participants into fitness quartiles determined by  $\dot{V}O_2$  peak, HOMA was higher in quartile one when compared with participants in quartiles three ( $p = 0.003$ ,  $d = 0.8$ ) and four [ $p = 0.001$ ,  $d = 1.1$ ; main effect:  $F_{(3,60)} = 5.7$ ,  $p = 0.002$ ; **Table 3**]. Participants in quartile two also had increased HOMA when compared with participants in quartile four ( $p = 0.019$ ,  $d = 0.7$ ). When considering the effect of sex, HOMA was higher in girls ( $1.50 \pm 0.13$ ) than boys [ $1.14 \pm 0.12$ ; main effect of sex:  $F_{(1,99)} = 4.1$ ,  $p = 0.046$ ,  $d = 0.4$ ], yet the effect of  $\dot{V}O_2$  peak on HOMA did not differ between boys and girls (all  $p > 0.05$ ). When separating participants into quartiles by blood lactate concentration at  $8.5 \text{ km h}^{-1}$  there was no difference in HOMA across quartiles (all  $p > 0.05$ , **Table 4**).

When separating participants into quartiles determined by adiposity, HOMA was higher in quartile one when compared with participants in quartiles two ( $p = 0.002$ ,  $d = 0.9$ ), three ( $p = 0.005$ ,  $d = 0.8$ ) and four [ $p < 0.001$ ,  $d = 1$ ; main effect:  $F_{(3,103)} = 5.6$ ,  $p = 0.001$ ; **Table 4**]. When considering the effect of sex, HOMA was higher in girls [ $1.52 \pm 0.12$  vs.  $1.10 \pm 0.12$ ; main effect of sex:  $F_{(1,103)} = 5.9$ ,  $p = 0.017$ ,  $d = 0.5$ ]. There was also an effect of adiposity on sex [interaction:  $F_{(3,103)} = 4.0$ ,  $p = 0.010$ ,  $d = 1.5$ ], in that girls in quartile one had higher HOMA ( $2.58 \pm 0.44$ ) than their male counterparts ( $1.22 \pm 0.19$ ). Girls with the highest adiposity also had increased HOMA when compared with girls in quartiles two ( $1.36 \pm 0.19$ ,  $p = 0.001$ ,  $d = 0.9$ ), third ( $1.18 \pm 0.25$ ,  $p < 0.001$ ,  $d = 1.1$ ) and four ( $0.94 \pm 0.15$ ,  $p < 0.001$ ,  $d = 1.3$ ). There was no difference in HOMA across adiposity quartiles in boys (all  $p > 0.05$ ). The multiple regression analysis (**Table 3**) revealed that adiposity was the only statistically significant predictor of HOMA, after adjustment for APHV, with a positive relationship observed between the two variables ( $\beta = 0.506$ ,  $p < 0.001$ ).

## Blood Pressure

### Systolic Blood Pressure

When separating participants into quartiles based on distance ran during the MSFT,  $\dot{V}O_2$  peak and blood lactate concentration at  $8.5 \text{ km h}^{-1}$  during the speed lactate test or adiposity, there was no difference in systolic blood pressure (all  $p > 0.05$ , **Table 4**). When considering the effect of sex there was no difference in systolic blood pressure between boys and girls (main effect of sex: all  $p > 0.05$ ; interaction: all  $p > 0.05$ ). The regression model for systolic blood pressure identified no statistically significant predictors.

### Diastolic Blood Pressure

When separating participants into adiposity quartiles, diastolic blood pressure was higher in quartile one when compared with participants in quartiles three ( $p = 0.003$ ,  $d = 0.7$ ) and four [ $p = 0.046$ ,  $d = 0.5$ ; main effect:  $F_{(3,116)} = 3.3$ ,  $p = 0.023$ ; **Table 4**]. There was no difference in diastolic blood pressure across quartiles when participants were separated by distance ran during the MSFT,  $\dot{V}O_2$  peak and blood lactate concentration at  $8.5 \text{ km h}^{-1}$  during the speed lactate

test (all  $p > 0.05$ ), nor was there any difference between boys and girls (main effect of sex: all  $p > 0.05$ ; interaction: all  $p > 0.05$ ). The multiple regression analysis (**Table 3**) revealed that adiposity was the only statistically significant predictor of diastolic blood pressure, after adjustment for APHV, with a positive relationship between the two variables ( $\beta = 0.259$ ,  $p = 0.042$ ).

### Mean Arterial Pressure

When separating participants into adiposity quartiles, mean arterial pressure was higher in quartile one when compared with quartiles two ( $p = 0.021$ ,  $d = 0.6$ ), three ( $p = 0.004$ ,  $d = 0.7$ ) and four [ $p = 0.017$ ,  $d = 0.6$ ; main effect:  $F_{(3,116)} = 3.5$ ,  $p = 0.018$ ; **Table 4**]. There was no difference in mean arterial pressure when participants were separated by distance ran during the MSFT,  $\dot{V}O_2$  peak or blood lactate concentration at  $8.5 \text{ km h}^{-1}$  during the speed lactate test (all  $p > 0.05$ ), nor was there any difference between boys and girls (main effect of sex: all  $p > 0.05$ ; interaction: all  $p > 0.05$ ). The multiple regression analysis (**Table 3**) revealed that adiposity was the only statistically significant predictor of mean arterial pressure, after adjustment for APHV, with a positive relationship observed between the two variables ( $\beta = 0.322$ ,  $p = 0.011$ ).

## DISCUSSION

The primary finding of the present study was that adolescents categorized below the 25th centile for distance ran on the MSFT in the current dataset exhibited increased concentrations of pro-inflammatory cytokines IL-6 and IL-1 $\beta$  and reduced concentrations of the anti-inflammatory mediator IL-10 when compared with those categorized above the 25th centile. The present study is the first to report that distance ran on the MSFT and the blood lactate response to exercise were the only measures to influence inflammatory cytokine concentrations in adolescents, both of which are deemed more sensitive measures of an individual's physical capacity to perform prolonged exercise. In addition, the multiple regression revealed that the MSFT was the only significant predictor of inflammation in adolescents (with no relationship observed for  $\dot{V}O_2$  peak or adiposity). Furthermore, adolescents categorized below the 25th percentile with the lowest distance ran on the MSFT and  $\dot{V}O_2$  peak exhibited increased metabolic risk factors (including fasted blood glucose, plasma insulin and HOMA), whilst adolescents with the highest adiposity also presented with increased diastolic and mean arterial blood pressure compared to adolescents in all other quartiles. These findings emphasize the importance of enhancing the physical capacity to perform prolonged exercise, as evidenced by performance on the MSFT, and maintaining a healthy body composition during adolescence in order to attenuate the risk of developing early onset cardiovascular disease and type 2 diabetes.

Adolescents with the lowest MSFT performance in the present study exhibited increased concentrations of pro-inflammatory



cytokines IL-6 and IL-1 $\beta$ , and reduced concentrations of anti-inflammatory mediator IL-10 in comparison to adolescents in all other quartiles. These findings are novel as the present study is the first to measure a range of inflammatory cytokines that are reflective of low-grade chronic inflammation in a heterogeneous population of male and female adolescents (Gleeson et al., 2011). The finding that adolescents with the lowest physical fitness have increased concentrations of pro-inflammatory mediators is consistent with previous studies, in that increased concentrations of IL-6 (Bugge et al., 2012; Buchan et al., 2015) and CRP (Buchan et al., 2015) are observed in participants in the lowest quartile for physical fitness. However, the present study is the first to report that participants with the lowest MSFT performance have reduced circulating levels of IL-10. These findings are in contrast to those of Jürimäe et al. (2017b) whereby IL-10 concentration was similar in female rhythmic gymnasts and untrained controls. These discrepant findings might relate to the different methods used to categorize participants, as the present study measured the participant's physical capacity to perform prolonged exercise and body composition, whereas Jürimäe et al. (2017b) categorized participants solely based on participation in rhythmic gymnastics or not. Therefore, the present study assessed the objective relationship between performance in submaximal and maximal exercise tests and anti-inflammatory mediator IL-10. Increased concentrations of IL-10 protect against risk factors for cardio-metabolic diseases, as *in vitro* studies report that IL-10 inhibits the synthesis of IL-1 $\beta$  and TNF- $\alpha$  which promote the development of low-grade chronic inflammation (Petersen and Pedersen, 2005). As acute bouts of physical activity transiently increase IL-10 concentrations (Petersen and Pedersen, 2005), it is not surprising that participants with the lowest performance on the MSFT had significantly reduced concentrations of the potent anti-inflammatory mediator. Furthermore, there were no differences between pro- or anti-inflammatory cytokine concentrations in adolescents categorized above the 25th percentile, which is consistent with previous research (Buchan et al., 2015). These findings suggest that an enhanced physical capacity to perform prolonged exercise protects against low-grade chronic inflammation in adolescents by reducing exposure to pro-inflammatory mediators and increasing systemic concentrations of the anti-inflammatory cytokine IL-10.

Performance on the MSFT and the blood lactate response to submaximal exercise were the only measures to influence inflammation in adolescents in the present study. This finding was also observed in the multiple regression model, which revealed that distance ran on the MSFT was the only significant predictor of inflammation in adolescents, whilst  $\dot{V}O_2$  peak and adiposity were not related to inflammation. Previous studies that assessed physical fitness based on the  $\dot{V}O_2$  peak test (Ischander et al., 2007; Steene-Johannessen et al., 2013) also reported no relationship between pro-inflammatory mediators (IL-6, TNF- $\alpha$ , and CRP) and physical fitness in adolescents. In contrast, inverse associations between pro-inflammatory mediators (IL-6, TNF- $\alpha$ , and CRP) and physical fitness have been observed when performance on the MSFT was the

preferred measure of physical fitness (Silva et al., 2014; Buchan et al., 2015).

To the authors' knowledge, the present study is the first to have considered that the methodology used to measure an individual's capacity to perform prolonged exercise influences the relationship between physical fitness and risk factors for cardio-metabolic diseases in adolescents. The acute anti-inflammatory response stimulated post-exercise reduces low-grade chronic inflammation in adolescents if repeated regularly (Mendham et al., 2015). Increased engagement with regular physical activity improves exercise tolerance and initiates peripheral adaptations in the muscle, including enhanced efficiency of mitochondrial biogenesis, increased fat oxidation and reduced blood lactate concentration at a given exercise intensity (Joyne and Carsten, 2018). The MSFT and blood lactate response to sub-maximal exercise measure such peripheral changes and are therefore considered to be sensitive to changes in the ability to perform prolonged exercise. In contrast, the  $\dot{V}O_2$  peak test is limited when measuring peripheral adaptations as it is predominantly determined by central systems (cardiovascular and respiratory) that have a strong genetic predisposition (Joyne and Carsten, 2018). Consequently, the MSFT and blood lactate response to sub-maximal exercise are better suited in the measurement of physical fitness specifically for metabolic risk in young people. These findings suggest that adolescents can reduce low-grade chronic inflammation by enhancing performance on the MSFT, and that improving the capacity to perform prolonged exercise is a potential therapeutic intervention to prevent the development of risk factors for cardio-metabolic diseases.

In the present study, adolescents categorized below the 25th centile for distance ran on the MSFT,  $\dot{V}O_2$  peak and adiposity exhibited increased blood glucose and plasma insulin concentrations, and HOMA when compared with adolescents in all other quartiles. The participants categorized below the 25th centile for HOMA, the chosen measure of insulin resistance in the present study, were above the reference cut off values for insulin resistance (>1.65 in girls and >1.9 in boys) in healthy young people (Rocco et al., 2011). Whereas, participants categorized above the 25th centile were below the reference cut off values for insulin resistance. These findings are in conjunction with Silva et al. (2014) whereby increased adiposity and reduced maximal oxygen uptake increased metabolic risk (calculated from traditional risk factors including; blood pressure, blood glucose, triglyceride and HDL cholesterol) in adolescents. The multiple regression model in the current study showed that adiposity was the best predictor for metabolic risk factors (plasma insulin and HOMA) and blood pressure in adolescents. This finding is consistent with previous research in adults, which reported the sum of skinfold thickness to be the strongest predictor of insulin resistance (Abate et al., 1995). Studies in rodents reported that increasing adiposity drives an influx of free fatty acids, which deactivate insulin receptors and reduce insulin sensitivity (Capurso and Capurso, 2012). The findings of the present study indicate that adolescents, categorized with the highest adiposity are more insulin resistant than participants

with lower adiposity in all other quartiles. Similarly, Paradis et al. (2004) observed an association between adiposity and both systolic and diastolic blood pressure in adolescents, yet the present study is the first to report the association between adiposity and mean arterial pressure in this population. The mechanisms relating adiposity with higher blood pressure in young people are relatively unknown, with disturbances in autonomic function being a potential mechanism (Paradis et al., 2004). These findings emphasize the importance of maintaining a healthy body composition in conjunction with enhancing physical performance to reduce the presence of risk factors for cardio-metabolic diseases in adolescents and slow the progression of chronic diseases, such as type 2 diabetes.

The present study also reports that girls with the highest adiposity had elevated plasma insulin concentration and reduced insulin sensitivity (HOMA) when compared with their male counterparts (boys categorized below the 25th centile for adiposity). These findings may be explained by the significantly increased adiposity of girls in quartile one when compared with boys and girls in all other quartiles (Table 1). These findings also support previous studies, which have reported that girls exhibited reduced postprandial insulin sensitivity when compared with boys of the same chronological age (Cooper et al., 2017). However, there was no difference in APHV between boys and girls in the present study and APHV was a covariate in the analysis to account for the potential confounding effects of maturation. Therefore, it is not feasible to suggest that the increased adiposity and insulin resistance observed in the girls in the present study was the result of differences in pubertal development. However, the potential confounding effect of puberty on the relationship between adiposity and insulin resistance in both males and females does warrant further research. Regardless of the mechanisms involved, it is apparent that adolescent girls with increased adiposity exhibit reduced insulin sensitivity when compared with their male counterparts. As such, future interventions should focus on promoting healthy body composition and physical fitness in adolescent girls, as the findings of the present study report that both variables can mediate improvements in insulin sensitivity.

The present study has several strengths including the measurement of a comprehensive panel of inflammatory cytokines in a heterogeneous sample of adolescents with diverse endurance capacities and adiposity, which allowed for the relationship between these variables and cardio-metabolic health to be determined. The heterogeneity of performance capacity (measured using the distance ran in the MSFT) in the present study ranged from the 30th–95th percentile for boys and the 20th–95th percentile for girls, when compared with normative data in European adolescents (Tomkinson et al., 2018). Similarly, the adiposity of the adolescents in the present study ranged from the 5th to >95th percentile for BMI, which also supports the heterogeneity of the participants recruited to the present study. The diversity of the adolescents analyzed in the present study allows for broad dissemination of the main findings of the study. However, a limitation to the present study was the lack of power for the blood lactate response to submaximal treadmill running and thus its exclusion from the multiple

regression model. Therefore, future research should determine more fully the effect of the blood lactate response to exercise (as a measure of adolescent training status) on adolescent cardio-metabolic health. Further limitation include the absence of measurements pertaining to the ethnicity, daily dietary habits and the typical physical activity levels of the participants. Each of these measures are potential confounders in the relationship between performance tests and the risk factors measured in the present study (Hardman and Stensel, 2009). Nevertheless, given the difficulties of data collection in this age group and population the present study is the most comprehensive yet to examine fitness and the risk factors for cardio-metabolic disease in adolescents.

## CONCLUSION

In conclusion, the present study shows that a higher ability to perform prolonged exercise (as indicated by performance on the MSFT) in adolescents protects against the development of cardio-metabolic risk indicators that increase the likelihood of early onset cardiovascular disease and type 2 diabetes. These findings suggest that all young people can benefit from enhancing their ability to perform prolonged exercise as evidenced by the differences across quartiles based on MSFT performance. Furthermore, these findings are particularly important for those categorized below the 25th centile, as the benefits for metabolic risk factors were observed for those categorized above the 25th centile and for markers of inflammation for those above the 50th centile based on distance ran on the MSFT. Although there were also benefits of a high  $\dot{V}O_2$  peak and low adiposity, these were not as marked as the benefits of enhanced performance on the MSFT. Thus, enhancing performance on the MSFT is a key factor in successfully reducing cardio-metabolic risk in young people and thus training interventions should be given substantial attention in public policy interventions for young people.

## ETHICS STATEMENT

This study was carried out in accordance with the recommendations of Nottingham Trent University's Ethical Advisory Committee with written informed consent and assent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Nottingham Trent University Ethical Advisory Committee.

## AUTHOR CONTRIBUTIONS

KD, SC, CS, JM, and MN designed the study and collected the data. KD, GF, and AP analyzed the data. All authors contributed to interpretation of the data, drafting and revision of the manuscript, and approved the final version of the manuscript.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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# Variations in Central Adiposity, Cardiovascular Fitness, and Objectively Measured Physical Activity According to Weight Status in Children (9–11 Years)

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The purpose of this study was twofold: first, to compare the central adiposity (CA), cardiovascular fitness (CF), and physical activity (PA) in children with different weight status, and second, to determine the associations between moderate to vigorous physical activity (MVPA) and measures of adiposity [CA and body mass index (BMI)] and CF. A sample of 244 children (boys = 120 and girls = 124), 9.7–10.8 years of age ( $10.3 \pm 0.3$  years), was measured for stature, body mass, waist circumferences, and 20-m multi-stage fitness test. PA was recorded with ankle mounted accelerometer. BMI groups were used to classify children as underweight (UW), normal weight (NW), and overweight (OW). The prevalence of being OW was 21.7 and 25% in boys and girls, respectively. Only 5.3% of the participants were found to accumulate recommended amount ( $\geq 60$  min/day) of MVPA. Boys were significantly outperformed girls in terms of CF. Moreover, they were significantly more engaged in moderate and vigorous physical activities than girls. Regardless of gender, results indicated that OW children had significantly higher values in all anthropometric parameters and lower level of CF than their UW and NW counterparts. In girls, OW children were found to accrue less time engaging in MVPA than the children in UW and NW groups. In boys, OW children were found to accrue less time engaging in vigorous activities than UW and NW children. Results also showed that there were no significant differences between UW and NW girls and boys in respect to CF. Besides, UW girls were found to accrue more time engaging in MVPA than NW girls. MVPA was found to be significantly and negatively correlated with BMI and waist circumference and significantly and positively correlated with CF in both boys and girls. These discrepancies and associations highlight the considerable influences of MVPA on weight status and CF in children.

**Keywords:** physical activity, adiposity, cardiovascular fitness, accelerometry, BMI

## INTRODUCTION

The progressive increase in childhood obesity is believed to result in pathophysiological consequences, including incidence of cardiovascular events in adulthood (Ayer et al., 2015; McCrindle, 2015). For that reason, among many, an early detection of adverse cardio-metabolic risk profiles may promote a beneficial effect to prevent or protect against future morbidity by adopting specific intervention programs that may help to reduce the risk factors (Bailey et al., 2015). Among other factors, an increase in physical activity (PA) levels, systematization, quality, and intensity can be a safe and effective contributor to ameliorate the risk of morbidity, concomitantly increasing the quality of life of the citizens (Clark et al., 2016; Higuera-Hernández et al., 2018).

Empirical evidence is persistently supportive of the assertion that more PA accrued, the greater the health benefits, particularly of moderate-to-vigorous intensity (Janssen and LeBlanc, 2010). However, it is commonly observed that overweight (OW) children are typically less active than their normal-weight counterparts (Stratton et al., 2007). Moreover, a lack of motor competency also putatively mediates low PA levels; thus, PA programs that promote an improvement in fundamental movement skills may facilitate improvement of PA levels (Utesch et al., 2018). In fact, it seems that a highly developed level of motor competency in childhood may have the potential to foster lifelong functional independence and quality of life (Robinson et al., 2015; Clark et al., 2018).

One of the capacities typically included in batteries of motor competence assessment is aerobic cardiovascular fitness (CF; Khodaverdi et al., 2016; Luz et al., 2019). Cardiovascular capacity seems to play an important role in sustaining a healthier cardiovascular profile in young children and adolescents (Ruiz et al., 2015). Moreover, aerobic fitness is closely related to PA levels. In fact, in two cross-sectional studies, it was found that children with high moderate to vigorous physical activity (MVPA) levels and low sedentary behaviors had higher odds of having a high CF (Martinez-Gomez et al., 2011; Santos et al., 2014).

Briefly, the evidence suggests that less active children presents higher odds of having excess weight and lower CF. Such hypotheses should be better analyzed, namely because they can serve as a “red flag” to control and implement monitoring strategies in the early stages of childhood. Previous studies have widely investigated the PA, aerobic fitness, and adiposity of normal weight (NW) and OW children. However, there is paucity of information for children who are underweight (UW). Thus, in this study, we examined whether standardized measure of PA, central adiposity (CA), and CF were different in UW, NW, and OW children in both genders. Furthermore, we analyze the associations between MVPA and markers of adiposity [CA and body mass index (BMI)] and CF.

## MATERIALS AND METHODS

### Participants

A total of 244 children (boys = 120 and girls = 124), 9.7–10.8 years of age ( $10.3 \pm 0.3$  years), participated in the study.

They were a voluntary sub-sample of 822 children from 30 publicly funded primary schools in Southern Wales, UK. Prior to research commencing, legal guardian informed consent and child assent were attained. This research was conducted in agreement with the guidelines and policies of the institutional ethics committee and in accordance with the Declaration of Helsinki.

### Anthropometric Measurements

Measurements were taken by a single observer in accordance with standardized procedures (Lohman et al., 1988). Stature was measured with a stadiometer (SECA, Hamburg, Germany) to the nearest 0.01 m. Body mass was measured with a digital scale (SECA, Hamburg, Germany) to the nearest 0.1 kg. Waist circumference was measured with a flexible steel tape to the nearest centimeter and at the smallest circumference between the ribs and iliac crest. BMI was calculated by dividing weight (kg) by the squared height (m). BMI centiles were used to classify children as either UW (<5th percentile), NW (5th to 85th percentile), and OW (>85th to <95th percentile) (Cole and Lobstein, 2012).

### Twenty-Meter Multi-Stage Fitness Test

Multi-stage fitness test (MSFT) was used to assess CF of the participants. All participants were familiar with the MSFT procedures, since they have routinely been involved in this test at school. Nevertheless, instructions were provided, verbatim, prior to the commencement of the test, according to the standardized guidance of Leger et al. (1988). Moreover, the test was conducted on an indoor training facility (50 m × 50 m) in Southern Wales (UK). The participants completed the MSFT by running back and forth along a 20 m course and were required to touch the 20 m line at the same time where a sound signal was emitted from a pre-recorded audio disk. The frequency of the sound emissions increased to produce a corresponding increase in running speed. The test stopped when the participant reached volitional exhaustion and was no longer able to follow the set pace, or participants were withdrawn after receiving two verbal warnings to meet the required pace (Leger et al., 1988).

### Physical Activity

Study participants wore an ActiGraph GT3X+ accelerometer (ActiGraph, Pensacola, FL, USA) for 7 consecutive days in summer (Fuemmeler et al., 2011; Ridgers et al., 2014). Participants were instructed to wear the accelerometer constantly except when bathing or swimming. The accelerometer dimensions are 4.6 cm × 3.3 cm × 1.5 cm and weigh 19 g. Its sampling frequency was set to 100 Hz, and the sampling interval (epoch) in the present study was set to be 1-s (Pate et al., 2006; Østbye et al., 2013). Participants wore their accelerometer on the waist, above the right hip, affixed using an elastic belt (Hesketh et al., 2014). Accelerometer data were analyzed to measure the following parameters: daily duration of sedentary behavior, light PA, moderate PA, vigorous PA, and MVPA (Migueles et al., 2017).

ActiGraph acceleration data were analyzed using a commercially available analysis tool (KineSoft version 3.3.67, KineSoft; www.kinesoft.org). Non-wear periods were defined as any sequence of >20 consecutive minutes of zero activity counts (Tudor-Locke et al., 2015). Sedentary behavior was defined as <100 counts per minute, while 100, 2,296, and 4,012 counts per minute were thresholds to define light, moderate, and vigorous PA, respectively (Evenson et al., 2008; Trost et al., 2011). All PA data were collected in week blocks, over a 1-month period.

## Statistical Procedures

All data were analyzed using SPSS for Windows. Descriptive statistics (mean  $\pm$  SD) were calculated for the variables. Due to non-normal distribution of the data, the Kruskal-Wallis test was used to analyze the discrepancies of variables in regard to BMI categories. Mann-Whitney *U* tests were used to follow-up pairwise comparisons and to examine gender differences. Spearman's rank correlation coefficient was conducted to examine the association between MVPA and other variables. Statistical significance level was settled at 0.05.

## RESULTS

Descriptive statistics are detailed in **Table 1**. The results revealed that boys were significantly ( $p < 0.05$ ) outperformed girls in terms of CF. Furthermore, they were significantly ( $p < 0.01$ ) more engaged in moderate and vigorous physical activities than girls. Other variables were found comparable between genders.

The descriptive statistics of boys by weight status and the Kruskal-Wallis test results are given in **Table 2**. The Mann-Whitney *U* test revealed that OW boys had significantly higher values in all anthropometric parameters than their UW and NW counterparts. Further results indicated that OW boys had significantly lower level of CF than boys in other BMI groups. Moreover, they were found to accrue less time engaging in vigorous activities than UW and NW boys. There were no

significant differences between UW and NW boys in respect to CF and PA variables.

The descriptive statistics of girls by weight status and the Kruskal-Wallis test results are presented in **Table 3**. The Mann-Whitney *U* test demonstrated that OW girls had significantly greater values in all anthropometric parameters than their UW and NW counterparts. Furthermore, they were significantly less engaged in moderate and vigorous physical activities than UW and NW girls. Besides, they were found to have significantly lower level of CF than the girls in other BMI groups. UW girls were found to accrue more time engaging in moderate and vigorous physical activities than NW girls. There were no significant differences between UW and NW girls in terms of CF.

The correlations between MVPA and the other variables were presented separately for each gender in **Table 4**. The results showed that MVPAs were significantly and negatively associated with BMI and waist circumference and significantly and positively associated with CF in both boys and girls.

## DISCUSSION

The main purpose of this cross-sectional study was to determine the variations in the CA, CF, and PA among children with different weight status. The results indicated that regardless of gender, OW children were found to have greater CA and lower values in CF and MVPA than their UW and NW counterparts. These findings are in line with the results of previous investigations, where significant differences among BMI groups have been reported in regard to CA (Ferreira and Marques-Vidal, 2008; Karppanen et al., 2012), CF (Aires et al., 2010; Niederer et al., 2012), and MVPA (Page et al., 2005; Decelis et al., 2014). Although current literature has not reached a consensus regarding the actual causes of child OW/obesity, such disparities among children with different weight status may be, at least in part, attributed to the unhealthy eating habits, physical inactivity, sedentary screen time, environmental factors, and psychological stress (Slyper, 2004; Pate et al., 2013; Ross et al., 2016).

The results showed that, regardless of gender, there were no significant differences between UW and NW children in respect to CF. This result is in accord with the finding of the earlier examinations (Artero et al., 2010; Gulías-González et al., 2014). On the other hand, UW girls were found to accrue more time engaging in moderate and vigorous physical activities than NW girls. Previous studies present inconsistent results in regard to PA level of UW and NW children (Chung et al., 2012; Fairclough et al., 2017). Therefore, further research is needed on this important subject.

The results in regard to gender effect demonstrated that boys scored significantly better than girls in terms of CF. In addition, they were found to be significantly more engaged in MVPA than girls. These results are in accord with the findings of antecedent studies (Hussey et al., 2007; Pereira et al., 2010; Ridgers et al., 2010; Martinez-Gomez et al., 2011). This difference in PA level was previously explained by social

**TABLE 1 |** Descriptive statistics of boys and girls and the Mann-Whitney *U* test results.

Variables	Male (n = 120)	Female (n = 124)	<i>U</i>	<i>p</i>
Age (years)	10.28 $\pm$ 0.30	10.27 $\pm$ 0.31	7.2	0.697
Height (cm)	140.7 $\pm$ 5.7	141.8 $\pm$ 6.6	6.9	0.294
Body mass (kg)	36.1 $\pm$ 8.7	38.6 $\pm$ 10.6	6601.0	0.128
BMI (kg/m <sup>2</sup> )	18.1 $\pm$ 3.3	18.9 $\pm$ 3.9	6.6	0.138
Waist circumference (cm)	64.1 $\pm$ 10.0	65.2 $\pm$ 10.0	7.4	0.914
20 m shuttle (laps)	31.6 $\pm$ 16.7	26.6 $\pm$ 12.2	6.3	0.039
Wear time (min/day)	1413.5 $\pm$ 49.8	1396.7 $\pm$ 72.9	6719.0	0.180
Sedentary time (min/day)	510.2 $\pm$ 80.7	503.1 $\pm$ 63.8	7306.0	0.808
Light activity (min/day)	351.8 $\pm$ 61.5	367.1 $\pm$ 55.6	6.6	0.133
Moderate activity (min/day)	20.9 $\pm$ 9.9	15.7 $\pm$ 7.0	5059.5	0.001
Vigorous activity (min/day)	15.1 $\pm$ 9.0	9.3 $\pm$ 5.7	4.5	0.001
MVPA (min/day)	36.1 $\pm$ 18.5	25.0 $\pm$ 12.3	4.8	0.001
Sleep time (min/day)	541.9 $\pm$ 31.9	544.7 $\pm$ 39.1	6.6	0.114

**TABLE 2** | Descriptive statistics of boys by weight status and the Kruskal-Wallis test results.

BMI categories	UW (n = 12)	NW (n = 82)	OW (n = 26)	H	p
Age (years)	10.03 ± 0.31	10.30 ± 0.28	10.31 ± 0.30	8.8	0.012
Height (cm)	135.6 ± 4.1	139.9 ± 4.7	145.2 ± 6.6	23.7	0.001
Body mass (kg)	25.6 ± 2.8	33.5 ± 3.8	49.2 ± 7.5	73.5	0.001
BMI (kg/m <sup>2</sup> )	13.9 ± 1.2	17.1 ± 1.5	23.2 ± 2.0	79.5	0.001
Waist circumference (cm)	56.7 ± 2.5	60.7 ± 6.9	78.2 ± 7.2	67.8	0.001
20 m shuttle (laps)	39.1 ± 22.8	34.1 ± 15.8	20.3 ± 10.6	17.7	0.001
Wear time (min/day)	1408.5 ± 45.8	1415.4 ± 44.8	1409.8 ± 65.9	0.4	0.829
Sedentary time (min/day)	485.2 ± 70.6	506.0 ± 74.6	534.9 ± 98.8	3.7	0.156
Light activity (min/day)	370.5 ± 65.2	355.5 ± 57.5	331.3 ± 69.2	4.5	0.106
Moderate activity (min/day)	21.4 ± 10.8	22.3 ± 8.9	16.6 ± 11.7	12.5	0.002
Vigorous activity (min/day)	17.8 ± 9.7	16.4 ± 8.8	9.9 ± 7.4	14.9	0.001
MVPA (min/day)	39.2 ± 20.5	38.7 ± 17.2	26.5 ± 18.9	14.1	0.001
Sleep time (min/day)	545.1 ± 38.9	539.8 ± 31.7	547.3 ± 29.4	0.607	1.5

**TABLE 3** | Descriptive statistics of girls by weight status and the Kruskal-Wallis test results.

BMI categories	UW (n = 10)	NW (n = 83)	OW (n = 31)	H	p
Age (years)	10.04 ± 0.18	10.26 ± 0.32	10.35 ± 0.30	7.4	0.024
Height (cm)	137.0 ± 3.5	140.6 ± 5.9	146.5 ± 6.6	25.6	0.001
Body mass (kg)	26.6 ± 1.1	34.4 ± 4.8	53.6 ± 8.4	79.0	0.001
BMI (kg/m <sup>2</sup> )	14.2 ± 0.3	17.3 ± 1.5	24.9 ± 2.8	84.1	0.001
Waist circumference (cm)	54.9 ± 2.6	61.3 ± 5.6	78.9 ± 7.2	70.5	0.001
20 m shuttle (laps)	33.8 ± 11.3	30.3 ± 11.0	14.4 ± 5.6	48.8	0.001
Wear time (min/day)	1370.4 ± 140.5	1399.8 ± 64.9	1397.0 ± 63.6	2.1	0.357
Sedentary time (min/day)	480.4 ± 70.9	505.9 ± 66.6	503.2 ± 53.6	0.4	0.803
Light activity (min/day)	391.2 ± 62.9	365.3 ± 57.2	364.4 ± 48.3	1.5	0.465
Moderate activity (min/day)	22.3 ± 9.1	16.0 ± 6.6	12.5 ± 5.7	12.8	0.002
Vigorous activity (min/day)	14.3 ± 5.9	9.8 ± 5.7	6.6 ± 3.8	19.0	0.001
MVPA (min/day)	36.6 ± 14.7	25.8 ± 11.9	19.1 ± 9.1	15.8	0.001
Sleep time (min/day)	531.9 ± 75.0	543.1 ± 37.5	553.4 ± 24.7	1.1	0.587

**TABLE 4** | Correlation results between MVPA and other variables by gender.

Variables	Boys		Girls	
	r <sub>s</sub>	p	r <sub>s</sub>	p
BMI	-0.230	0.012	-0.333	0.001
Waist circumference	-0.179	0.049	-0.292	0.001
20 m shuttle run	0.490	0.001	0.236	0.008

support (Edwardson et al., 2013), perceived enjoyment of physical education (Cairney et al., 2012), and biological maturation (Wickel et al., 2009). Gender differences in CF may be due to the disparity in body mass, where girls were found to be significantly heavier than boys (Armstrong and Welsman, 1994; Martinez-Gomez et al., 2011).

The results of the correlation analysis demonstrated small to medium associations between MVPA and BMI, waist circumference, and aerobic fitness in both girls and boys. Similar observations were reported with the previous studies (Gutin et al., 2005; Ruiz et al., 2006; Jiménez-Pavón et al., 2010). It seems that participating in MVPA plays not only an important role on having a healthy central and total adiposity but also on aerobic fitness in childhood.

The overall prevalence of OW was found to be 25.4% (boys = 21.7% and girls = 25.0%). These percentages were

higher than the previously measured children of similar age; for example, Chinn and Rona (2001) studied the secular trends in OW among British children aged between 9 and 11 years. In boys, prevalence of OW in 1974, 1984, and 1994 was reported as 6.2, 5.8, and 12.7, respectively. In girls, it was noted as 9.9, 9.9, and 16.7, respectively. Correspondingly, lower level of OW prevalence was observed for the children living in Wales (girls = 15.8% and boys = 17.9%) (Elgar et al., 2005) and living in Ireland (girls = 20.7% and boys = 20.2; Hussey et al., 2007). Additionally, in a recent paper (Skinner et al., 2018), lower prevalence of being OW (18.5%) was found in US children (9–11 years). This result might be due to that only 5.3% of the participants were found to accumulate recommended amount (≥60 min/day) of MVPA (World Health Organization, 2010). In summary, these findings undoubtedly indicate the existence of excessive adiposity in children.

## LIMITATIONS

It should be acknowledged that the present study has several limitations. First, the cross-sectional observational design employed in this study precludes cause and effect interpretations. Second, biological maturity status, one of the major potential confounding factors for physical and functional characteristics



of children in these age groups, was not evaluated. Third, in addition to BMI, estimation of body fat percentage would help to improve the interpretation of data in regard to body composition. Fourth, weather-related variables (temperature, rain, wind, etc.) were not evaluated. Lastly, rather than fractionation (intermittent versus continuous), only cumulative amounts of MVPA were considered in this study.

## CONCLUSIONS

In this study, we examined whether children with different weight status differ in respect to their PA, CA, and CF. It is evident that, regardless of gender, OW children had significantly lower CF than their UW and NW counterparts. In girls and boys, OW children accrued less MVPA than the children in UW and NW groups. This study is original in the sense that it also provides data not only for NW and OW children but also for their UW counterparts. Our findings indicated that there were no significant differences between UW and NW girls and boys in terms of CF. Moreover, UW girls were found to accrue more time engaging in moderate and vigorous physical activities than NW girls. MVPA was found to be significantly and negatively correlated with BMI and waist circumference and significantly and positively correlated with CF in both boys and girls. These discrepancies and associations highlight the considerable influences of MVPA on weight status and

CF in children, highlighting the need for sustained and longitudinal monitoring, in addition to greater effort given employing interventions, relevant to PA and CF.

## DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

## ETHICS STATEMENT

Ethical approval prior to research commencing, legal guardian informed consent and child assent were attained. This research was conducted in agreement with the guidelines and policies of the institutional ethics committee and in accordance with the Declaration of Helsinki. The article was approved by a local ethical committee with the number IPVC-ESDL13092018.

## AUTHOR CONTRIBUTIONS

MS, FC, and CC designed the study. CC collected the data. MS analyzed and interpreted the data and drafted the manuscript. PN, TR, and BK critically revised the paper. All authors discussed the results and contributed to the final version of the article.

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# Clarity and Confusion in the Development of Youth Aerobic Fitness

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Peak oxygen uptake ( $\dot{V}O_2$ ) is internationally recognized as the criterion measure of youth aerobic fitness, but flawed laboratory assessments and fallacious interpretations of peak  $\dot{V}O_2$  in ratio with body mass have confused our understanding of the development of aerobic fitness. Moreover, the recent emergence of specious predictions of peak  $\dot{V}O_2$  from performance tests and the promotion of spurious “clinical red flags” and cardiometabolic cut-points have confused our understanding of the relationship between youth aerobic fitness and health. Recent longitudinal studies of 10–18-year-olds using multilevel allometric modeling have empirically demonstrated that peak  $\dot{V}O_2$  increases in accord with sex-specific, concurrent changes in age- and maturity status-driven morphological covariates with the timing and tempo of changes specific to individuals. During both cycle ergometry and treadmill running age- and maturity status-driven changes in fat free mass have been revealed as the most powerful morphological influences on the development of youth aerobic fitness. To bring some clarity to current confusion, this paper argues that future studies must be founded on rigorous assessment and interpretation of peak  $\dot{V}O_2$  and ensure that they address the development of youth aerobic fitness and its relationship with present and future health in relation to appropriate sex-specific morphological covariates governed by individual biological clocks.

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## INTRODUCTION

Aerobic fitness defines the ability to deliver oxygen from the atmosphere to the skeletal muscles and to use it to generate energy to support muscle activity during exercise. Peak oxygen uptake ( $\dot{V}O_2$ ), the highest rate of oxygen consumed during an incremental exercise test to exhaustion, limits the capacity to perform aerobic exercise and is internationally recognized as the best single measure of youth aerobic fitness. Peak  $\dot{V}O_2$  is the most researched physiological variable in pediatric exercise physiology but understanding of the development of youth aerobic fitness is embedded in confusion with shoddy assessments and fallacious interpretations of peak  $\dot{V}O_2$  during growth and maturation. Moreover, youth aerobic fitness and its relationship with current and future health is shrouded in confusion through a resurgence of specious predictions of peak  $\dot{V}O_2$  from performance tests and the promotion of spurious “cardiometabolic cut-points” and “clinical red flags.” To bring some clarity to current confusion, this mini-review critically reviews

the evidence relating peak  $\dot{V}O_2$  to changes in age, maturity status, body size, and body composition with reference to health.

## CONFUSION IN THE DEVELOPMENT OF YOUTH AEROBIC FITNESS

### Assessment

Antoine-Laurent Lavoisier was the first to experiment with the measurement of  $\dot{V}O_2$  during exercise in the 1770s, but it is Hill and Lupton (1923), who introduced the concept of a near-linear relationship between  $\dot{V}O_2$  and running speed until, despite an increase in running speed, a plateau in  $\dot{V}O_2$  emerges at the point of maximal  $\dot{V}O_2$ . In the first laboratory-based study of boys Robinson (1938) ran 6–17-year-olds on a treadmill at a speed of 7 miles·h<sup>-1</sup> up an 8.6% gradient until they were “exhausted” and reported their  $\dot{V}O_2$  as “maximal.” In his seminal study of boys and girls, Åstrand (1952) criticized Robinson’s (1938) methodology and adopted a more rigorous discontinuous, incremental exercise protocol over several days. He noted that the  $\dot{V}O_2$  plateau proposed by Hill and Lupton (1923) was found in only 50% of schoolchildren. This phenomenon was subsequently confirmed in large studies with both pre-pubertal (Armstrong et al., 1995) and pubertal (Armstrong et al., 1991) youth but generally ignored for decades. When it was addressed and the term peak  $\dot{V}O_2$  introduced (Armstrong and Davies, 1981) scientific journals confused understanding of youth aerobic fitness for several years by often rejecting papers reporting peak  $\dot{V}O_2$  on the basis that maximal values were not attained.

Peak  $\dot{V}O_2$  is now recognized as the “gold standard” measure of youth aerobic fitness but researchers continue to wrestle with factors related to its rigorous determination. In cardiopulmonary exercise tests children and adolescents normally exercise to voluntary exhaustion but there is no way to confirm, in the single tests typical of most studies, whether an individual has delivered a maximal effort. The experience of the testing team, supported by subjective criteria of intense effort (e.g., facial flushing, sweating, hyperpnoea, and unsteady gait), is critical in deciding whether a maximal value has been attained. However, to verify efforts as maximal, secondary criteria such as pre-set (and often submaximal) values of heart rate (HR), respiratory exchange ratio, and blood lactate accumulation at the termination of exercise are widely used. However, all secondary criteria exhibit large individual variations and are exercise protocol and ergometer dependent (Armstrong and McManus, 2017). The growing tendency to rely on what are clearly submaximal criteria such as HR  $\geq$  85% of predicted maximum to confirm maximal values has confused understanding of both the development of aerobic fitness and its purported relationship with other health-related variables. Barker et al. (2011) reported that terminating a test with secondary criteria can underestimate a child’s “true” peak  $\dot{V}O_2$  by ~10–22%.

Both treadmills and cycle ergometers are used routinely in pediatric exercise laboratories. Due to the greater muscle mass, enhanced venous return, higher stroke volume (SV), and

reduced peripheral resistance during running, mean treadmill-determined values are ~11–14% higher than those determined on a cycle ergometer. Ergometer-dependent differences in peak  $\dot{V}O_2$  at specific ages vary with sex, age, maturity status, and morphological covariates (Armstrong and Welsman, 2019a) but some reviewers have confused understanding of the development of aerobic fitness by combining treadmill- and cycle ergometer-determined peak  $\dot{V}O_2$  values (e.g., Bar-Or and Rowland, 2004). Other authors have added to the confusion by “correcting” for ergometer differences by multiplying cycle ergometer values by fixed percentages regardless of sex, age, or maturity-status (e.g., Stavnsbo et al., 2018) or assuming that increasing cycle ergometer values “by ~2–3 mL·kg<sup>-1</sup>·min<sup>-1</sup> would make them equivalent to values obtained by a treadmill protocol” (Aadland et al., 2019, p. 248).

### Development

Robinson (1938) initially reported boys’ “maximal”  $\dot{V}O_2$  in L·min<sup>-1</sup> before “referring them to body weight” (p. 280), analyzing his data in ratio with body mass (i.e., in mL·kg<sup>-1</sup>·min<sup>-1</sup>), and initiating an approach for “controlling” for growth that has confused pediatric exercise physiology for over 80 years.

Tanner (1949) unequivocally established that expressing peak  $\dot{V}O_2$  in ratio with body mass was fallacious. Subsequent reviews have explained the statistical assumptions underlying ratio scaling of peak  $\dot{V}O_2$  with body mass and demonstrated that they are seldom (if ever) met (Welsman and Armstrong, 2008, 2019). Yet the vast majority of pediatric exercise studies still interpret peak  $\dot{V}O_2$  in ratio with body mass and reports of spurious correlations with indicators of cardiovascular health are common (Mintjens et al., 2018). Purported relationships between ratio-scaled peak  $\dot{V}O_2$  and other health-related variables have confused the association of youth aerobic fitness with current and future health. For example, any relationship between cardiovascular risk factors in overweight/obese youth with ratio-scaled peak  $\dot{V}O_2$  is more likely to reflect overweight/obese status than aerobic fitness (Loftin et al., 2016).

In practice, ratio-scaled peak  $\dot{V}O_2$  favors lighter (e.g., clinically underweight or delayed maturing) youth and penalizes heavier (e.g., overweight or advanced maturing) youth. Literature reviews (e.g., Krahenbuhl et al., 1985) and textbooks (e.g., Bar-Or and Rowland, 2004) reporting ratio-scaled peak  $\dot{V}O_2$  have confused understanding of developmental exercise physiology for decades. For example, peak  $\dot{V}O_2$  data ratio-scaled with body mass indicates that boys’ aerobic fitness decreases slightly or remains unchanged from 10 to 18 years, whilst in girls a progressive decline is apparent over the same time scale. However, when body mass is appropriately controlled using log-linear regression boys’ peak  $\dot{V}O_2$  increases with age and girls’ peak  $\dot{V}O_2$  increases at least until 13 or 14 years and then levels-off (Welsman et al., 1996). Similarly, ratio-scaled data indicate that once body mass is controlled for maturity status has no effect on peak  $\dot{V}O_2$  (e.g., Fahey et al., 1979) whereas allometric (log-linear regression) scaling has demonstrated positive effects of maturity status in addition to those of age and body mass in both boys and girls (Armstrong et al., 1998).

## Performance Tests and Health-Related Cut-Points

A recent resurgence of interest in estimating/predicting peak  $\dot{V}O_2$  from 20-m shuttle run test (20mSRT) performance, has confused understanding of youth aerobic fitness (Armstrong and Welsman, 2018; Welsman, 2019). The 20mSRT is not a measure of aerobic fitness but a function of willingness to run between two lines 20 m apart whilst keeping pace with audio signals which require the running speed to increase each minute until participants are unwilling or unable to maintain the pace. The number of shuttles/stages completed is converted into an estimate of peak  $\dot{V}O_2$  in ratio with body mass through one of at least 17 published prediction equations (Tomkinson et al., 2017). A recent meta-analysis reported that over half of published correlation coefficients between 20mSRT scores and “true” peak  $\dot{V}O_2$  explain less than 50% of the total variance in peak  $\dot{V}O_2$  and concluded, “testers must be aware that the performance score of the 20MSR test is simply estimation and not a direct measure of cardiorespiratory fitness” (Mayorga-Vega et al., 2015, p. 545). The capacity for confusion created by uncritical application of 20mSRT data to relationships with indicators of health is revealed by the 95% range for a “true” peak  $\dot{V}O_2$  value estimated from 20mSRT performance being  $\sim 10 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  or  $\sim 24\%$  (Tomkinson et al., 2019a).

Further confusion has arisen over the introduction and promotion of “cardiometabolic cut-points” “to define children with poor cardiometabolic health” (Aadland et al., 2019, p. 240) and “clinical red flags” to identify “children and adolescents who may benefit from primary and secondary cardiovascular prevention programming” (Ruiz et al., 2016, p. 1451). The validity of these “cut points” and “clinical red flags,” both based on ratio-scaled peak  $\dot{V}O_2$ , is also challenged through them being derived from combined cycle ergometer- and treadmill-determined peak  $\dot{V}O_2$  values with a fixed 5% added to cycle ergometer values (Stavnsbo et al., 2018); an amalgam of data from treadmill- and cycle ergometer (+5%)-determined peak  $\dot{V}O_2$  and peak  $\dot{V}O_2$  predicted from 20mSRTs (Aadland et al., 2019); and solely from 20mSRT predictions of peak  $\dot{V}O_2$  (Ruiz et al., 2016). None of the proposed “cut points” consider maturity status. “Clinical red flags” also take no account of age with the indefensible assumption that a pre-pubertal 8-year-old is comparable to a post-pubertal 18-year-old with the same peak  $\dot{V}O_2$  ratio-scaled with body mass.

## CLARITY IN THE DEVELOPMENT OF YOUTH AEROBIC FITNESS

### Assessment

The laboratory measurement of peak  $\dot{V}O_2$  has been progressively developed and refined as new technology has replaced the classic Douglas bag method initially with mixing chambers and more recently with breath-by-breath analyses. The importance of appropriate exercise test protocols, ergometers, breathing interfaces, size of components of respiratory gas collection systems, and sampling intervals during growth and maturation

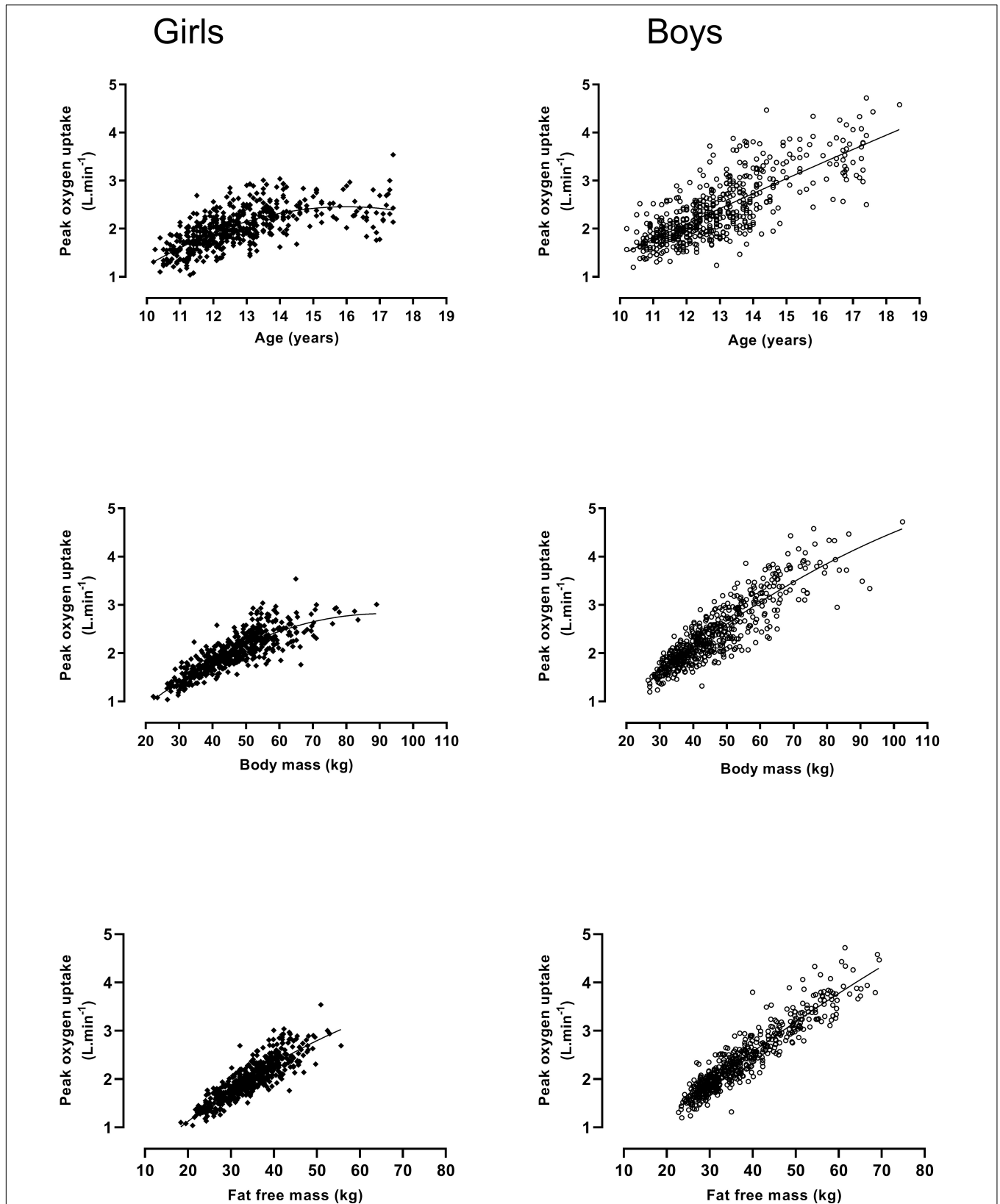
cannot be overemphasized and all methodology, apparatus, and calibration techniques should be carefully reported (see McManus and Armstrong, 2017; Falk and Dotan, 2019 for comprehensive reviews).

The typical error of youth peak  $\dot{V}O_2$  rigorously determined in three tests each a week apart is in our hands  $\sim 4\%$  (Welsman et al., 2005). To increase confidence in obtaining a “true” peak  $\dot{V}O_2$  in a single session an initial exercise test can be confirmed with a validation test. For example, a cycle ergometer ramp test to exhaustion followed  $\sim 15$  min later by a validation test consisting of a 2 min warm-up before a step change to 105% of the peak power elicited at the end of the initial test. On the few occasions (in our hands  $< 5\%$ ) that the peak  $\dot{V}O_2$  is higher than in the initial test the validation test can be repeated at 110% of peak power following full recovery (Barker et al., 2011). This protocol is facilitated by children’s ability to recover from heavy exercise faster than adults (Ratel et al., 2006; Armstrong, 2019).

### Development

Youth peak  $\dot{V}O_2$  has been comprehensively documented but flawed experimental designs, statistical analyses, and data interpretation have limited insights into the development of aerobic fitness. Use of equipment and exercise protocols designed for adults, small sample sizes, combining of data from boys and girls, only reporting peak  $\dot{V}O_2$  ratio-scaled with body mass, and serious concerns over whether true maximal values have been attained make peak  $\dot{V}O_2$  data from young children difficult to interpret (Armstrong and Welsman, 1994). The focus herein will therefore be on the more secure database of 10–18-year-olds.

The snapshot moments in time reflected by cross-sectional studies provide few insights and rigorous examination of the development of aerobic fitness requires longitudinal studies (Armstrong and McManus, 2017). Longitudinal data based on 1057 treadmill determinations of peak  $\dot{V}O_2$  have revealed that aerobic fitness is significantly correlated with age ( $r = 0.78$ , boys and  $0.64$ , girls), body mass ( $r = 0.89$ , boys and  $0.83$ , girls), and fat-free mass (FFM) ( $r = 0.94$ , boys and  $0.87$ , girls) (Armstrong and Welsman, 2019b). As can be seen in **Figure 1**, there is a near-linear increase in boys’ peak  $\dot{V}O_2$  from 10 to 18 years. In girls, a near-linear increase in peak  $\dot{V}O_2$  until  $\sim 13$ – $14$  years of age is followed by a leveling-off from  $\sim 14$  to 18 years. Boys’ peak  $\dot{V}O_2$  almost doubles from 10 to 18 years while girls’ values increase by  $\sim 50\%$  over the same time period. Pre-pubertal boys’ peak  $\dot{V}O_2$  values are, on average,  $\sim 12\%$  higher than those of similar aged pre-pubertal girls and the mean sex difference in peak  $\dot{V}O_2$  increases as young people progress through adolescence reaching  $\sim 50\%$  in post-pubertal 18-year-olds. **Figure 1** illustrates the relationships of body mass and FFM with peak  $\dot{V}O_2$  with sex differences evident throughout the age range. Girls’ peak  $\dot{V}O_2$  in relation with body mass tends to taper-off from  $\sim 60$  kg. Body mass includes both fat mass which is metabolically inert (Goran et al., 2000) and FFM which reflects the active muscle. The relationship of peak  $\dot{V}O_2$  with FFM (estimated from the equations of Slaughter et al., 1988) is remarkably linear from 10 to 18 years in both sexes (Armstrong and Welsman, 2019b).



**FIGURE 1** | Peak oxygen uptake by age, body mass, and fat free mass in 10–18-year-old girls and boys: Figure founded on 1057 determinations of peak oxygen uptake, age, body mass, and fat free mass; girls (*n* = 501), boys (*n* = 556). Data from Armstrong and Welsman (2019b).

In a parallel longitudinal study 72 boys and 64 girls also had their peak  $\dot{V}O_2$  determined on a cycle ergometer. The pattern of peak  $\dot{V}O_2$  relationships with age, body mass, and FFM were similar to those on a treadmill although the magnitude of the covariates varied. Mean treadmill values of peak  $\dot{V}O_2$  were significantly ( $p < 0.05$ ) higher in boys on each testing occasion but the percentage difference varied with age, peaking at 13 years. Moreover, some individuals in both sexes demonstrated higher values on a cycle ergometer than a treadmill on at least one test occasion further illustrating the folly of predicting treadmill-determined peak  $\dot{V}O_2$  by adding a fixed percentage to cycle ergometer values regardless of age, sex, and maturity status (Armstrong and Welsman, 2019a).

Collectively these longitudinal data also unequivocally show the fallacy of ratio scaling peak  $\dot{V}O_2$  with body mass, regardless of whether it is determined during mass-supporting or mass-supported exercise. Statistical assumptions underlying ratio scaling include a perfect correlation (i.e.,  $r = 1.0$ ) between peak  $\dot{V}O_2$  and body mass which was not met and an allometric exponent of 1.0 for body mass, a value which fell outside the 95% confidence limits. Moreover, significant negative correlations between ratio-scaled peak  $\dot{V}O_2$  and body mass demonstrated that body mass was not controlled for by ratio scaling (Armstrong and Welsman, 2019a,b).

The application of multilevel regression modeling to trained (Nevill et al., 1998) and untrained youth (Armstrong et al., 1999) and the technique's on-going refinement (Rasbash et al., 2018) has enabled multiple, individual growth trajectories to be examined in relation to the development of aerobic fitness. The effects of sex, age, maturity status, body size, and body composition on peak  $\dot{V}O_2$  can be partitioned concurrently within an allometric

framework to provide a sensitive interpretation of youth aerobic fitness. Armstrong and Welsman (2019a,b) adopted this approach and explored the development of peak  $\dot{V}O_2$  through a series of models which are shown in **Table 1**. In contrast to traditional ratio-scaled interpretations, the initial models 1.1 (girls) and 2.1 (boys) show that with body mass controlled for, peak  $\dot{V}O_2$  increases with age in both sexes. The negative age<sup>2</sup> term indicates that the size of the age effect reduces as the rate of growth decreases. Stature was not a significant ( $p > 0.05$ ) covariate in any of the models. In conflict with ratio-scaled data, the addition of maturity status, in the form of the stages of pubic hair described by Tanner (1962), showed each stage to have a positive effect on peak  $\dot{V}O_2$  independent of those from body mass and age (models 1.2 and 2.2). The introduction of sum of triceps and subscapular skinfolds to act with body mass as a surrogate of FFM (Roemmich et al., 1997) resulted in the effects of maturity status being negated but age and age<sup>2</sup> remained significant covariates. These models (1.3 and 2.3) were the best statistical fit ( $p < 0.05$ ) of the data and demonstrate the powerful effects of FFM on the development of aerobic fitness.

Boys' FFM increases, on average, by ~90% from 11 to 16 years. It is, however, maturation which drives changes in FFM. This is evidenced by percentage changes in FFM being at their zenith around the time of peak height velocity (PHV) when FFM increases by ~83% over the period 2 years pre-PHV to 2 years post-PHV. Girls' FFM increases by ~40% over the same age range with the greatest increase (~31%) occurring over a 2 year period centered on PHV, before it levels-off in accord with the development of peak  $\dot{V}O_2$  (Armstrong, 2019; Baxter-Jones et al., 2003).

**TABLE 1** | Multilevel regression models for peak oxygen uptake.

	Girls model 1.1	Girls model 1.2	Girls model 1.3	Boys model 2.1	Boys model 2.2	Boys model 2.3
	Log <sub>e</sub> peak oxygen uptake	Log <sub>e</sub> peak oxygen uptake	Log <sub>e</sub> peak oxygen uptake	Log <sub>e</sub> peak oxygen uptake	Log <sub>e</sub> peak oxygen uptake	Log <sub>e</sub> peak oxygen uptake
Fixed part						
Constant	-1.701 (0.119)	-1.657 (0.127)	-2.004 (0.117)	-1.861 (0.121)	-1.694 (0.123)	-2.273 (0.099)
Log <sub>e</sub> body mass	0.631 (0.031)	0.609 (0.034)	0.815 (0.038)	0.713 (0.032)	0.655 (0.033)	0.964 (0.031)
Age	0.035 (0.004)	0.024 (0.006)	0.020 (0.005)	0.051 (0.005)	0.031 (0.005)	0.023 (0.004)
Age <sup>2</sup>	-0.010 (0.001)	-0.008 (0.001)	-0.007 (0.001)	-0.004 (0.001)	ns	-0.003 (0.001)
Pubic hair 2	-	0.038 (0.013)	ns	-	0.030 (0.011)	ns
Pubic hair 3	-	0.046 (0.015)	ns	-	0.063 (0.013)	ns
Pubic hair 4	-	0.052 (0.018)	ns	-	0.091 (0.015)	ns
Pubic hair 5	-	0.055 (0.023)	ns	-	0.091 (0.023)	ns
Log <sub>e</sub> skinfolds	-	-	-0.129 (0.018)	-	-	-0.185 (0.013)
Random part						
Level:2						
Var (cons)	0.006 (0.001)	0.006 (0.001)	0.004 (0.001)	0.007 (0.001)	0.006 (0.001)	0.003 (0.000)
Level: 1						
Var (cons)	0.004 (0.000)	0.004 (0.000)	0.004 (0.000)	0.005 (0.000)	0.004 (0.001)	0.004 (0.000)
-2 × loglikelihood	-1060.443	-951.197	-1107.768	-1088.073	-1028.937	-1238.092

*Vales are model estimates (standard error); boys' data centered on mean age 12.9 years; girls' data centered on mean age 12.8 years; ns, not significant ( $p > 0.05$ ); -, not entered; Table founded on 1057 determinations of peak oxygen uptake (boys, 556; girls, 501) and 972 (boys, 516; girls, 456) assessments of maturity status. Data from Armstrong and Welsman (2019b).*

For ethical and technological reasons the physiological mechanisms underpinning the development of youth aerobic fitness remain to be fully elucidated (Warburton and Bredin, 2017). HR at peak  $\dot{V}O_2$  is independent of sex, age, maturity status, body size, and body composition at least until the late teens (Rowland, 2017). Developmental changes in peak  $\dot{V}O_2$  are therefore a function of increases in SV and/or arterio-venous oxygen difference. Both oxygen delivery and oxygen utilization are facilitated by increases in FFM (Armstrong and McManus, 2017). Data on the development of arterio-venous oxygen difference are not available but the positive effects of FFM on the longitudinal development of SV has been demonstrated with multilevel allometric modeling (Armstrong and Welsman, 2002). Moreover, it has been reported that sex differences in SV disappear when it is expressed relative to allometrically scaled FFM (Vinet et al., 2003).

## Performance Tests and Health-Related Cut-Points

The evidence base outlined herein shows that is simplistic to describe aerobic fitness in a cross-sectional snapshot in relation to a single morphological covariate. Analyses of the development of aerobic fitness must take account not only of age but also of sex-specific, maturation-driven changes in FFM which are governed by individual biological clocks. 20mSRT predictions of peak  $\dot{V}O_2$  have confused our understanding of youth aerobic fitness with proposals to establish international age-related norms (Tomkinson et al., 2019b), to provide “reference standards” for

children as young as 2 years (Cadenas-Sanchez et al., 2019), to initiate fitness surveillance programs (Lang et al., 2018), and to promote inter-country comparisons of “who are the fittest?” (Lang et al., 2016). Moreover, youth who raise a “clinical red flag” or cross an age-related “cardiometabolic cut point” are more likely to be suffering from what Tanner (1949) identified as, “no more formidable a disease than statistical artifact” (p. 3) than warranting medical attention.

## CONCLUSION

Peak  $\dot{V}O_2$  increases in accord with sex-specific, concurrent changes in age- and maturity status-driven morphological covariates with the timing and tempo of changes specific to individuals. For clarity future studies should ensure that they address youth aerobic fitness and its relationship with present and future health with this firmly in mind.

## AUTHOR CONTRIBUTIONS

Both authors conceived the review, drafted the manuscript, and reviewed and approved the final version of the manuscript.

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# Effects of the Order of Physical Exercises on Body Composition, Physical Fitness, and Cardiometabolic Risk in Adolescents Participating in an Interdisciplinary Program Focusing on the Treatment of Obesity

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The main objective of this study was to investigate the effects of the order of physical exercises on body composition, physical fitness, and cardiometabolic risk in adolescents participating in an interdisciplinary program focusing on the treatment of obesity. The final 12-week analyses involved 33 female adolescents who were split into two groups of concurrent training (CT): resistance plus aerobic training and aerobic plus resistance training, with equalization performed in all physical exercises. The only difference between the two groups was the order in which the exercises were performed. The results showed reductions in fat mass, body fat, and waist circumference, as well as increases in musculoskeletal mass and resting metabolic rate ( $p < 0.05$ ) following the multiprofessional intervention period. However, no significant differences were observed in regard to body mass, body mass index, neck circumference, or arm circumference ( $p > 0.05$ ). Maximal isometric strength and maximal oxygen consumption showed significant increases after the intervention period ( $p < 0.05$ ). There were reductions in insulin, HOMA-IR, total cholesterol, triglycerides, and low-density lipoproteins ( $p < 0.05$ ), and an interaction within the resistance plus aerobic training group showed lower values for triglycerides when compared to itself ( $p = 0.002$ ). No difference was found in fasting glycemia for either group ( $p > 0.05$ ). It is worth noting that the equalization training variables presented no differences between the two groups ( $p > 0.05$ ). Based on these results, both CT methods were found to be effective in promoting health parameters in overweight and obese female adolescents, and triglyceride values decreased more in the resistance plus aerobic group. Future studies with larger samples and feeding control should be conducted to confirm or refute our findings.

**Keywords:** adolescent health, cardiometabolic risk in childhood, childhood obesity, exercise physiology, health promotion, interdisciplinary research

## INTRODUCTION

Obesity is substantially associated with increased caloric intake, low levels of physical activity, and reduced energy expenditure (Blüher, 2019). The literature has indicated that, by 2015, more than 100 million children and adolescents worldwide were classified as obese in both developed and developing countries (Lee and Yoon, 2018). Furthermore, obesity in adolescence exponentially increases the risks of developing other diseases, including diabetes mellitus and hypertension and other cardiovascular diseases in adulthood (Ho et al., 2019).

Currently, the scientific literature still lacks controlled studies seeking to identify the effects of physical exercise (PE) intensity on weight loss in adolescents since most studies present a low volume of interventions (Stoner et al., 2019). Therefore, consensus among researchers regarding the most efficient physical training method for reducing body fat (BF) in adolescents is lacking. Among the recommended strategies, multiprofessional intervention has been noted for the treatment of obesity (Branco et al., 2018).

In addition, Petridou et al. (2019) indicated that resistance exercises (RE) are relevant for the treatment of obesity due to increased resting metabolic rate (RMR) and fat-free mass, which consequently increase the day-to-day energy expenditure. The same authors suggested that aerobic exercises (AE) should be incorporated in weight-loss programs since, depending on volume and intensity, the energy expenditure during the session may be greater than that in resistance training (RT), especially when the resting intervals are very long.

Recently, Branco et al. (2018) compared the effects of two different concurrent training methods on body composition, physical fitness, and cardiometabolic risk of obese adolescents. The two physical-training protocols were similar in volume, intensity, and types of exercises. The only difference was the means by which the exercises were performed, i.e., one group used body weight and the other group used machines, and when possible, the exercises were equalized by the primary muscle groups involved in achieving the activity. The results of all analyzed variables were similar since the volume, intensity, and exercises performed were equalized.

Consistent with Kang and Ratamess (2014) regarding the choice of the protocol used to conduct concurrent training, AE or RE at the beginning or end of the session will depend on the first objective of physical training for athletes of different modalities. However, this condition is recommended for athletes but not for sedentary individuals or for the treatment of certain chronic diseases. The same authors noted that the choice of the order of PE within the same training session can be organized according to the specificity of the modality, i.e., for athletes with aerobic resistance modalities, performing aerobic training first to increase the quality of their training is recommended. However, for athletes seeking muscular hypertrophy, it is recommended that RE be performed at the beginning of the training session for the same reason mentioned above. The World Health Organization [WHO], (2010) suggested that children and adolescents need to improve cardiorespiratory fitness and muscular endurance and strength. Thus, a strategy that may

be used is concurrent training (Coffey and Hawley, 2017). Furthermore, Vilaça et al. (2011) emphasized that a combination of AE and RE (regardless of order) is beneficial for reducing BF and increasing energy expenditure during and after PE sessions.

To the best of the author's knowledge, the chronic physiological and metabolic responses under the order of concurrent training in obese sedentary adolescents are unknown. Regardless of the order of the execution of PE, there is no consensus or indication regarding which model/order of PE should be followed in order to enhance improvement in components related to physical fitness in terms of health. Considering these aspects, the identification of physiological and metabolic responses during respective intervention models could direct the interventions and even prevent paradigms or concepts based on the common sense of health professionals, especially those involved in prescribing PE. Therefore, the aim of the present study was to investigate the effects of the order of physical exercises on body composition, physical fitness, and cardiometabolic risk in female adolescents participating in an interdisciplinary program focusing on the treatment of obesity. Regarding the hypothesis, it is believed that the responses observed in the different parameters analyzed will be similar since only the order of the exercises was changed.

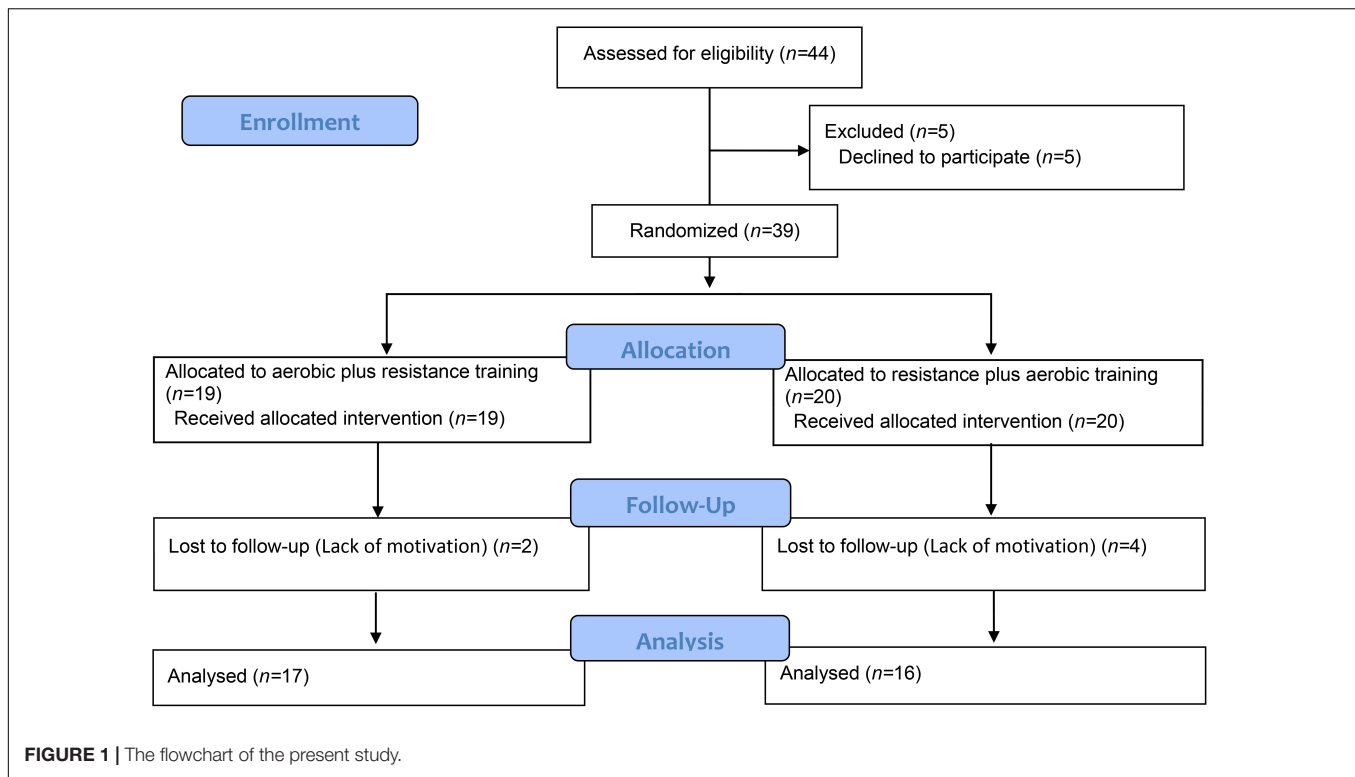
## MATERIALS AND METHODS

### Participants

A total of 44 female adolescents were recruited according to the following established inclusion criteria: (1) age between 13 and 17 years; (2) overweight or obesity according to the cutoff ranges established by Cole and Lobstein (2012); and (3) availability to participate in multiprofessional interventions 3× per week during the evening for a period of 12 weeks. The exclusion criteria were as follows: (1) participating in sports practices systematized outside of school, (2) orthopedic, cardiovascular, or cognitive deficits that impede practicing PE, (3) some type of restrictive diet, i.e., low-carb, low-fat or hypocaloric, (4) the use of a psychotropic or appetite-regulating medicine, and (5) completion of less than 75% of the multiprofessional interventions during the 12-week intervention. It is emphasized that the adolescents were encouraged to be more physically active during the intervention (outside the university setting). In relation to the variables, a sample size with nine participants in each group intervention was enough to detect eventual changes in dependent variables, with standard deviation smaller when compared to previous study, with 80% confidence (Branco et al., 2018). The details of the process are provided in **Figure 1**, which depicts the flowchart for the present study.

### Ethics Statement

This study was approved by the Committee for Ethics in Research of the University Center of Maringá (UniCesumar) through the opinion n° 2,505,200/2018. The research followed all recommendations proposed by resolution 466/2012 of the Ministry of Health of the Brazilian Government and the Declaration of Helsinki. All parents or guardians of



the adolescents were informed in detail about the purposes of the study and subsequently signed an informed consent form. In addition, the adolescents signed a consent term agreeing to participate.

## Study Design

This study adopted an experimental, longitudinal, parallel-group, and repeated-measures design. Previously, the adolescents were randomly allocated to one of the following two experimental groups: G1 = aerobic plus resistance group or G2 = resistance plus aerobic group. The only distinction between the groups was the order of performing the activities, i.e., G1 performed AE followed by RE, whereas G2 performed the activities in the inverse order, i.e., RE followed by AE. The details of the aerobic and resistance exercises in regard of volume and intensity are presented in the discussion below. The participants performed the interventions three times a week for 12 weeks guided by a team of professionals in physical education, nutrition, and psychology. PE was performed 3× per week divided into training A and B, as theoretical and practical classes related to nutrition were conducted 2× per week, and cognitive-behavioral therapy was provided 1× per week over the course of the 12-week intervention. On the first day of evaluations, the adolescents underwent a medical consultation involving the following: (a) anamnesis, (b) measurement of resting blood pressure, (c) pulmonary and cardiac auscultation, (d) completion of the International Physical Activity Questionnaire (IPAQ), and (e) release for venous blood collection. On the second day, the following were performed: (a) venous blood collection following ~ 12 h of fasting, (b) body

composition assessment, (c) anthropometry, and (d) explanation and collection of food records. On the third day, physical tests were performed in the following order: (a) maximal isometric handgrip test (MIHT), (b) maximal isometric lumbar-traction strength (MILTS), (c) maximal isometric lower-body strength (MILBT) and (d) aerobic fitness. The 3 days of evaluations were separated by a 48-h interval. Considering that the interventions were performed on Mondays, Wednesdays, and Fridays, the evaluations began on the same days, and re-evaluations were conducted after the weekend. The girls rested for 2 days, and the process followed the same days of evaluation in the beginning of this study.

## Exercise Training Protocol

The training sessions were divided into two mesocycles performed in circuit form with series A and B. The two series were performed alternately to promote the physiological recovery of the muscle groups previously worked. The training schedule was divided into two mesocycles of 6 weeks duration each. Regarding the exercises, we chose to perform PE without repetition counting and to control the exercises by timing the activities. During the first mesocycle, the effort: pause ratio was 30'' by 30'', while for the second mesocycle, the effort: pause ratio was 40'' by 20'' using a 1:1 ratio between the concentric and eccentric phases. In addition, before the session, the adolescents performed a 10-min warm-up as follows: light walking and stretching of the main muscle groups (with low muscle tension). To periodize the training, promote anatomical adaptation, and reduce any lesions, the intensity of the sessions was controlled by the Borg (1982), which includes values of 6 arbitrary units (a.u.)

up to 20 a.u., i.e., from very easy to exhausting. **Table 1** shows the AE performed by both experimental groups.

**Table 2** shows the intensity of the resistance efforts during the 12-week intervention.

**Table 3** presents the training program groups A and B performed during the first and second RT mesocycles.

## Training Monitoring

To monitor the physical training sessions, several non-invasive and practical instruments were used by the physical trainer. Prior to each training session, the rating of the perceived recovery (RPR) scale as proposed by Laurent et al. (2011), the rating of perceived exertion (RPE session) as proposed by Foster et al. (2001), and the internal training load (ITL) were assessed according to the considerations proposed by Nakamura et al. (2010). The RPR indicates the recovery status and is used before each training session. The scale comprises numerical values ranging from 0 a.u. (very poor recovery/extremely tired) to 10 a.u. (very good recovery/extremely well-disposed). The RPE session was applied 30 min after the end of the training sessions. This scale comprises values ranging from 0 a.u. (extremely light effort) to 10 a.u. (extremely heavy stress). The ITL was calculated by multiplying the value of the RPE by the duration of the training session in minutes. The girls were familiarized with the scales before and during interventions. The scales were administered to the girls, and explanations were provided during the theoretical classes and before the PE sessions.

## IPAQ Questionnaire

The IPAQ questionnaire adapted for adolescents was used to identify the levels of physical activity before and after the interdisciplinary interventions and to determine whether the adolescents had already engaged in other moderate/intense physical activities before starting the program (Guedes et al., 2005).

## Nutrition Intervention

The nutritional interventions aimed to guide the adolescents in nutritional aspects such as: (a) introduction to healthy eating: food manufacturers, regulators, energy, and the food pyramid, (b) frequency and portioning of food, (c) soluble and insoluble fibers, (d) the importance of fiber consumption, (e) a list of food replacements, according to the food groups, (f) pre- and post-physical activity feeding, (g) minimally processed, processed, and ultra-processed foods, (h) amounts of sugar, salt, and fat in foods, (i) how to read and interpret food labels, (j) diet and “light” foods, (k) eating disorders, (l) the dangers of “fad diets”, and (m) ingesting food for health and quality of life using food re-education as an instrument. Those responsible were advised to note, in specific forms, all foods and beverages consumed throughout the day, and also to record the size of portions consumed; the name of the preparations, their ingredients, and the method preparation. They were also instructed to note details such as the addition of salt, sugar, oil, sauces, etc., and whether the food or drink consumed was regular, diet, or light. For the

**TABLE 1** | Systematization of aerobic training during 12 weeks of intervention.

Order	Exercises	Effort time	Type of exercise	Intensity
1	Light jogging	4 min	Continuous	9–10 a.u.
2	Running	4 min	Continuous	13–14 a.u.
3	Sprints during 40 m and active self-selected recovery for 20 m	8 min	HIIE	All-out mode
4	Group activities	4 min	Interval activities	Self-selected
	Total per session	20 min		

Group activities involved collective games and recreation; HIIE, high-intensity interval exercise; a.u., arbitrary units of Borg scale (6–20).

**TABLE 2** | Intensity of the resisted efforts during the 12 weeks of intervention.

Week	Intensity	Series	Time per session
1st and 2nd	10–12 a.u.	2×	18 min + 3 min of rest + 5 min of slowdown = 26 min
3rd and 4th	10–12 a.u.	3×	27 min + 6 min of rest (3 min per series) + 5 min of slowdown = 38 min
5th and 6th	12–14 a.u.	2×	18 min + 3 min of rest + 5 min of slowdown = 26 min
7th and 8th	12–14 a.u.	3×	27 min + 6 min of rest (3 min per series) + 5 min of slowdown = 38 min
9th and 10th	15–17 a.u.	2×	18 min + 3 min of rest + 5 min of slowdown = 26 min
11th and 12th	15–17 a.u.	3×	27 min + 6 min of rest (3 min per series) + 5 min of slowdown = 38 min

After each series that was performed in a circuit format, the adolescents rested for 3 min and then the exercises continued until the series were completed, according to the training weeks. In slowdown, it was performed low intensity aerobic exercises and light intensity stretching exercises were used for the most used muscle groups during the session; a.u., arbitrary units of Borg scale (6–20).

**TABLE 3** | First and second mesocycle of resistance training of both experimental groups.

Order	Training program A	Order	Training program B
<b>First mesocycle of RT (effort: pause ratio 30'' per 30'')</b>			
1	Push-ups (on knees)	1	Suspended row
2	Half squat	2	Hip bridge
3	Plank	3	Crunch abdomen
4	Medicine ball chest throw (with partner)	4	Pulling tire with rope
5	Box march	5	Standing calf raise
6	Medicine ball alternating side rotation throw	6	Crunch abdomen throwing medicine ball
7	Medicine ball overhead throw	7	Upright Row with dumbbells
8	Half squat in isometric position	8	Stiff with dumbbells
9	Twisting Sit-up	9	Abdomen with Swiss ball
<b>Second mesocycle of RT (effort: pause ratio 40'' per 20'')</b>			
1	Push-ups (on knees) using a step (progression of the previous physical exercise)	1	Suspended row - neutral grip
2	Thruster with medicine ball	2	Hip bridge
3	Plank	3	Twist abdomen with medicine ball
4	Throw wall-ball on the wall	4	Rope tsunami + half isometric squat
5	Low skipping	5	Agility ladder: lateral displacement exercises
6	Medicine ball alternating side rotation throw	6	Crunch abdomen throwing medicine ball
7	Half squat in isometric position with Swiss ball between the legs	7	Suspended row - pronated grip
8	High skipping	8	Flexion and knee extension with Swiss ball
9	Plank changing arms and legs	9	Abdomen with Swiss ball

RT, resistance training; each mesocycle presented 6 weeks of duration.

best estimation of the portion size, they used home measurements (Slater et al., 2002). The classes, which were conducted by means of theoretical activities and practical dialogues, lasted approximately 1 h and took place 2× per week. A 3-day food recall was applied on two non-consecutive days of the week and on 1 day at the end of the week. All data were tabulated using the program Avanutri (Avanutri Equipamentos de Avaliação Ltda, Três Rios, Brazil). Another point that needs to be highlighted was the absence of feeding control. It was suggested that the teenagers change their dietary habits according to theoretical and practical classes on nutrition and psychological interventions. However, a control of percentages of macronutrient ingestion or the quality of the food consumed was not performed during this study. Finally, the kilocalories/day ingested by the adolescents were calculated, and the values pre- and post-intervention were compared. The intervention model and food-recall analysis procedures were the same as those performed in the study conducted by Branco et al. (2018).

## Psychological Intervention

The primary themes of the psychological intervention were cognitive and behavioral strategies used to improve the quality of food consumption among the adolescents, including self-monitoring, control of anxiety, negative feelings, and health

education. The activities also aimed to reduce negative emotions regarding the participants' relationships with food consumption and strategies used to improve the assessment of body self-image. Psychological intervention was divided into four large groups of subjects, and a cognitive-behavioral methodology in a group setting was used once a week for 1 h.

## Eating Habits

Discussions about food re-education explored the eating habits of the participants and their families.

## Self-Monitoring

Self-monitoring is an important variable to be discussed in the behavioral treatment of obesity. The meetings held with this theme were conducted with the following characteristics: group activities to increase mutual knowledge and foster interaction among the participants, discussions about the advantages of having a healthy lifestyle for the whole family and alluding to the practice of exercise and its advantages.

## Control of Anxiety and Negative Feelings

The purpose was to promote reflection on anxiety and to explore the fears, difficulties, and expectations of the participants. The purpose of the meetings with this theme was to discuss issues related to the acquisition of behaviors considered healthy

and to reduce the negative feelings related to anxiety, as well as to promote the participants' self-knowledge so that they could reflect on positive and negative thoughts that permeate their daily lives.

### Health Education

The purpose of the meetings was to raise awareness about issues related to the reality of the group, for example, to demystify beliefs, prejudices, and stereotypes associated with obesity. The interventions followed the model proposed by Branco et al. (2018).

### Medical Consultation

Prior to all activities, the adolescents consulted with a medical team. They were interviewed following a structured anamnesis to investigate their clinical history, previous and current pathologies, and their use of medications. In addition, pulmonary and cardiac auscultation and blood pressure measurements were performed according to the 7th Brazilian Guideline of Arterial Hypertension (Malachias et al., 2016).

### Biochemical and Hormonal Tests

The adolescents arrived at the biochemistry laboratory of the teaching institution after fasting for approximately 12 h. After local asepsis of the arm, a puncture was performed on the antecubital veins of the evaluated adolescents. All analyses were performed by a biomedical team blinded to the details of the interventions, i.e., the team did not have access to the intervention model realized by the adolescents during the 12-week intervention. All analyses were performed in triplicate using Siemens reagents (Frimley, Camberley, United Kingdom) in accordance with the manufacturer's specifications. Subsequently, the samples were centrifuged at 3,600 rpm for 11 min at a controlled temperature (24°C) for the separation of the serum and plasma. Vacuum tubes (Becton Dickinson Vacutainer®, Plymouth, United Kingdom) were used for all collections, namely a tube with potassium fluoride and ethylenediaminetetraacetic acid (EDTA) was used for the analysis of the fasting plasma glucose level, and a clot activator tube (silica) was used for the analysis of the insulin, total cholesterol (TC), high-density lipoprotein (HDL-c), low-density lipoprotein (LDL-c), and triglyceride (TG) serum levels. For the biochemical analyses, Siemens equipment (Advia 1800 Chemistry Analyzer®, Siemens Healthcare Diagnostics, Illinois, United States) was used. The formula  $HOMA-IR = \text{fasting glucose (nmol/L)} \times \text{fasting insulin test (HOMA-IR)}$  was used to calculate the homeostasis model assessment (HOMA) to evaluate insulin resistance [ $\mu\text{U/mL}/22.5$ ] according to Matthews et al. (1985).

### Body Composition Assessment

Electrical bioimpedance (BIA) was conducted using the eight-tailed InBody four-quadrupole multifrequency apparatus (model 570® Body Composition Analyzers, Seoul, South Korea). On the first day of evaluations, all participants received an informative document containing the protocol used to perform the assessment, including (a) 12-h fasting, (b) no moderate or vigorous physical exercise within the 24 h preceding the test,

(c) urination and evacuation for the evaluation, (d) the absence of metallic objects during the evaluation, (e) postponement of the assessment in menstruating participants until after their period, (f) at least 8 h of sleep, and (g) no ingestion of caffeinated beverages or foods during the 12 h prior to the measurement (Heyward, 2001; Branco et al., 2018b). The following BIA variables were evaluated: body weight, body mass index (BMI), musculoskeletal mass (MME), fat mass (FM), %BF, and RMR.

### Anthropometry

Stature was measured by an electronic scale with a stadiometer (Filizola®, São Paulo, Brazil) with a capacity of 200 kg, with a measuring capacity of 2 m and accuracy of 0.1 cm. Waist (WC), relaxed right arm (AC), and neck (NC) circumferences were measured following the protocol proposed by Heyward (2001). An extensible measuring tape of the WISO brand (model T87-2®, Florianópolis, Santa Catarina, Brazil) was used with a measuring capacity of 2 m and precision within 0.1 cm.

### Physical Assessment

Before the measurements were obtained, all adolescents were familiarized with the MIHS, MILTS, MILBS and aerobic fitness tests (Branco et al., 2018a).

#### MIHS

A Jamar dynamometer (Asimow Engineering, Los Angeles, CA, United States) was used to evaluate MIHS. The teenager remained standing while holding the device close to her body with her arm extended with a neutral grip. Following a signal by the evaluator, the adolescent squeezed the dynamometer as hard as possible while maintaining the isometric contraction for 3 to 5 s. The measurement was performed with both hands. It was used the sum of right and left hand (sum of maximum isometric handgrip strength of right and left hand – SMHS, in the statistical analysis), for After three attempts on each side, the maximum isometric strength of the handgrip of the right and left hands was recorded. A 60-s interval separated each attempt (Branco et al., 2018a).

#### MILTS

A Kratos dynamometer (Kratos Industrial Equipment, Model DS®, São Paulo, Brazil) was used to evaluate MILTS. The teenager walked with both feet on the device with her knees extended, trunk flexed approximately 120°, head and neck aligned with the trunk, and fingers (holding the bar) positioned in front of the patella bone. The maximum contraction over 3 to 5 s was recorded, and the highest value of the measurement after three attempts with 60 s of rest between attempts was recorded (Branco et al., 2018a).

#### MILBS

To evaluate the maximal isometric strength of the lower limbs, a Kratos dynamometer also used (the same equipment of evaluation of MILTS). The teenager stood on a platform with her knees bent at approximately 120° and trunk upright while holding the traction bar lined up in the inguinal fold. Following the evaluator's signal, the adolescent performed the maximum

isometric contraction for 3 to 5 s, and the highest measured value after three attempts with 60-s resting intervals was recorded (Branco et al., 2018a).

### Aerobic Fitness

To measure aerobic fitness, the maximal oxygen consumption ( $\text{VO}_2\text{max}$ ) test, which was proposed by Léger and Lambert (1982), was used. This test has 21 stages; the initial velocity is 8.5 km/h, which increases by 0.5 km/h per stage. The  $\text{VO}_2\text{max}$  was calculated by the following equation:

$$\text{VO}_2\text{max} = 31.025 + (3.288 * X) - (3.248 * A) \\ + (0.1536 * A * X)$$

where X = velocity in the stage reached, and A = age in years.

### Statistical Analysis

Previously, the normality of the data was tested using the Shapiro–Wilk test. Similarly, the homogeneity of the data was tested by Levene’s test. After confirming normality and homogeneity, the data were expressed as the mean and ( $\pm$ ) standard deviation. Mauchly’s test of sphericity was used to test as well as the Greenhouse-Geisser correction, if necessary. A two-way mixed-measures analysis of variance (ANOVA) was used to compare the groups and phases (pre- and post-intervention). Bonferroni *post hoc* test was used when a significant difference was found. A significance level of 5% was adopted for all analyses. In addition, the effect size was calculated using Cohen’s d (1992) as follows: 0.20 (*small effect*), 0.50 (*moderate effect*), and 0.80 (*large effect*). Furthermore, the partial eta-squared:  $\eta^2$  was calculated with the following classification: 0.10 (*small effect*), 0.25 (*moderate effect*), and 0.40 (*large effect*), according to Cohen (1992). All statistical analyses were performed using the statistical package SPSS 22.0 (IBM, Inc., United States).

## RESULTS

Information regarding age, body weight, and stature is provided in **Table 4**.

### IPAQ Responses

For the IPAQ, no significant differences were observed in the level of physical activity among the adolescents after the 12-week multiprofessional intervention ( $p > 0.05$ ). The only difference observed was an increase in the level of physical activity performed on Mondays, Wednesdays, and Fridays. However, this result was expected since the adolescents participated in the activities 3  $\times$  per week during the 12-week intervention.

### Training Responses

For the RPE, ITL, and RPR, no significant differences were identified during the same microcycles and mesocycles of training performed by the adolescents ( $p > 0.05$ ). These responses suggest that both training groups were similarly equalized in recovery, volume, and intensity.

## Food Recall

For the caloric intake in kcal, no significant differences were observed before and after the 12-week intervention ( $p > 0.05$ ). These responses suggest that the 12-week nutritional intervention is incipient for promoting significant changes in caloric intake.

**Table 5** presents the body composition and anthropometric measurements of the adolescents participating in the interdisciplinary project for the treatment of obesity before and after the 12-week intervention.

Age, body weight, stature, BMI, CN, and AC did not show group, time or interaction effects. However, specifically regarding MME [ $F_{(1,31)} = 5.71$ ,  $p = 0.02$ ,  $\eta^2 = 0.15$ , *small*] and RMR [ $F_{(1,31)} = 5.06$ ,  $p = 0.03$ ,  $\eta^2 = 0.14$ , *small*] a time effect was identified, with higher values after the intervention period when compared to the pre-intervention values, while FM [ $F_{(1,31)} = 7.79$ ,  $p = 0.008$ ,  $\eta^2 = 0.20$ , *small*], %BF [ $F_{(1,31)} = 19.43$ ,  $p = 0.001$ ,  $\eta^2 = 0.38$ , *medium*] and WC [ $F_{(1,31)} = 11.28$ ,  $p = 0.002$ ,  $\eta^2 = 0.28$ , *medium*] showed significantly lower values after the intervention period.

**Table 5** shows the physical tests performed by the adolescents participating in the interdisciplinary project for the treatment of obesity before and after the 12-week intervention.

For MIHS [ $F_{(1,31)} = 4.13$ ,  $p = 0.05$ ,  $\eta^2 = 0.11$ , *small*], MILTS [ $F_{(1,31)} = 6.18$ ,  $p = 0.01$ ,  $\eta^2 = 0.16$ , *small*], MILBS [ $F_{(1,31)} = 13.49$ ,  $p = 0.0008$ ,  $\eta^2 = 0.30$ , *medium*] and  $\text{VO}_2\text{max}$  [ $F_{(1,31)} = 116.35$ ,  $p < 0.001$ ,  $\eta^2 = 0.79$ , *large*] a time effect was identified, with higher values after the intervention period when compared to the pre-intervention values.

**Table 6** shows the biochemical examination results of the adolescents participating in the interdisciplinary project for the treatment of obesity before and after the 12-week intervention.

Fasting glycemia levels, did not show group, time or interaction effects. Regarding insulin [ $F_{(1,25)} = 9.67$ ,  $p = 0.004$ ,  $\eta^2 = 0.28$ , *medium*], HOMA-IR, [ $F_{(1,25)} = 6.44$ ,  $p = 0.01$ ,  $\eta^2 = 0.22$ , *small*], TG [ $F_{(1,25)} = 12.12$ ,  $p = 0.001$ ,  $\eta^2 = 0.33$ , *medium*], TC [ $F_{(1,25)} = 22.59$ ,  $p = 0.000$ ,  $\eta^2 = 0.47$ , *large*] and LDL-c [ $F_{(1,24)} = 1.56$ ,  $p = 0.003$ ,  $\eta^2 = 0.06$ , *small*] a time effect was identified, with lower values after the intervention period when compared to the pre-intervention values. In addition, it was observed an interaction effect for TG [ $F_{(1,25)} = 4.29$ ,  $p = 0.048$ ,  $\eta^2 = 0.15$ , *small*] with lower values in the resistance plus aerobic group in post-intervention period when compared to the pre-intervention values ( $p = 0.002$ ). Besides, for HDL-c [ $F_{(1,25)} = 29.43$ ,  $p < 0.001$ ,  $\eta^2 = 0.54$ , *large*] a time effect was identified, with higher values after the intervention period when compared to the pre-intervention values.

## DISCUSSION

The main results of the present study indicated the following differences after 12 weeks in the two intervention groups: (a) increase in MME and RMR, (b) reduction in FM, %BF, and WC, (c) increase in MIHS, MILTS, and MILBT, (d) increase in  $\text{VO}_2\text{max}$ , (e) reduction in insulin, HOMA-IR, TG, TC, and LDL-c levels, and (f) increase in HDL-c. In addition, an interaction was detected with lower values of TG in the resistance



**TABLE 4 |** Body composition and anthropometric measurements of female of adolescents participating in the study.

Variables	Aerobic plus resistance training group			Resistance plus aerobic training group		
	Pre	Post	Cohen's d	Pre	Post	Cohen's d
Age (years old)	13.4 ± 2.0	13.9 ± 2.1	0.2	13.1 ± 3.0	13.1 ± 2.5	0.0
Body mass (kg)	90.8 ± 23.7	87.5 ± 19.3	-0.1	86.5 ± 21.1	85.8 ± 21.0	-0.0
Stature (cm)	162.0 ± 8.7	164.4 ± 9.6	0.3	161.1 ± 11.4	161.2 ± 10.5	0.0
BMI (kg/m <sup>2</sup> )	34.1 ± 7.1	32.2 ± 5.8	-0.3	33.6 ± 6.2	32.1 ± 5.4	-0.2
MME (kg)*	27.6 ± 6.6	28.3 ± 6.6	0.1	27.0 ± 6.6	27.3 ± 7.0	0.0
FM (kg) *	41.7 ± 14.9	36.5 ± 11.9	-0.3	40.9 ± 13.8	36.5 ± 11.8	-0.3
BF (%) *	44.7 ± 6.4	41.3 ± 6.8	-0.5	44.6 ± 7.4	41.9 ± 6.8	-0.4
RMR (Kcal) *	1414.3 ± 216.1	1470.6 ± 237.6	0.3	1427.8 ± 241.3	1434.2 ± 235.6	0.0
WC (cm)*	95.6 ± 13.1	91.7 ± 10.4	-0.3	93.6 ± 12.9	92.2 ± 11.3	-0.1
NC (cm)	35.9 ± 3.4	35.8 ± 3.0	0.0	36.2 ± 3.4	36.1 ± 3.0	0.0
AC (cm)	34.7 ± 4.5	34.7 ± 3.8	0.0	35.1 ± 5.2	35.5 ± 5.3	0.1

Data are expressed as mean and (±) standard deviation; BMI, body mass index; MME, musculoskeletal mass; FM, fat mass; BF, percentage of body fat; RMR, resting metabolic rate; WC, waist circumference; NC, neck circumference; AC, relaxed right arm circumference; \*time effect ( $p < 0.05$ ).

**TABLE 5 |** Physical tests of female of adolescents participating in the study.

Variables	Aerobic plus resistance training group			Resistance plus aerobic training group		
	Pre	Post	Cohen's d	Pre	Post	Cohen's d
SMIHS (kg) *	51.4 ± 13.2	55.0 ± 14.0	0.3	51.6 ± 16.5	53.1 ± 18.8	0.1
MILTS (kg) *	59.5 ± 14.5	64.1 ± 17.3	0.3	56.4 ± 24.5	65.0 ± 24.1	0.4
MILBS (kg) *	59.9 ± 15.6	70.8 ± 22.3	0.7	57.8 ± 28.2	75.2 ± 31.1	0.6
VO <sub>2</sub> max (mL/kg/min) *	35.4 ± 4.2	43.7 ± 6.6	2.0	37.9 ± 5.1	46.0 ± 8.4	1.6

Data are expressed as mean and (±) standard deviation; SMIHS, sum of maximum isometric handgrip strength of right and left hand; MILTS, maximum isometric lumbar-traction strength; MILBS, maximum isometric lower-body strength; VO<sub>2</sub>max, maximal oxygen consumption; \*time effect ( $p < 0.05$ ).

**TABLE 6 |** Hormonal and biochemical tests of female of adolescents participating in the study.

Variables	Aerobic plus resistance training group			Resistance plus aerobic training group		
	Pre	Post	Cohen's d	Pre	Post	Cohen's d
Fasting glycemia (mg/dL)	82.6 ± 4.4	82.0 ± 3.9	-0.1	81.7 ± 6.2	82.0 ± 8.8	0.0
Insulin (μ/mL)*	13.7 ± 4.2	11.2 ± 3.4	-0.6	14.0 ± 6.7	11.3 ± 5.5	-0.4
HOMA-IR *	2.84 ± 0.94	2.28 ± 0.71	-0.6	2.93 ± 1.35	2.34 ± 1.38	-0.4
Triglycerides (mg/dL)*	96.5 ± 30.2	86.9 ± 35.9	-0.3	113.9 ± 38.2	76.1 ± 27.2 <sup>#</sup>	-1.0
Total cholesterol (mg/dL)*	169.6 ± 25.5	150.3 ± 25.0	-0.8	171.9 ± 23.0	144.1 ± 30.5	-1.2
LDL-c (mg/dL)*	100.3 ± 23.1	90.1 ± 17.4	-0.4	99.9 ± 20.8	88.3 ± 22.4	-0.6
HDL-c (mg/dL)*	42.8 ± 6.9	50.1 ± 8.6	1.1	40.7 ± 9.6	48.6 ± 9.5	0.8

Data are expressed as mean and (±) standard deviation; HOMA-IR, homeostatic model assessment; LDL-c, low density lipoprotein; HDL-c, high density lipoprotein; \*time effect ( $p < 0.05$ ); <sup>#</sup>interaction with lower values at the post-intervention for the same experimental group ( $p = 0.001$ ).

plus aerobic group after the intervention period. However, no differences were observed in fasting glycemia levels in the two experimental groups after the 12 weeks. As a result, the study's hypothesis was confirmed.

In the condition that the concurrent protocols are somehow equalized, the training responses have a tendency to be similar (Branco et al., 2018). The findings of the present study indicate that MME increased significantly after 12 weeks of concurrent training concomitant with multiprofessional care in obese adolescents. Thus, muscle hypertrophy is intimately associated with the optimal volume and intensity of RE

(Schoenfeld, 2010). A study conducted by Branco et al. (2018) did not identify muscle hypertrophy after 12 weeks of concurrent training in obese male adolescents. However, the volume of the resistance exercises conducted by Branco et al. (2018) was relatively lower than that in the present study. Regarding this discrepancy, Figueiredo et al. (2018) noted that volume can be considered the most effective variable in RE for muscle hypertrophy. However, in a letter to the editor, Souza et al. (2018) explained that RE is very complex, and due to methodological differences among scientific studies, determining the decisive variables for establishing what volume is critical for muscle

hypertrophy is impossible. In light of these different findings and because scientific studies sometimes employ different intervention models with dissimilar body-composition analyses, it is believed that the key to the process of muscle hypertrophy is the optimal interdependence of volume and intensity based on the uniqueness of individuals. Thus, the differences observed between the study conducted by Branco et al. (2018) and the present study may be related to the higher volume of RE performed by the adolescents in this study.

Considering absolute FM and BF, significant reductions were identified after 12 weeks. However, it is suggested that the reduction in %BF is mainly related to the increase in energy expenditure resulting from the concurrent training practiced since no significant differences in caloric intake were detected during the 12-week period. In addition, no differences among IPAQ responses were identified during the 12-week intervention. Branco et al. (2018) identified no alterations in caloric intake inferred from food records for 2 days during the week and one weekend day and IPAQ responses over the course of 12-week multiprofessional interventions that focused on reducing body fat and cardiometabolic risk and improving the components of physical fitness related to health in adolescents. Despite the limitations of overestimation or underestimation during the completion of food records and the IPAQ, especially by adolescents, considering the above-mentioned aspects is recommended to determine whether the reduction in absolute and relative fat is closely linked to an increase in energy expenditure resulting from physical exercises performed daily for a duration of ~60 min, reaching ~180 min weekly. Thus, Donnelly et al. (2004) suggested that the addition of regular physical exercise over the medium and long term can result in additional energy expenditure (between 1000 and 2000 kcal per week depending on volume and intensity). However, regarding energy expended to be converted to reduce body fat, it cannot be compensated by increasing daily caloric consumption.

Well-controlled studies indicate that an elevation in RMR is closely related to increased levels of physical activity if dietary restrictions with a negative energy balance are not incorporated (Stiegler and Cunliffe, 2006). Thus, overall, the gain of MME directly affect elevation in the RMR (Stiegler and Cunliffe, 2006). However, studies in the literature note that MME accounts for only 20% of oxygen consumption at basal conditions (Brozek and Grande, 1955; Durnin and Passmore, 1967; Wahrlich and Anjos, 2001). Thus, importantly, the energy expenditure of the MME represents only 13 kcal/kg/day (McClave and Snider, 2001). Consequently, the increase in MME has a very low impact on the total energy expenditure/day of individuals. Therefore, it is essential to increase the performance of structured and unstructured physical activities and decrease the time spent performing low-intensity activities to increase energy expenditure throughout the day.

Regarding WC, significant reductions were identified after the multiprofessional intervention period. Branco et al. (2018) also identified a reduction in WC in overweight or obese adolescents after a 12-week intervention. Reduction in WC

has a considerable impact on reduction in cardiometabolic risk (Freedman et al., 1999). As a result, despite the significant reduction in WC in the two intervention groups, the values are considered high (Patnaik et al., 2017). However, NC and AC did not exhibit significant differences, which is likely due to the short duration of the 12-week intervention. These two measures, NC and AC, have been associated with cardiometabolic risk (Jaiswal et al., 2017; Patnaik et al., 2017). Thus, both anthropometric measures can also be used in clinical practice for the evaluation of adolescents with overweight or obesity. From this perspective, Patnaik et al. (2017) identified that WC and NC are strongly correlated ( $r = 0.69$ ), suggesting that both may be used to identify possible conditions related to cardiometabolic risk. In addition, Jaiswal et al. (2017) reported accuracy between 0.92 and 0.98 of the ROC curve of the association between arm circumference and overweight in children and adolescents. In summary, it has been identified that 12 weeks of multiprofessional interventions are insufficient to reduce WC and AC. These responses can be interrelated with a higher deposition of fat mass in WC compared to NC and AC. Thereby, NC and AC can be used to verify the applicability of these measures in longer-term obesity-treatment interventions.

Regarding the variables of health-related physical fitness, significant improvements were observed in MIHS, MILTS, MILBT, and  $VO_2$ max in the two experimental groups, results that were expected. A relevant point highlighted by Nardo Júnior et al. (2018) discuss that success criteria for the multiprofessional treatment of obesity. The physical fitness variable selected in the study as substantial was aerobic fitness. Improvement in cardiorespiratory fitness reduces risks associated with morbidity and mortality and is considered a key component of the cardiovascular health of adolescents (Nardo Júnior et al., 2018).

Fasting glycemia did not show significant changes in either experimental group after the intervention period. However, the values found in the adolescents before and after the respective time were well below (from 81.7 to 82.6 mg/dL) the cutoff points proposed by the American Diabetes Association, i.e., < 100 mg/dL after fasting for 8 h (American Diabetes Association [ADA], 2018). Nevertheless, significant reductions were observed in the fasting insulin levels in both experimental groups. Thus, it was verified that physical exercise activates the insulin-signaling pathways, facilitating the process of glucose diffusion via GLUT-4 (Röhling et al., 2016). Therefore, considering the mechanisms occurring via GLUT-4, by performing exercise, sensitivity to insulin action is increased and lower levels of insulin release are required for glucose uptake. In regard to lipid profiles, significant reductions in TG, total cholesterol, and LDL-c levels and an elevation in HDL-c were identified in both experimental groups. A systematic review and meta-analysis published by García-Hermoso et al. (2018) identified that concurrent training tends to be more effective for the treatment of variables associated with the lipid profile, particularly in pediatric obesity. A substantial difference was observed for TG levels in this study, and decreases in TG are closely linked to reductions in cardiovascular diseases (Keech and Jenkins, 2017). TG decreases may be associated with PE

and suggest changes in feeding during the interdisciplinary intervention (Hartley et al., 2016; Surampudi et al., 2016); such dietary changes may be related to the increased consumption of fibers and omega-3 (Surampudi et al., 2016; Siscovick et al., 2017). However, this is only a hypothesis since feeding control was not conducted during the 12-week interdisciplinary interventions. Considering that, in the present study, the authors did not control food intake over the course of the intervention, it is not possible to confirm that one model is better than the other to further reduce TG levels. Therefore, the differences between the groups can be attributed to a spurious relationship because other variables can influence the responses that are interrelated. Acute studies have identified similar global responses between the order of exercise during concurrent training (Coffey and Hawley, 2017). Nevertheless, in accordance with the author's knowledge, these are still incipient studies that tested the responses among these biochemical and hormonal variables in order to verify the effectiveness of the order of the concurrent training on these variables. In this way, new longitudinal studies that include food control can be tested to verify the responses between the order of exercise.

Finally, considering that the two models of intervention resulted in similar responses, the choice of the order may be suggested by adolescents in order to maintain adherence, enjoyment, and satisfaction and to reduce dropouts. In addition, another point that can be highlighted is the low cost of the materials needed to conduct the PE routine and to control volume, intensity, and recovery. In light of these, this methodology may be replicated in larger studies in different contexts, e.g., activities against the school shift, sports centers, clubs, and basic health units, among others. Accordingly, this methodology may represent a strategy for health promotion that costs less, considering that the materials are cost effective.

Two limitations may be highlighted: (a) the lack of controlled feeding during the 12-week intervention, and (b) the utilization of perceptual effort and recovery scales in order to quantify training load and recovery, respectively. Despite this, Haddad et al. (2017) suggested that RPE presents good reliability for monitoring training load in teenagers. The same approach was used by Branco et al. (2018) with the purpose of monitoring the training load with PE sessions. Thus, it is believed that familiarization can be incorporated to minimize eventual errors in the utilization of perceptual scales.

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## CONCLUSION

Based on the results presented, the two models of concurrent training within a multiprofessional context focusing on the treatment of obesity positively impacted the body composition and anthropometric variables, reduced FM, %BF, and WC, and elevated MME and RMR. Moreover, regardless of the order in which the concurrent exercise was performed, physical fitness related to health presented significant improvements in maximum isometric strength and cardiopulmonary fitness tests. Finally, in regard to glycemic and lipid profiles, reductions in insulin, HOMA-IR, triglycerides, total cholesterol, and LDL-c and increases in HDL-c were observed in the two experimental groups.

## DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

## ETHICS STATEMENT

This study was approved by the Committee of Ethics in Research of the University Center of Maringá (UniCesumar) through the opinion n 2,505,200/2018.

## AUTHOR CONTRIBUTIONS

BB, DV, IC, LdO, and SB contributed to conception and design of the study. BB, FdO, DM, and AC organized the database. BB performed the statistical analysis. BB, DV, FdO, IC, DM, AC, LdO, and SB wrote the first draft of the manuscript. BB, IC, LdO, and SB wrote the sections of the manuscript. All authors contributed to the manuscript revision, and read and approved the submitted version.

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# Sedentary Thresholds for Accelerometry-Based Mean Amplitude Deviation and Electromyography Amplitude in 7–11 Years Old Children

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We investigated the ability of energy expenditure, movement sensing, and muscle activity to discriminate sedentary and non-sedentary activities in children. Thirty-five 7–11-year-old children participated in the study. Simultaneous assessment of oxygen uptake ( $\dot{V}O_2$ ), triaxial accelerometry, and thigh muscle electromyography (EMG) were performed during eight different sedentary and non-sedentary activities including lying down, sitting-, standing-, and walking-related activities, which were performed in a random order. Mean values of  $\dot{V}O_2$ , accelerometry, and EMG from the concurrent 2 min epochs during each activity were computed. Resting energy expenditure (REE) was measured during 30 min supine rest. Directly measured metabolic equivalent of tasks (METs,  $\dot{V}O_2$  in activities/ $\dot{V}O_2$  in REE) were calculated for each activity. Mean amplitude deviation (MAD) was computed for accelerometry. EMG was normalized for mean muscle activity during self-paced walking. The classification accuracy of METs, MAD, and EMG to discriminate sedentary activities from physical activities was investigated by receiver operating characteristic curves and optimal cut-offs based on maximal sensitivity and specificity. Mean (SD) REE was  $5.0 \pm 0.8$  ml/kg/min. MET, MAD, and EMG values ranged from 1.0 to 4.9, 0.0020 to 0.4146 g, and 4.3 to 133.9% during lying down and walking at 6 km/h, respectively. Optimal cut-offs to discriminate sedentary activities from non-sedentary activities were 1.3 for METs (sensitivity = 82%, specificity = 88%), 0.0033 g for MAD (sensitivity = 80%, specificity = 91%), and 11.9% for EMG (sensitivity = 79%, specificity = 92%). In conclusion, this study provides applicable thresholds to differentiate sitting and standing and sedentary and non-sedentary activities based on METs, MAD, and EMG in young children.

**Keywords:** resting energy expenditure, accelerometry, electromyography, sitting, standing, posture

## INTRODUCTION

Sedentary lifestyle has reached pandemic levels among children across the world (Carson et al., 2016). The evidence from studies using accelerometry to assess sedentary behavior consistently suggests that children and adolescents spend most of their waking hours being sedentary (Cooper et al., 2015; LeBlanc et al., 2015). However, the prevalence of sedentary behavior and the magnitude of the associations between sedentary behavior and health outcomes are modified by the utilized accelerometer cut-offs (Atkin et al., 2013; Banda et al., 2016). Therefore, accurate assessment and definition of sedentary behavior are necessary in the studies on the associations of sedentary behavior with different health outcomes and when creating sedentary behavior and physical activity surveillance systems in children (Salmon et al., 2011).

Sedentary behavior is defined as any waking behavior in a sitting, reclining, or lying posture with energy expenditure less than 1.5 metabolic equivalents of task (MET; Tremblay et al., 2017). One MET is usually considered as equal to an oxygen uptake ( $\dot{V}O_2$ , ml/kg/min) during peaceful sitting or lying down (Jetté et al., 1990). MET of 1.5 has been found to relatively accurately discriminate sitting from standing in adults (Mansoubi et al., 2015). Furthermore, some previous studies suggest that energy expenditure alone is not accurate in assessment of sedentary activities and including postures would enhance the discrimination accuracy (Pesola et al., 2016; Gao et al., 2017).

Because free-living measurement of  $\dot{V}O_2$  is not feasible, accelerometry has become the most common method to assess sedentary behavior (Migueles et al., 2017). The mean amplitude deviation (MAD) method is used to compare data gathered by different types of accelerometers because it utilizes universal  $g$  values instead of arbitrary counts (Vähä-Ypyä et al., 2015b). To the best of our knowledge, few studies have studied the validity of MAD in classification of sedentary and physical activities (Aittasalo et al., 2015; Vähä-Ypyä et al., 2015a), and none of them had utilized  $\dot{V}O_2$  to cross-validate MAD values against  $\dot{V}O_2$  in children. Furthermore, instead of measuring physiological parameters such as energy expenditure and muscle activity or inactivity, accelerometry only captures movement (Godfrey et al., 2008). That is, it is well established that changing from a lying or sitting posture to a standing posture increases energy consumption by about a 50% due to muscles having to overcome the pull of gravity. Standing is a stationary activity, which therefore does not register on an accelerometer, and this increased in energy expenditure is not reflected in the accelerometer readings. Low energy expenditure and muscle inactivity are the underlying mechanisms in the relationships between high levels of sedentary behavior and impaired health (Hamilton et al., 2007; Hamilton, 2017).

Measuring muscle activity using electromyography (EMG) may provide more direct information on sedentary behavior and physical activity than accelerometry (Hamilton et al., 2007; Hamilton, 2017). We have previously found that EMG may provide superior accuracy in the assessment of low intensity physical activity and to better capture typical short-lasting sporadic activity bouts than accelerometry in children (Gao et al., 2018).

Furthermore, previous studies from our laboratory have determined EMG thresholds for sedentary activities using data derived from adults (Tikkanen et al., 2013, 2014; Pesola et al., 2016), but such thresholds have not been developed for children.

Our understanding of sedentary behavior of children is still limited because of the lack of comprehensive studies with concurrent assessment of energy expenditure, accelerometry, and muscle activity. The primary aim of the present study was to establish the optimal cut-offs for sedentary activities in children using energy expenditure, accelerometry, and EMG. We therefore investigated the ability and accuracy of energy expenditure defined as METs, accelerometry-derived MAD, and thigh muscle activity to discriminate sedentary and non-sedentary activities in children. We hypothesized that (1) MAD can be used to differentiate sitting and standing, and sedentary and non-sedentary activities in children (Mansoubi et al., 2015) but (2) energy expenditure and muscle activity will be more sensitive to discriminate different sedentary activities from each other and sedentary activities from non-sedentary activities than accelerometry (Pesola et al., 2016).

## MATERIALS AND METHODS

### Participants

This study is a part of the Children's Physical Activity Spectrum (CHIPASE) study. Children were recruited from local schools. Forty-five children and their families were interviewed in the familiarization session, and 10 of them withdrew due to scheduling difficulties. Finally, 35 healthy children aged 7–11 years who volunteered to participate and were included in the study. All aspects of the CHIPASE study were approved by the Ethics Committee of the University of Jyväskylä. All children gave their assents, and their parents/caregivers gave their written informed consents. The study was conducted in agreement with the Declaration of Helsinki.

### Power Calculations

A sample size of 30 was estimated to provide sufficient statistical power for differentiating METs between sitting ( $1.33 \pm 0.24$ ) and standing ( $1.59 \pm 0.37$ ) based on the data of Mansoubi et al. (2015) with 80% power and 5%  $\alpha$ -error level.

### Overview of the Protocol

The participants visited laboratory for familiarization session and for two measurement sessions.

### Familiarization Session

The participants and their parents were introduced to the study protocol and got familiarized to the laboratory environment and measurement devices. They also provided written informed consent during the visit.

### Measurement Visit 1

The participants arrived at the laboratory in the morning after 10–12 h overnight fast. Stature was measured to the nearest

0.1 cm using a stadiometer. Body mass, skeletal muscle mass, fat mass, fat free mass, and percent body fat were measured with a bioelectrical impedance device (InBody 770, Biospace Ltd., Seoul, Korea). Body mass index standard deviation score (BMI-SDS) was computed using the Finnish reference values (Saari et al., 2011). After these assessments, participants were helped to dress in EMG shorts (Myontec Ltd., Kuopio, Finland), and an elastic belt with an accelerometer (X6-1a, Gulf Coast Data Concepts Inc., Waveland, USA) worn on the right hip. Resting energy expenditure (REE) was measured over 30 min when children were lying down in a supine position in a quiet room with a stable temperature. Children were allowed to watch a children's program from a digital device, and the program was the same for all children. Respiratory gases were collected using a pediatric face mask (Hans Rudolph, Inc., Kansas, USA) and recorded using a respiratory gas analyzer (Oxycon mobile, CareFusion Corp, USA). After the assessment of REE, a breakfast was served for children. The validation against Douglas Bag method has shown that Oxycon mobile is reliable and valid in respiratory gas exchange analysis (Rosdahl et al., 2010).

### Measurement Visit 2

At the second visit, the arrival time was not standardized, and the participants arrived at the laboratory when it suited to their schedule. Children were asked to perform the following activities for 4.5 min in a random order interspersed with 1-min rest (Saint-Maurice et al., 2016): sitting quietly, sitting while playing a mobile game, standing quietly, standing while playing a mobile game, walking on a treadmill at 4 and 6 km/h, and self-paced walking around an indoor track (on an average of  $5.0 \pm 0.8$  km/h).  $\dot{V}O_2$ , MAD, and EMG were concurrently recorded during the tasks.

### Measurement of Oxygen Uptake, Accelerometry, and Electromyography

All activities were timed and recorded in a log sheet. Devices were synchronized using a custom-written Matlab (MathWorks, MA, USA) script based on the recording sheets. Synchronization was confirmed visually and re-synchronized manually if necessary. The raw data of  $\dot{V}O_2$ , MAD, and EMG were averaged into non-overlapping 1 s epochs for each activity prior to calculating the 2-min mean values that were used as the outcome measures.

### Indirect Calorimetry

The respiratory gas analyzer was calibrated according to manufacturer's guidelines before assessments. Dead space was adjusted to 78 ml for the petite size of the face mask following the manufacturer's recommendations.  $\dot{V}O_2$  (ml/kg/min), carbon dioxide production ( $\dot{V}CO_2$ , ml/kg/min), and respiratory exchange ratio (RER) were collected breath by breath and computed in non-overlapping 1 s epoch length. Data collected during third and fourth minute when plateau in  $\dot{V}O_2$  and  $\dot{V}CO_2$  was observed.  $\dot{V}O_2$  was then averaged over 2 min and used for analyses (Saint-Maurice et al., 2016).  $\dot{V}O_2$  in different activities was converted

to MET values. Those values were calculated based on individual REE measured METs ( $\dot{V}O_2$  measured during the activities/ $\dot{V}O_2$  in REE). REE was determined for the mean value between the 15th and 25th minute of 30 min laying down when the steady state was reached (Ventham and Reilly, 1999). Otherwise, the steady state was visually selected for further analysis.

### Triaxial Accelerometry

The triaxial accelerometry was provided as the raw acceleration data in actual g units, where the high range up to 6 g with 16-bit A/D conversion and sampling at 40 Hz. The resultant acceleration of the triaxial accelerometer signal was calculated from  $\sqrt{x^2 + y^2 + z^2}$ , where  $x$ ,  $y$ , and  $z$  are the measurement sample of the raw acceleration signal in  $x$ ,  $y$ , and  $z$  directions. The number of consecutive data points was 40, and corresponding epoch length was 1 s. The X6-1a accelerometer has been confirmed concurrent validity with ActiGraph GT3X accelerometer in children (Laukkanen et al., 2014). The universal analysis of MAD was calculated from the resultant acceleration in non-overlapping 1 s epoch. MAD described as the mean distance of data points about the mean ( $\frac{1}{n} \sum_{i=1}^n |\hat{r}_i - \bar{r}|$ ), where  $n$  is the number of samples in the epoch,  $\hat{r}_i$  is the  $i$ th resultant sample within the epoch, and  $\bar{r}$  is the mean resultant value of the epoch; Aittasalo et al., 2015; Vähä-Ypyä et al., 2015b). Thus, the mean of MAD values (g) was calculated in the certain 2-min time window for each activity and 10 min for lying down.

### Textile Electromyography

Textile EMG electrodes embedded into elastic garments were used to assess muscle activity from the quadriceps and the hamstring muscles. Four different sizes of EMG shorts (120, 130, 140, and 150 cm) with using zippers located at the inner sides of short legs and adhesive elastic band in the hem ensured proper fit in every child. The conductive area of the electrodes over the muscle bellies of the left and the right quadriceps was  $9 \times 2$  cm<sup>2</sup> (length  $\times$  width) in all short sizes, while the corresponding sizes for the hamstring muscles were  $6 \times 2$  cm<sup>2</sup> in sizes of 120, 130, and 140 cm and  $6.5 \times 2$  cm<sup>2</sup> in size of 150 cm. The conductive area of the reference electrodes was  $11 \times 2$  cm<sup>2</sup>, and they were located longitudinally over the iliotibial band. Water or electrode gel (Parker Laboratories Inc., Fairfield, NJ, USA) was used on the electrode surfaces to minimize the skin-electrode impedance.

EMG signal was stored in a small waist-mounted module (Finni et al., 2007) and sampled at 1,000 Hz after which the data were pre-processed into non-overlapping 40 ms root-mean-squared values. This technology has been reported to be valid, reproducible, and feasible in adults (Finni et al., 2007; Pesola et al., 2014) and to have good day-to-day reliability in children (Gao et al., 2018). Data were downloaded to Muscle Monitor software provided by the manufacturer (Myontec Ltd., Kuopio, Finland) and visually checked for possible artifacts and non-physiologic signals. If the artifacts lasted more than the analyzed duration in a specific activity, then it was manually discarded from the particular channel. Baseline shifts were

corrected based on a moving 5-min window (Tikkanen et al., 2013). The 5-min window was determined to be the best to correct for minor baseline fluctuations without distorting the physiological signal (Pesola et al., 2014). In the signal analysis, EMG data were identified from different activities in the certain time windows simultaneously according to the steady state in respiratory gases. Individual EMG activities were normalized channel by channel to EMG amplitude measured during self-paced walking (%EMG<sub>self-paced walking</sub>). The normalized EMG data were averaged for quadriceps from right and left side and hamstring muscles from right and left side, then the mean amplitude of the average normalized data was computed as the intensity of muscle activity level for each activity.

## Statistics

Statistical analyses were conducted using IBM SPSS for Windows 24.0 (IBM Corp., Armonk, NY, USA). The data were described as mean  $\pm$  standard deviation (SD) or mean with 95% confidence interval (CI) unless otherwise indicated. Normality of the data was investigated with Shapiro-Wilk test. Independent samples *t* test was used to compare sex differences. METs, MAD, and EMG were normalized for corresponding measure during self-paced walking to allow comparison between methods.

Two-way repeated measures analysis of variance (ANOVA) was used to compare differences between the measures of METs, MAD, and EMG within specific activities including lying down vs. standing quietly, sitting quietly vs. standing quietly, and during sitting or standing quietly vs. while playing mobile game. When ANOVA revealed significant main effects, *post hoc* comparisons by a Bonferroni correction were used to localize the difference. A probability level of  $p \leq 0.05$  (two-tailed) was considered statistically significant.

Receiver operating characteristics (ROC) curves were used to investigate the optimal cut-offs for METs, MAD, and EMG to discriminate sedentary activities from non-sedentary activities. Sedentary activities were pre-determined based on measured energy expenditure ( $\leq 1.5$  METs) and non-upright postures. We also performed ROC curves analyses excluding walking-related activities from non-sedentary activities to discriminate lying down or sitting from standing-related activities. The area under the curve (AUC) with their 95% CI is considered a measure of the utility of the predictor variable and represents the trade-off between the correct identification of sedentary activity (sensitivity) and the correct identification of non-sedentary activity (specificity). The cut-off that maximized the norm of sensitivity and specificity (that is, the cut-off that resulted in the maximum value of the square root of the sum of the sensitivity squared and specificity squared) is reported. An AUC of 1 represents the ability to perfectly identify sedentary activities from non-sedentary activities, whereas an AUC of 0.5 indicates no greater predictive ability than chance alone (Fan et al., 2006).

Spearman's rho (*r*) was individually determined for all tasks and activities between METs and MAD, METs, and EMG. Mean correlation coefficient was averaged from individual correlation coefficients. The strength of correlation was interpreted as weak ( $<0.30$ ), low (0.30–0.49), moderate (0.50–0.69), strong (0.70–0.89), or very strong ( $>0.90$ ) (Pett, 1997).

## Missing Values

Data were initially screened for missing values for each activity. In one case, we observed an abnormal REE value, which was then predicted from others based on age, sex, height, body mass, and fat free mass. Of the 280 activities (35 participants  $\times$  8 activities), acceptable data were obtained for a total of 242 activities. Full datasets of concurrently recorded both measured and adults METs, MAD and EMG were obtained for 84 pre-determined sedentary activities and 158 non-sedentary activities.

## RESULTS

Boys were heavier ( $p = 0.009$ ) and had more skeletal muscle mass ( $p = 0.009$ ) and more fat-free mass ( $p = 0.012$ ) than girls (Table 1). There were no other differences between boys and girls.

## Metabolic Equivalent of Tasks, Mean Amplitude Deviation, and Electromyography During Sedentary and Non-sedentary Activities

The mean (SD) of REE in children was  $5.0 \pm 0.8$  ml/kg/min. The results of METs, MAD (g), and EMG (%) for each activity are presented in Table 2. When we compared METs, MAD, or EMG between lying down and sitting- and standing-related activities, we found significant main effects (all  $p < 0.001$ ) for METs, MAD, and EMG in all activities (Figure 1). METs, MAD, and EMG were lower during lying down and sitting quietly than during standing quietly (both  $p < 0.05$ ). METs and EMG were also lower during sitting quietly than sitting while playing a mobile game (both  $p < 0.001$ ) and during standing quietly than during standing while playing a mobile game (both  $p \leq 0.05$ ). There were no statistically significant differences in MAD between either sitting quietly and sitting while playing a mobile game or between standing quietly and standing while playing a mobile game (both  $p > 0.05$ ).

TABLE 1 | Characteristics of participants.

Mean $\pm$ SD	All ( $n = 35$ )	Girl ( $n = 21$ )	Boy ( $n = 14$ )
Age (years)	9.6 $\pm$ 1.4	9.6 $\pm$ 1.5	9.7 $\pm$ 1.4
Stature (cm)	137.6 $\pm$ 9.2	135.7 $\pm$ 9.3	140.4 $\pm$ 8.7
Body mass (kg)	32.6 $\pm$ 6.9	30.2 $\pm$ 6.0	36.2 $\pm$ 6.8 <sup>†</sup>
Skeletal muscle mass (kg)	14.0 $\pm$ 2.9	13.0 $\pm$ 2.5	15.5 $\pm$ 2.8 <sup>†</sup>
Body fat mass (kg)	5.7 $\pm$ 3.6	4.9 $\pm$ 3.0	6.8 $\pm$ 4.2
Fat free mass (kg)	26.9 $\pm$ 4.8	25.2 $\pm$ 4.2	29.4 $\pm$ 4.6 <sup>†</sup>
BMI standard deviation score*	-0.2 $\pm$ 1.2	-0.5 $\pm$ 1.1	0.3 $\pm$ 1.2
Percent body fat (%)	16.6 $\pm$ 8.1	15.7 $\pm$ 7.3	18.0 $\pm$ 9.3
RER during REE	0.883 $\pm$ 0.124	0.884 $\pm$ 0.145	0.882 $\pm$ 0.089
$\dot{V}O_{2\text{REE}}$ (ml/kg/min) <sup>#</sup>	5.0 $\pm$ 0.8	4.9 $\pm$ 0.6	5.1 $\pm$ 1.1

\*BMI standard deviation score was calculated based on Finnish age and sex specific growth charts (Saari et al., 2011).

<sup>#</sup>One case of abnormal resting energy expenditure (REE) value was predicted from others based on age, gender, height, body mass, and fat free mass.

<sup>†</sup>Significant difference between genders,  $p < 0.05$ .



**TABLE 2** | The directly measured metabolic equivalent of tasks (METs), mean amplitude deviation (MAD), and mean muscle activity (EMG) in different sedentary and non-sedentary activities.

All activities (mean ± SD)	METs	MAD (g)	EMG (%)
Lying down (REE; $n = 35/34/34$ )	1.0 ± 0.0	0.0020 ± 0.0011	4.3 ± 3.6
Sitting quietly ( $n = 34/32/32$ )	1.2 ± 0.2	0.0021 ± 0.0012	4.3 ± 2.8
Sitting while playing mobile game ( $n = 34/33/32$ )	1.3 ± 0.2	0.0024 ± 0.0009	7.4 ± 5.1
Standing quietly ( $n = 33/33/32$ )	1.3 ± 0.2	0.0046 ± 0.0033	14.1 ± 10.1
Standing while playing mobile game ( $n = 34/33/32$ )	1.5 ± 0.3	0.0041 ± 0.0022	18.3 ± 15.3
Walking on a treadmill at 4 km/h ( $n = 33/33/32$ )	3.2 ± 0.7	0.1932 ± 0.0363	75.2 ± 43.9
Walking on a treadmill at 6 km/h ( $n = 34/33/32$ )	4.9 ± 1.0	0.4146 ± 0.0718	133.9 ± 58.1
Self-paced walking* ( $n = 31/31/30$ )	4.1 ± 1.0	0.3353 ± 0.0705	100.0 ± 0.0

\*Self-paced walking around an indoor track, individual speed was an average of 5.0 ± 0.8 km/h.

## Optimal Cut-Offs for Sedentary Thresholds in Different Measures

The AUCs with their 95% CI for METs, MAD, and EMG for classifying sedentary activities are shown in **Figure 2A**. The optimal cut-offs for discriminating sedentary and non-sedentary activities were 1.3 for measured METs (sensitivity = 81.6%, specificity = 88.1%), 0.0033 g for MAD (sensitivity = 80.4%, specificity = 90.5%), and 11.9% EMG (sensitivity = 79.1%, specificity = 91.7%).

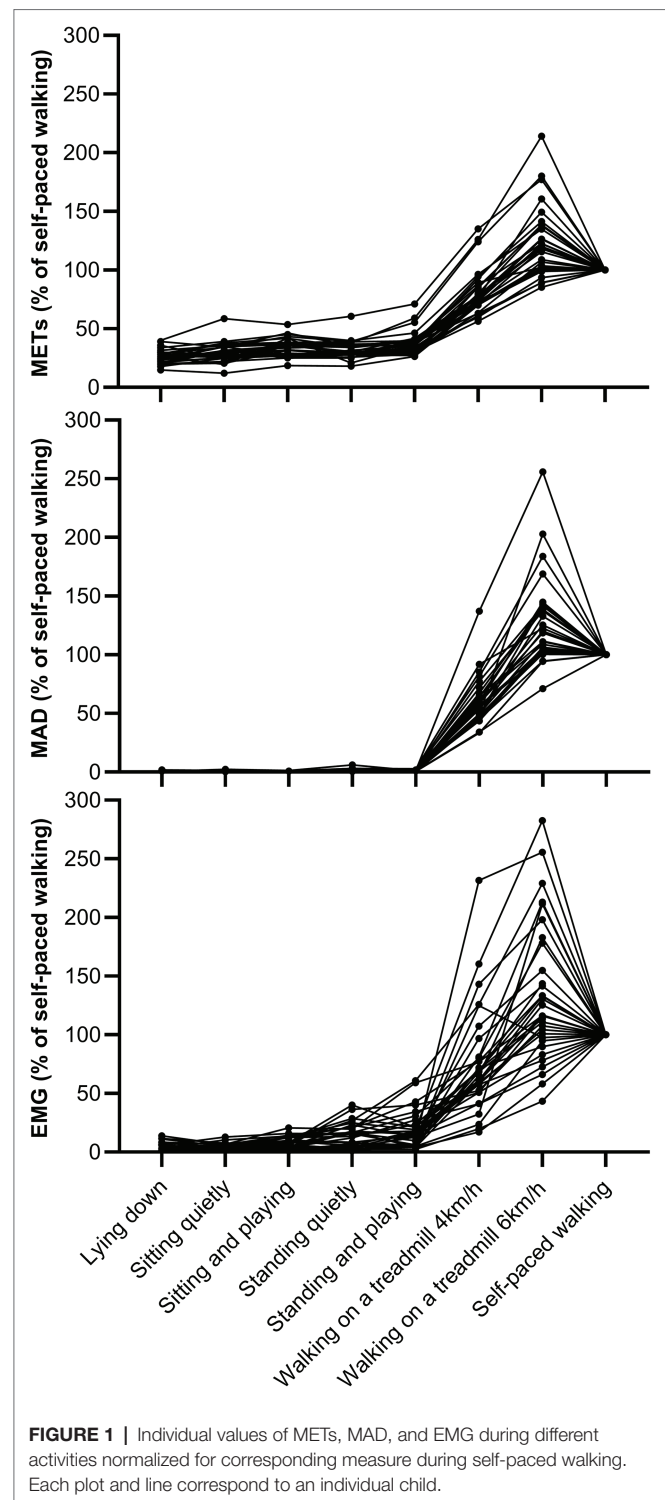
The corresponding AUC with their 95% CI when walking-related activities were excluded from the analyses is presented in **Figure 2B**. The optimal cut-offs to discriminate lying down or sitting from standing were 1.2 for measured METs (sensitivity = 77.5%, specificity = 71.4%), 0.0025 g for MAD (sensitivity = 76.1%, specificity = 71.4%), and 9.5% for EMG (sensitivity = 56.3%, specificity = 88.1%).

## Individual Correlations of Mean Amplitude Deviation and Electromyography to Metabolic Equivalent of Tasks

Within individuals, a strong positive mean correlation was found between METs and MAD ( $r = 0.982$ ) and between METs and EMG ( $r = 0.950$ ; **Figure 3**). In all participants, the MAD or EMG was increased with increasing METs for all activities (all  $p < 0.05$ ).

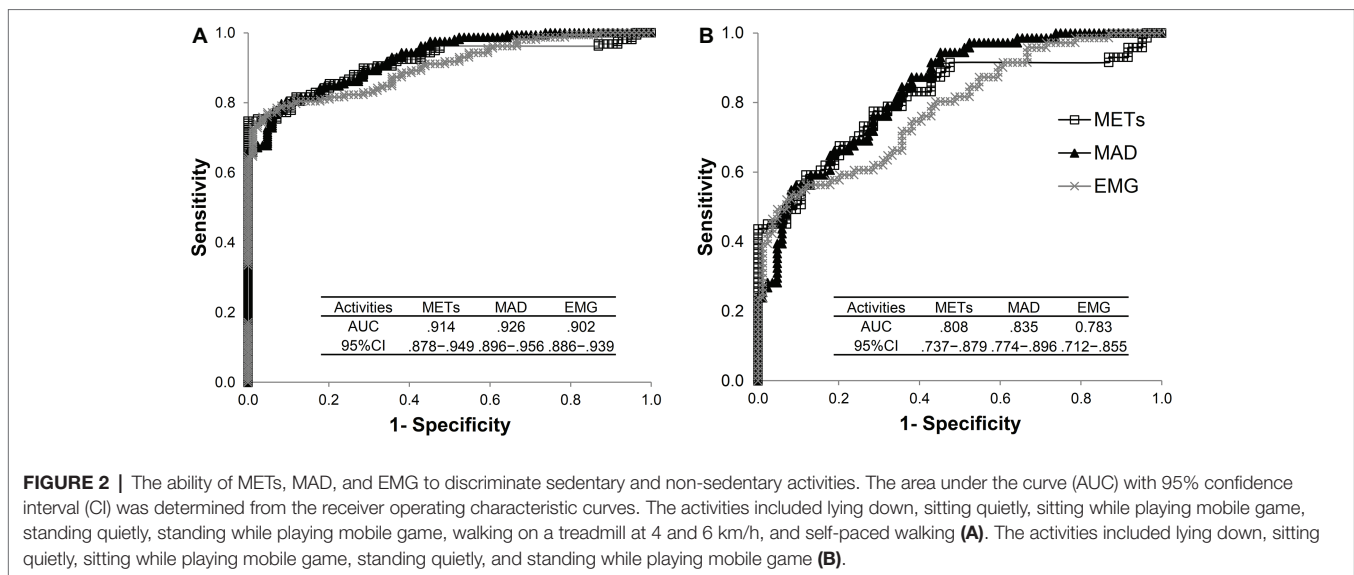
## DISCUSSION

We found that energy expenditure, movement sensing, and muscle activity were able to discriminate sedentary from non-sedentary activities with acceptable sensitivity and specificity. However, their ability to discriminate sedentary activity from standing was poorer, and the probability for false positive and false negative classification increased. Nevertheless, somewhat reasonable classification performance was still maintained, and

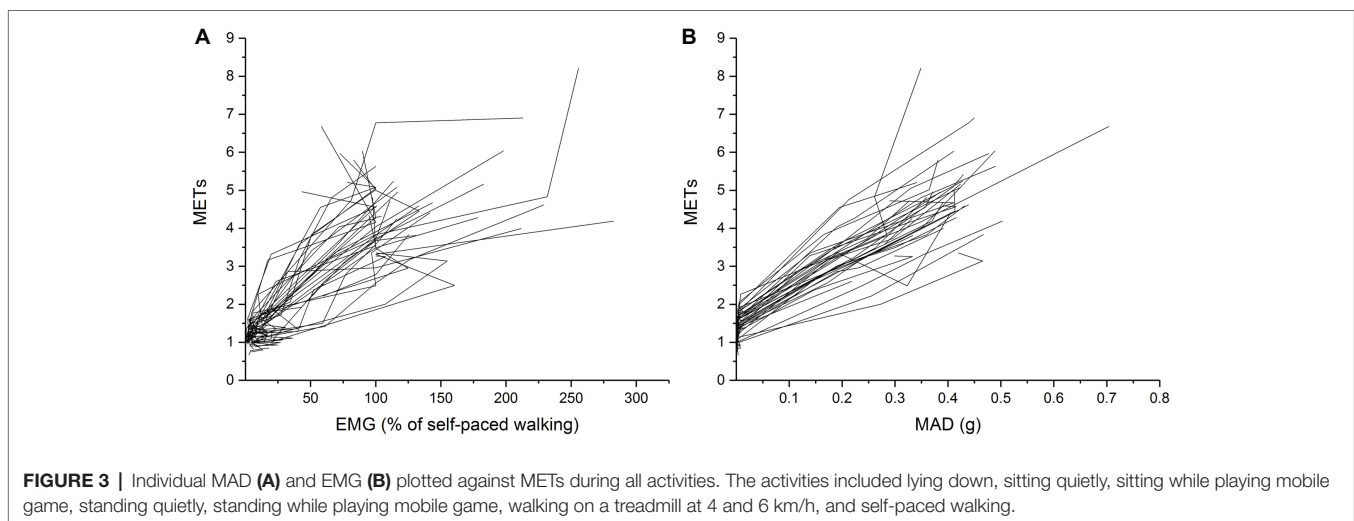


relatively similar cut-off was found as compared to when all non-sedentary activities were considered.

In line with previous studies (Evenson et al., 2008; Trost et al., 2011), we found that movement sensing had acceptable sensitivity and specificity to differentiate sedentary activities from non-sedentary activities. However, comparison of physical



**FIGURE 2 |** The ability of METs, MAD, and EMG to discriminate sedentary and non-sedentary activities. The area under the curve (AUC) with 95% confidence interval (CI) was determined from the receiver operating characteristic curves. The activities included lying down, sitting quietly, sitting while playing mobile game, standing quietly, standing while playing mobile game, walking on a treadmill at 4 and 6 km/h, and self-paced walking (A). The activities included lying down, sitting quietly, sitting while playing mobile game, standing quietly, and standing while playing mobile game (B).



**FIGURE 3 |** Individual MAD (A) and EMG (B) plotted against METs during all activities. The activities included lying down, sitting quietly, sitting while playing mobile game, standing quietly, standing while playing mobile game, walking on a treadmill at 4 and 6 km/h, and self-paced walking.

activity and sedentary outcomes between different studies is not straightforward because different devices utilize different metrics and algorithms (Migueles et al., 2017). Our study is one of the first providing cut-off for sedentary activity using MAD in children less than 13 years of age. MAD may overcome many problems related to arbitrary counts reported in previous studies. MAD is based on the raw acceleration data and allows a direct comparison between different accelerometer brands (Vähä-Ypyä et al., 2015b). However, to the best of our knowledge, only two studies have investigated the cut-off values of MAD to separate sedentary activities from non-sedentary activities in adolescents and adults (Aittasalo et al., 2015; Vähä-Ypyä et al., 2015b). Those studies included standing-related activities into sedentary activities (Aittasalo et al., 2015; Vähä-Ypyä et al., 2015b). This has obscured our understanding on thresholds of sedentary activities in children because standing should be considered separate element from

sedentary behavior as it has been found to exhibit higher energy expenditure and muscle activity than sitting (Mansoubi et al., 2015; Gao et al., 2017). Previous study reported 0.0167 g as an optimal cut-off to differentiate between sedentary and non-sedentary behaviors (Vähä-Ypyä et al., 2015b), which is larger than that was observed at 0.0033 g in the present study. Further, this value slightly decreased to 0.0025 g when we considered only non-sedentary activities without walking-related activities. While our results suggest a lower cut-off for sedentary activities in children than in adolescents and adults, it is unclear to what extent this reflects actual differences between children and adults, e.g., the wider pelvis of adults and associated higher accelerations caused by any rotational pelvic movement, or if this is caused by the differences between measurement protocols.

Complexity and large inter-individual variation of sedentary behavior in children with often short intermittent bouts of

different sedentary activities at different activity and energy expenditure levels interspersed with non-sedentary activities make the assessment of sedentary activities using movement sensing challenging (Mansoubi et al., 2015). We found that METs, MAD, and EMG were higher during standing than sitting or lying down. Furthermore, METs, MAD, and EMG were able to separate sedentary activities from non-sedentary activities with good sensitivity and specificity, but the ability to separate lying down and sitting from standing was much weaker. These results suggest that different methods can be used to differentiate sedentary activities from non-sedentary activities including movement with relatively good accuracy, but the discrimination between lying down or sitting and standing is much less precise. Furthermore, we found that MAD was increased with increasing walking velocity, while for lying down, sitting, and standing-related activities, MAD values remained consistently low. Importantly, we found more variation between children in METs and EMG than in MAD in lying down and sitting- and standing-related activities. This observation suggests that one fixed cut-off based on movement sensing may not completely capture sedentary behavior in children, and therefore, studies investigating whether individualized cut-offs for sedentary behavior based on posture, energy expenditure, and accelerometry improve the classification accuracy are warranted. To this end, we have also presented MAD values as percentage of self-paced walking (**Figure 1**), and this approach should be further investigated whether it could take into account individual's functional capacity and therefore better reflect the individual's energy requirement.

Because of a strong positive correlation of MAD and EMG with METs, our results suggest that MAD and EMG can be used as surrogates of energy expenditure in activities with varying intensity mimicking activities found in free-living conditions. When we evaluated during sitting or standing quietly vs. while playing mobile game, EMG, but not MAD, was able to discriminate quiet sitting or standing from playing in a sitting or standing position. On the other hand, both EMG and MAD were similarly sensitive and specific to discriminate sedentary from non-sedentary behavior with cut-off values of 11.9% of EMG during self-paced walking and 0.0033 g, respectively. It is important to notice that MAD is an absolute measure, while EMG threshold is related to individual's effort (as percentage during self-paced walking), suggesting the EMG can supplement accelerometry recordings providing individualized approach to the threshold values. Furthermore, in the present study, MAD values were obtained from hip-worn accelerometry, whereas thigh-worn devices, particularly when utilizing the device orientation to indicate upright/horizontal, may better distinguish postures like sitting and standing compared to hip-worn devices (Edwardson et al., 2016).

Muscle activity has been hypothesized to be a key physiological stimulus in preventing the detrimental effects associated with sedentary behavior, sitting in particular (Hamilton et al., 2007). Accordingly, standing, which requires activation of the anti-gravity muscles, should be considered

a non-sedentary behavior (Mansoubi et al., 2015; Gao et al., 2017) and be differentiated from sitting. For example, some of the cardio-metabolic benefits of replacing sitting with standing may be accounted for by (1) a higher muscle activation during standing vs. sitting; (2) a higher muscle activation in overweight vs. normal weight people (the overweight get larger benefits from these trials); and (3) inter-individual variability in muscle activation during sitting and standing (Pesola et al., 2016). Anecdotally, and as seen in the present study, it is not entirely trivial to differentiate sitting from standing in free-living conditions using contemporary wearable devices and analysis methods, but being able to differentiate between the two is a key requirement in order to develop a nuanced understanding of the consequences of sedentary and non-sedentary behaviors. Thus, differentiating between sedentary and non-sedentary behaviors may yield in-depth information for future interventions targeting sedentary behavior. Importantly, a similar volume of total energy expenditure can be accumulated with wildly varying combinations of sedentary and non-sedentary behaviors, and the effects of specific combinations on health outcomes are, thus far, poorly understood.

The strengths of the present study include the use of three different methods to assess sedentary threshold and their ability to discriminate sedentary activities from non-sedentary activities with and without standing-related activities. However, we did not evaluate the usefulness of wrist-worn accelerometers to assess sedentary threshold, and therefore, the thresholds provided in the present study are not translatable for studies using only wrist-worn accelerometry. We also directly measured REE, which allowed us to use child-specific MET values. Because previous studies have not collected data on individual REE, their analyses are based on adult MET value (Aittasalo et al., 2015; Saint-Maurice et al., 2016). However, our study sample was relatively lean and included children aged 7–11 years, which may hinder the generalizability of our results to overweight or obese children and to adolescents. Furthermore, because our sample pooled children aged 7–11 years, we cannot exclude the possibility that the sedentary threshold varies between different age-groups. Younger children have been found to have higher REE than older children (Harrell et al., 2005). We also used MET values normalized for body mass, which may have influenced our results because body mass includes fat mass that has smaller effect on energy expenditure than muscle mass (Tompuri, 2015).

## CONCLUSION

We found that measured METs, open-source accelerometry analysis, and EMG can be used to differentiate sitting and standing, and sedentary behaviors from physical activities with appropriate sensitivity and specificity. When validated thresholds are used, we can gain understanding of the specific constructs of sedentary behavior, which link it to several health and development outcomes already at childhood.

## DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

## ETHICS STATEMENT

The Ethics Committee of the University of Jyväskylä.

## AUTHOR CONTRIBUTIONS

YG, EH, AS, and TF, conceived and designed the experiments. YG and AV performed the experiments. YG, AV, and TR analyzed the data. YG wrote the first draft

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# Performance Development From Youth to Senior and Age of Peak Performance in Olympic Weightlifting

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A total of 3,782 performance results for male and female weightlifters, ages 14–30 from 123 countries, from Youth, Junior, and Senior World Championships and Olympic Games 2013–2017 were used to estimate the age at peak performance in Olympic weightlifting and quantify performance development from adolescence to adulthood. The age at peak performance was estimated for men and women globally and for different geographic regions. Overall, male and female weightlifters achieve their peak performance in weightlifting at similar ages. The median peak age is 26.0 years (95% CI: 24.9, 27.1) for men and 25.0 years (95% CI: 23.9, 27.4) for women, at the 90th percentile of performances. The median peak age was 26.3 years for men (95% CI: 24.5, 29.6) and 26.4 years for women (95% CI: 24.5, 29.6), at the 50th percentile. It is a novel finding that the age at peak performance varies for male and female athletes from different geographic regions (Western Europe, Eastern Europe, Middle East, Far East, North- and South America). For some regions men reach peak performance at a younger age than women, while this relationship is reversed for other regions. A possible explanation could be that socio-economic factors influence the pool of available athletes and thus may under- or overestimate the true peak age. Unlike in track and field where the discipline might determine specific body types, weightlifters at all ages compete in body weight classes, enabling us to compare performance levels and annual rate of change for athletes of different body mass. We quantified increases in performance in Olympic weightlifting for male and female adolescents. Sex-specific differences arise during puberty, boys outperform girls, and there is a rapid increase in their performance levels before the further growth slows down. The largest annual rate of increase in the total weight lifted was achieved between 16 and 17 years of age for both sexes with lower body mass and between 21 and 22 years with higher body mass. Such new information may help to establish progression trajectories for young athletes.

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## INTRODUCTION

Olympic weightlifting training is comprised of high-speed resistance exercises. It requires technical skills, speed, balance, coordination, and strength. Since muscles exert maximal forces in a fraction of a second, the power output exceeds that of other strength athletes such as bodybuilders and powerlifters (Garhammer, 1993; Storey and Smith, 2012). Performance development in youth

depends on changes due to puberty and differ for male and female athletes. Physical fitness and growth during puberty are directly related to hormonal changes, and bone development, muscle strength, and body composition are most effected (Goswami et al., 2014). Participants in Youth World Championships in Olympic weightlifting are 13–17 years old. This is the age range when sex differences in functional capacities for strength, muscular power, and speed become more pronounced (Malina et al., 2010). Despite the highly technical component of Olympic weightlifting the changes in performances are expected to mirror such age and sex related differences. A prior study estimated the age at peak performance from Senior World Championships results (Solberg et al., 2019), but to our knowledge performance curves during the competitive lifespan have not been documented in weightlifting.

Several studies have established performance increases and age at peak performances in track and field disciplines (Hollings et al., 2014; Tønnessen et al., 2015; Boccia et al., 2017; Ganse et al., 2018; Haugen et al., 2018). The performance development varied by discipline and can be attributed to physical growth and learning highly technical skills (Tønnessen et al., 2015). Throwers have higher body mass than other track and field athletes and achieve peak performance at a later age (Hirsch et al., 2016; Haugen et al., 2018). Unlike in track and field, athletes in weightlifting compete in different body weight classes. At all age categories there is one class with no upper limit for the body weight<sup>1</sup>. Thus, athletes can increase their body weight to possibly achieve a higher performance in the lift, although with less efficiency in the body mechanics (Duncan et al., 2013). This enables us to compare performance levels for athletes of different body mass within one sport discipline.

Athletes may discontinue training at competitive levels due to various reasons. Socio-economic factors and availability of public support differ between countries. This impacts athlete development and athlete career termination (Alfermann et al., 2004; Moesch, 2012). In particular, ages of retirement from elite training differ between countries (Kuetzel et al., 2017). However, changes in cultural and political landscapes enable athletes to participate, or participate longer, in a sport, in particular women, which has led to a shift in the age of athletes at the Olympic games over time (Elmenshawy et al., 2015). Such factors impact the pool of available athletes and thus may result in differences in peak age for athletes from different geographic and cultural regions.

The aim of this study was twofold. First, we estimated the age at peak performance in Olympic weightlifting for male and female weightlifters globally and in different geographic regions. Since the performance decline in the younger masters age classes (ages 35–45) is similar between men and women (Huebner et al., 2019), we hypothesized that men and women achieve peak performance at the same age and investigated this globally and for different geographic regions. Second, we quantified the age-associated performance development in adolescent athletes stratified by sex, bodyweight, and performance level as measured by percentiles. Due to the differences in peak age in track and field disciplines (Haugen et al., 2018) we hypothesized

that athletes with higher body mass would achieve peak performance at a later age.

There are several novel contributions in this study. We quantified the performance growth in Olympic weightlifting for male and female adolescents and established an age range when maximum performance was reached both globally and stratified by geographic regions. We estimated the age at peak performance for athletes of different body mass within one competitive sport discipline for an internationally diverse group. Estimating the rate of performance increase for girls and boys and the age at which the rate of increase is at its maximum stratified by body mass and by performance level may help to establish progression trajectories for youth weightlifters.

## MATERIALS AND METHODS

### Study Population

Competition results from the International Weightlifting Federation (IWF) Championships and from Olympic Games were included. The competitions are for Youth (ages 13–17), Junior (ages 15–20), and Senior (15 and older) age groups. Data were obtained from the IWF database<sup>2</sup> from 2013 to 2017. Only a few athletes older than age 30 compete in senior championships, thus leading to sparse matrices for estimation. Therefore, only weightlifters up to age 30 were included in the study. All results from weightlifters who received a sanction due to doping offenses were removed. Sanctioned athletes are listed on the IWF website<sup>2</sup>. Exclusions are described in the study flow diagram (Figure 1).

In competitions the total weight lifted is the sum of the best snatch weight and best clean and jerk weight, if there was at least one valid attempt among three attempts in each of these lifts. The lifts are judged by three referees according to the same rules at all ages. Podium awards are decided within age and body weight categories. Since snatch and clean and jerk results are highly correlated, we analyze the total weight lifted.

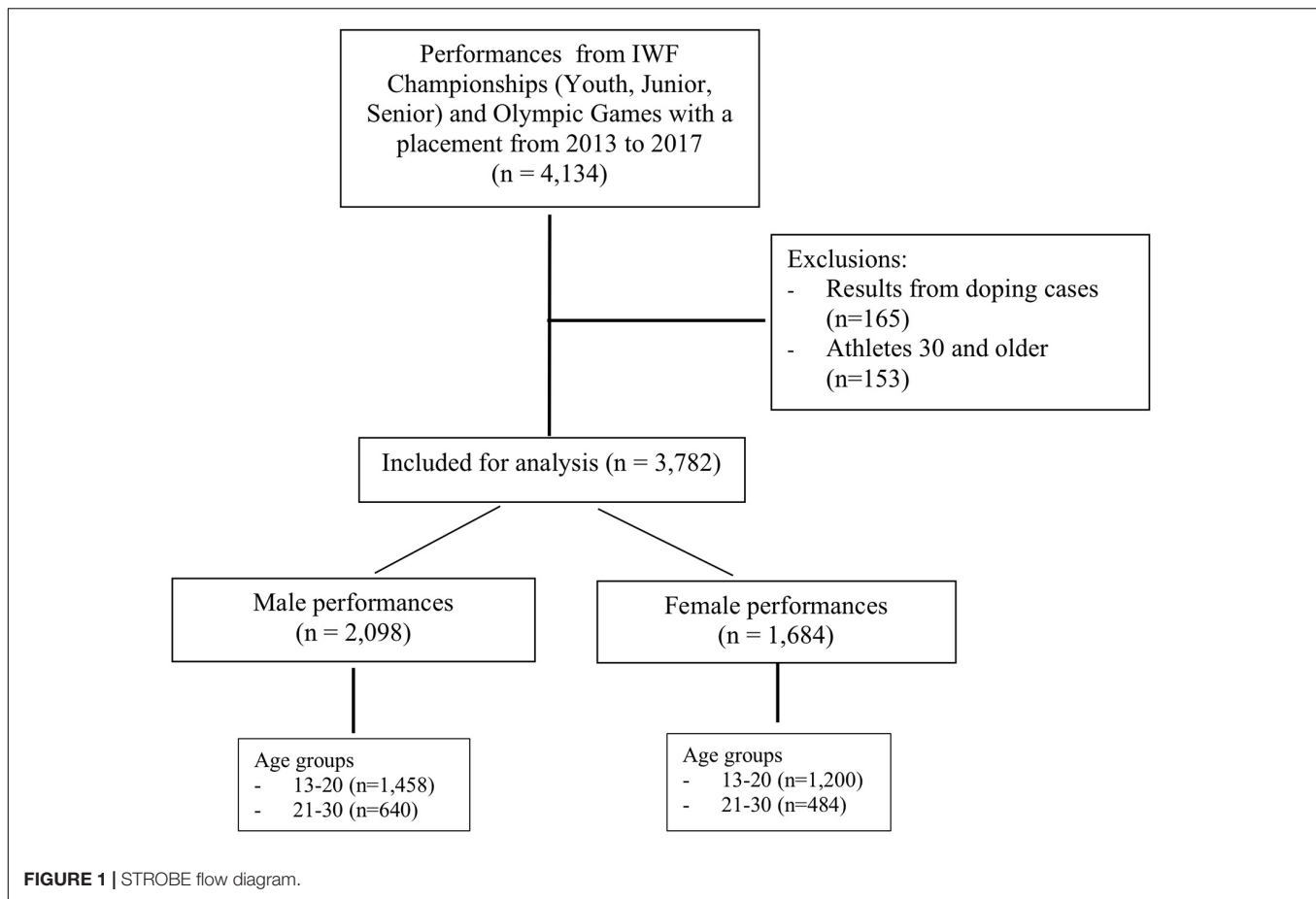
The project protocol was reviewed by the Internal Review Board at Michigan State University and was granted an exempt status, since the data are publicly available.

### Statistical Analysis

The performance was defined as the total weight (kg) lifted. Exact body weights were available for each competition result. For comparisons of the age at peak performance overall and in different geographical regions quantile regression models for the performance as a quadratic function of age were fitted with bootstrap sampling to estimate a confidence interval for age at peak performance. There were 500 iterations with random samples of 200 athletes drawn at each iteration. The model for the total weight lifted =  $a \times \text{Age} + b \times \text{Age}^2 + c$  used the exact age at the first day of the competition rather than the competition age which is the age as of December 31 or the year of the competition. The maximum was calculated for the 90th and 50th percentiles, and the age at peak performance between ages 19

<sup>1</sup><https://www.teamusa.org/usa-weightlifting/weightlifting101/weight-classes>

<sup>2</sup><http://www.iwf.net/>



and 31 was estimated with a 95% bootstrap confidence interval. We are interested in the performance of the top 10% of athletes at the world championships who may have different training ages or different training variables than athletes at lower percentiles. The median (50th percentile) is less likely to be influenced by extreme observations as the mean would be.

Countries with at least 10 results in two of three age categories 16–17, 18–20, and 21–30, or more than 20 results were grouped by geographic regions, so that the combined group was larger than 100. Smaller European countries were included in the West European region. Six regions were considered, Western Europe (Belgium, Denmark, Finland, France, Germany, Ireland, Italy, Netherlands, Norway, Spain, Sweden, United Kingdom), Eastern Europe (Armenia, Azerbaijan, Belarus, Bulgaria, Georgia, Latvia, Poland, Russia, Turkey, Turkmenistan, Ukraine, Uzbekistan), Far East (China, India, Indonesia, Japan, Korea, Malaysia, Mongolia, North Korea, Taiwan, Thailand, Vietnam), Middle East (Egypt, Iran, Iraq, Saudi Arabia, Turkey, Tunisia), North America (Canada, United States), Central/South America (Brazil, Chile, Colombia, Ecuador, Mexico, Peru, Venezuela). Australia and all other countries were labeled as “Other.”

Quantile sheets are an extension of quantile regression models and are an effective tool to estimate curves for median, quartiles, or other quantiles under consideration of other covariates, such as age or body mass. The sheets are based on asymmetrically

weighted squares regression but with an extra smoothing dimension to prevent curves at different quantiles from crossing (Schnabel and Eilers, 2013). This enables us to study changes in the conditional distribution of the response variable, namely the total weight lifted, with age as a covariate. Quantile sheets are implemented in R as a special case of a Generalized Additive Model for Location Shape and Scales (GAMLSS) within the R package *gamlss* (Rigby and Stasinopoulos, 2005). We extended this approach to quantile foliation, to estimate quantiles from 0.05 to 0.95, for two covariates, age and body mass. In order to smooth the data across three dimensions, quantiles, age, and body mass, P-splines are utilized to ensure a smooth function along the axes.

Our findings were reported according to the STROBE statement (von Elm et al., 2007). All analyses were performed using the statistical software R v. 3.5.1 (R Core Team, 2017) and the package *gamlss* v.5.1.2.

## RESULTS

A total of 3,782 performance results for male and female weightlifters aged 14–30 were used in the analyses (Figure 1). There were 44.6% ( $n = 1,684$ ) female and 55.4% ( $n = 2,098$ ) male weightlifting results 2013–2017 (Table 1). There were 1,085 youth



**TABLE 1** | Age and body mass distribution stratified by sex in World Championships 2013–2017.

	Women ( <i>n</i> = 1684)	Men ( <i>n</i> = 2098)
<b>Age groups [<i>n</i>, %]</b>		
13–15	84 (5.0%)	40 (1.9%)
16–17	448 (26.6%)	513 (24.5%)
18–20	546 (32.4%)	726 (34.6%)
21–30	606 (36.0%)	819 (39.0%)
<b>Geographic regions [<i>n</i>, %]</b>		
West Europe	133 (7.9%)	146 (7.0%)
East Europe	378 (22.4%)	501 (23.9%)
Middle East	117 (7.0%)	249 (11.9%)
Far East	446 (26.5%)	490 (23.3%)
North America	121 (7.2%)	122 (5.8%)
Central/South America	297 (17.6%)	288 (13.7%)
Other	192 (11.4%)	302 (14.4%)
<b>Body mass by age groups (median, range in kg)</b>		
13–15	51.7 (40.6 – 92.0)	55.3 (48.3 – 83.2)
16–17	57.5 (39.9 – 129.5)	67.9 (45.9 – 151.0)
18–20	60.5 (43.4 – 133.1)	76.2 (49.3 – 166.4)
21–25	62.2 (47.1 – 155.4)	83.3 (55.2 – 171.1)
26–30	62.5 (47.3 – 124.7)	84.4 (55.5 – 173.7)

athletes up to age 17 with 51.0% (*n* = 553) males and 49.0% (*n* = 532) females. Athletes from 123 countries participated in the IWF competitions. Most athletes were from East European (23.2%) and Far East countries (24.7%).

## Age at Peak Performance in Different Geographic Regions

From the cross-sectional analysis of world championship results, at the 90th percentile, the age at peak performance was 26.0 years for men (95% CI: 24.9, 27.1) and 25.0 years for women (95% CI: 23.9, 27.4). At the 50th percentile, the peak age was 26.3 years for men (95% CI: 24.5, 29.6) and 26.4 years for women (95% CI: 24.5, 29.6). There was a discrepancy in age of peak performance for different geographical regions. Men from Middle East and North American countries were older with a median of 27.6 and 27.2 years, respectively, while men from Western European countries are younger, 24.6 years, with a wide confidence interval (95% CI: 19.7, 28.9). Women from Western European countries were younger than the average at peak performance with 21.4 years (95% CI: 18.3, 29.1), while women from Far East countries were older with 26.8 years (95% CI: 23.5, 30.5) (Figure 2).

## Rate of Performance Increase by Body Mass and Sex

As they grow older youth weightlifters move into higher body weight classes. The median body weight changed from 49.8 kg at age 14 to 81.5 kg at age 21 for males, and 47.1 kg to 62.0 kg, for females, respectively (Table 1). The total weight lifted increased for male athletes in the middle body mass range (75, 80] kg by

22% from age 16 to peak performance (from 312 to 380 kg total) and for female athletes in the middle body mass range (60, 65] kg by 19% (from 221 to 262 kg total). Using quantile volumes the performance development of adolescent weightlifters can be quantified. Athletes with a higher body mass were able to lift more weight across all ages for both sexes, and performance levels increased with older age (Figures 3, 4). There was a rapid annual rate of increase until ages 16–17 for male and female athletes with lighter body mass (Figures 5, 6 and Table 2). In the unlimited body weight class the age-associated increase was highest at ages 22.1 for females (Figure 5 and Table 2) and 21.3 for males (Figure 6 and Table 2). At age 16 the average annual rate of improvement was 2.2% for boys and 1.6% for girls, respectively. At age 21 the average annual rate of improvement was 1.4 and 1.2% for male and female athletes, respectively.

## Age at Peak Performance by Performance Level

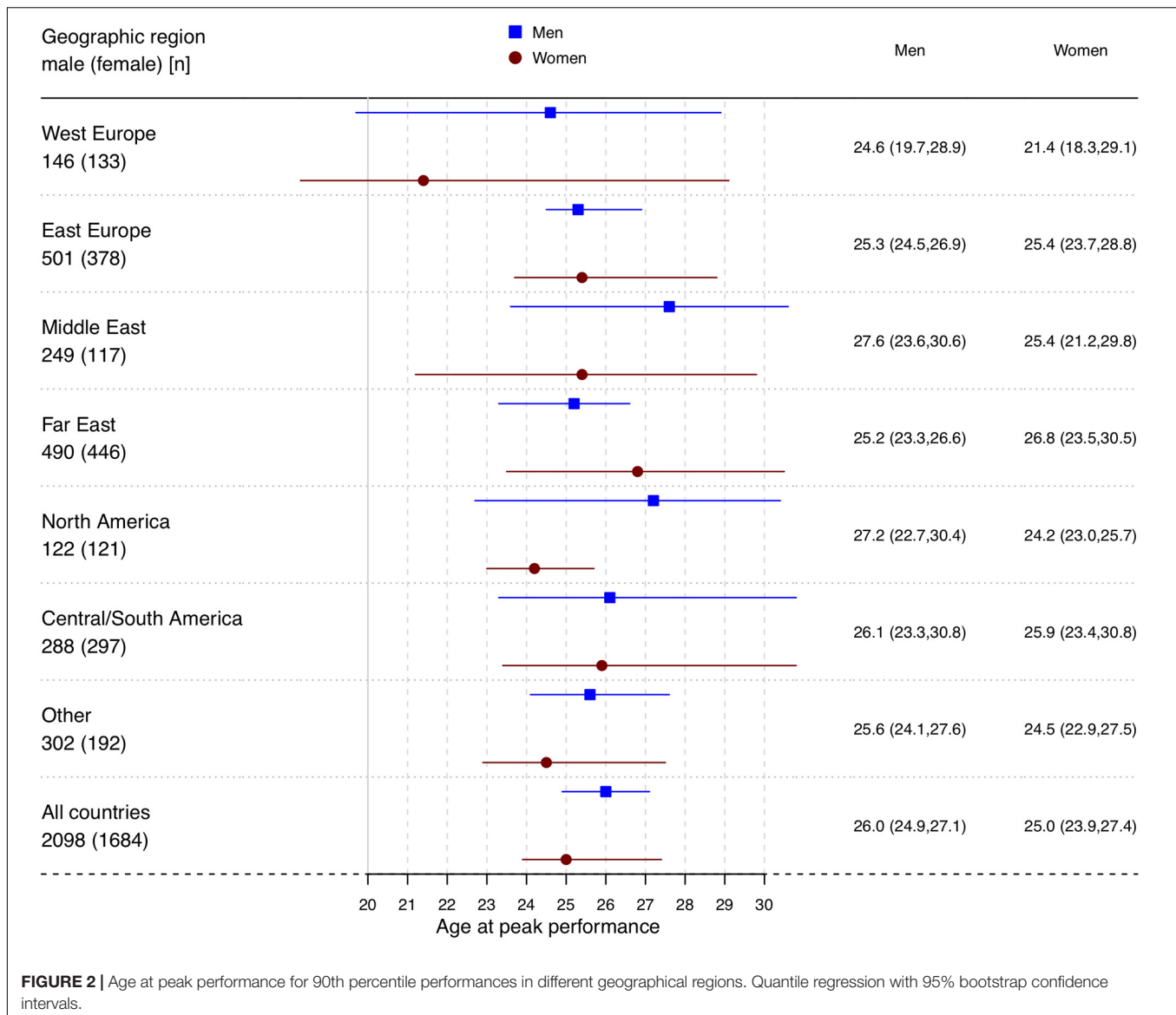
Female athletes with heavier body mass (>80 kg) reached the peak performance at an earlier age at the 90th percentile (25.5 years) compared to female athletes at the 50th percentile (27.8 years). The peak ages were comparable for the 90th and 50th percentile in the lighter body mass groups (24–29 years). Male athletes at the 50th percentile reached the peak performance at an older age (24–29 years) compared to those at the 90th percentile (25–26 years). Female athletes at the 50th percentile reached the peak performance at an older age (24–29 years) compared to those at the 90th percentile (24–25 years). Women and men reached the age at peak performance at similar ages. In the unlimited body weight category men and women were 25.5 years at peak performance (Table 2). At the 50th percentile male athletes with higher body mass reached their peak performance at a later age than athletes with a lower body mass, but ages were more similar for weightlifters with different body masses at the 90th percentile.

## DISCUSSION

We described performance development in competitive athletes in Olympic weightlifting from adolescence to career peak. We focused on analyzing performances in recent years, 2013–2017, from World Championships and Olympic Games since there has been an influx of youth and women into the sport of weightlifting, and improvements in performances were seen over time (Elmenshawy et al., 2015; Huebner et al., 2019). A total of 3,782 performances were analyzed with athletes from 123 countries (females 42–44%).

## Age at Peak Performance

Performance decline in weightlifting after age 30 has been studied (Meltzer, 1994; Huebner et al., 2019). It has been shown that women's decline in performance mirrors that of men, except for an accelerated decline from late 40's to late 50's coinciding with a transition into menopause. This raises the question whether men and women achieve peak performance prior to age 30 at the same age. In our study the age at peak performance, at

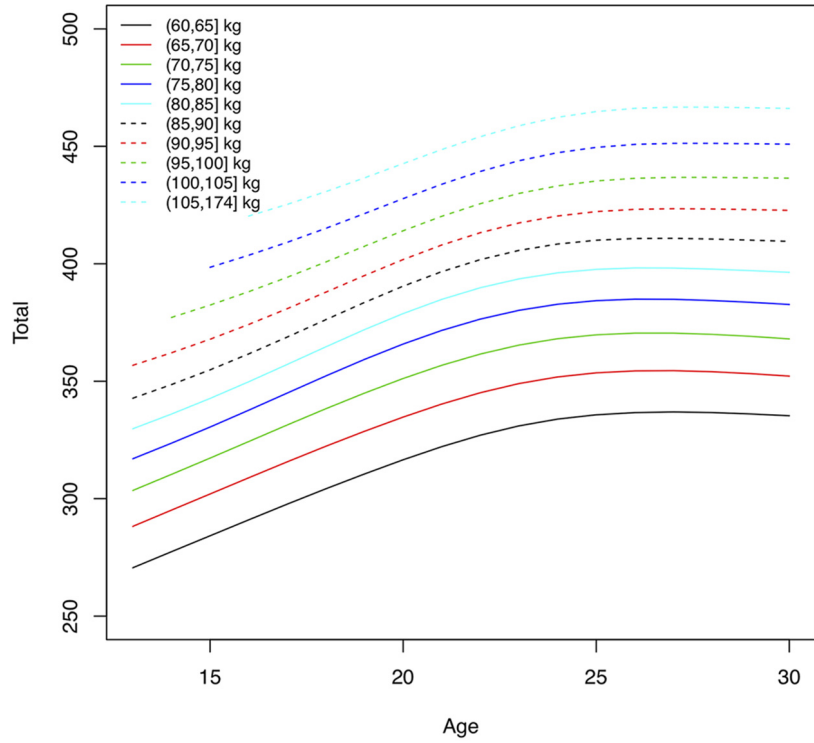


the 90th percentile, was similar, with overlapping confidence intervals, for men and women, namely 26.0 years for men (95% CI: 24.9, 27.1) and 25.0 years for women (95% CI: 23.9, 27.4), respectively. The corresponding peak age for the 50th percentile of performances was 26.3 years for men (95% CI: 24.5, 29.6) and 26.4 years for women (95% CI: 24.5, 29.6). This is comparable to the mean age at peak performance ( $\pm 90\%$  confidence limits) of  $26 \pm 3$  years which was estimated using Senior World Weightlifting Championships data from 1998 to 2017 (Solberg et al., 2019). In comparison, in track and field disciplines the mean age at peak performance ( $\pm 90\%$  confidence limits) was also similar for men and women in sprint and hurdles with  $25.2 \pm 0.3$  and  $25.7 \pm 0.3$  years and in jumping disciplines with  $25.8 \pm 0.3$  and  $25.6 \pm 0.4$  years, respectively. In throwing disciplines men reached their peak at an older age,  $28.0 \pm 0.4$  years, both in comparison to women with  $26.7 \pm 0.6$  years, and in comparison to athletes from other disciplines (Hollings et al., 2014). Thus the

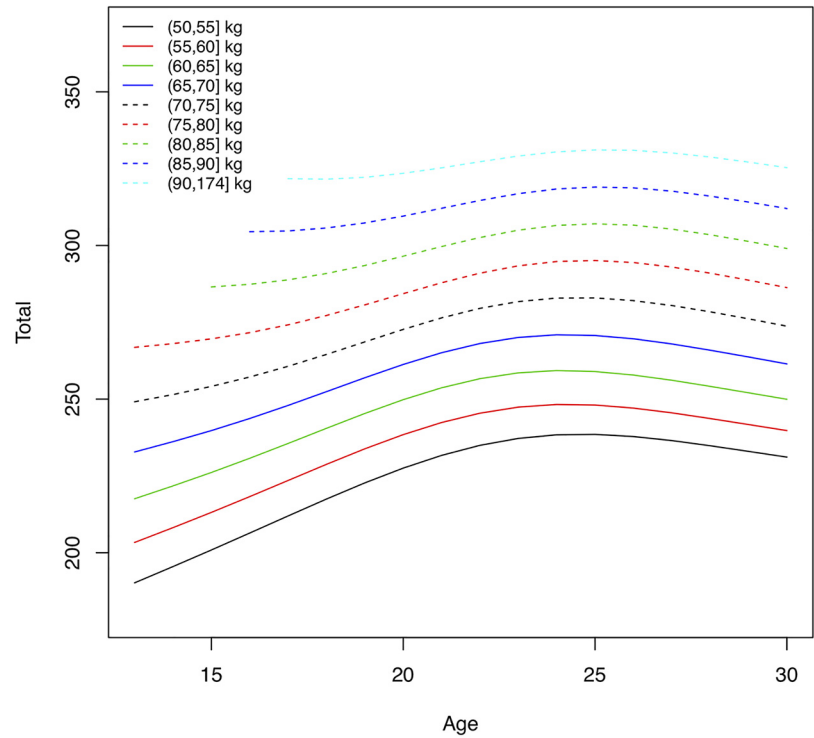
overall age at peak performance in weightlifting was comparable to that of sprint and jumping disciplines in track and field.

## Geographic Differences for Male and Female Athletes

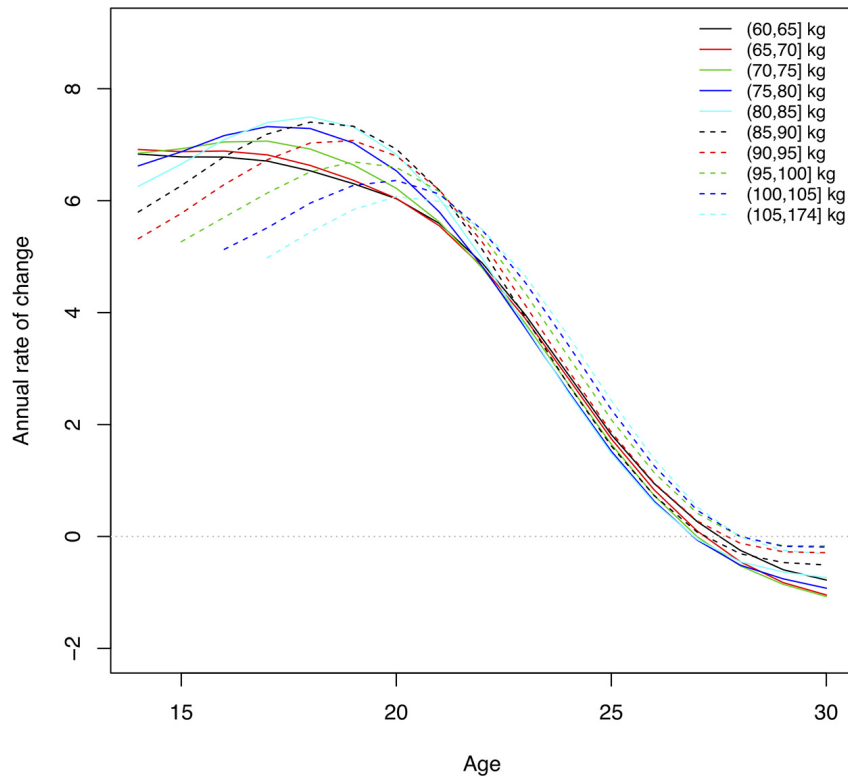
The estimated median age at peak performance varied for athletes from different countries. This is a new finding about weightlifters from an international database. The oldest age at peak performance was 27.6 years for men in the Middle East, 27.2 years for men in North America, and 26.8 for women in the Far East. West European women were younger at peak performance than East European women with 21.4 and 25.4 years, respectively. This corresponds to observed younger age at peak performance for German and Italian track and field athletes. German athletes reached the maximal performance at an average age of 20.0 years for men and 21.6 years for



**FIGURE 3 |** Absolute performance levels in total weight lifted (kg), at the 90th percentile, by age and body mass for men. Lines represent total weight lifted for athletes with different body mass.



**FIGURE 4 |** Absolute performance levels in total weight lifted (kg), at the 90th percentile, by age and body mass for women. Lines represent total weight lifted for athletes with different body mass.



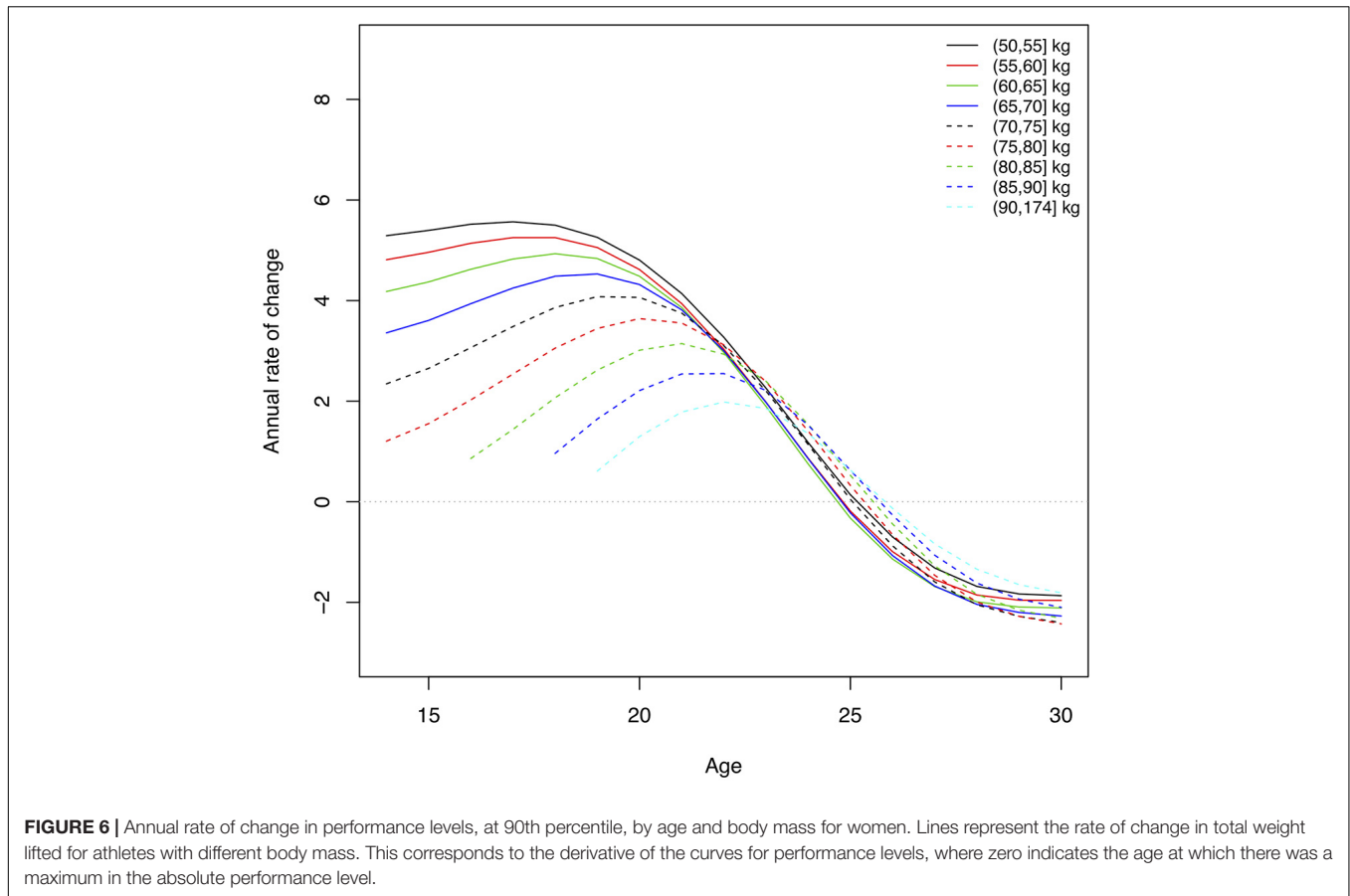
**FIGURE 5 |** Annual rate of change in performance levels, at the 90th percentile, by age and body mass for men. Lines represent the rate of change in total weight lifted for athletes with different body mass. This corresponds to the derivative of the curves for performance levels, where zero indicates the age at which there was a maximum in the absolute performance level.

women (Ganse et al., 2018), while Italian top-level high jumpers were 21.6 and 21.1 years for men and women, respectively (Boccia et al., 2017). It is unlikely that such differences between geographic regions are solely due to biological reasons or training variables. Participation requires financial resources for travel and fees, and many countries have favored boys in organized sports (Tremblay et al., 2014). Athlete development and career termination is impacted by public support, cultural expectations, and life transitions (Alfermann et al., 2004; Moesch, 2012; Kuettel et al., 2017). Women are more likely to discontinue than men because of family related reasons (Moesch, 2012). Such factors change over time, therefore we used recent data from 2013 to 2017. In North America and Western Europe women were younger than men at peak performance with 24.2 years and 27.2 years, respectively, in North America and 21.4 and 24.6 years for women and men, respectively, in West European countries. However, women were older than men in Far East countries with 26.8 years for women compared to 25.2 years for men. Using all countries to estimate the age at peak performance results in overlapping confidence intervals for men, 24.9–27.2 years, and women, 23.9–27.1 years. The observed differences in peak age may be due to participation levels of women in different countries. Although confidence intervals for peak ages in the geographic regions overlap, the width of the confidence intervals and the estimated median peak ages differ for different regions.

This may be an indicator that the global median age at peak performance may be an under- or overestimate, if the pool of active athletes does not reflect the physical capabilities of the population due to participation levels. This may be true for estimated age at peak performance in other sport disciplines that did not take geographic differences into account.

### Rate of Performance Increase by Body Mass and Sex

Performance levels improve rapidly from adolescence to adulthood. In this study the total weight lifted increased for male athletes in the middle body mass range (75, 80] kg by 22% from age 16 to peak performance and for female athletes in the middle body mass range (60, 65] kg by 19%. At age 16 the average annual rate of improvement across body weight groups was 2.2% for boys and 1.6% for girls, at the 90th percentile. At age 21 the average annual rate of improvement was 1.4 and 1.2% for male and female athletes, respectively. A higher annual rate of improvement for boys compared to girls is not surprising, since the total performance increases dramatically in puberty for boys compared to girls (Figures 3, 4). However, at the 50th percentile, the average annual improvement was 2.4% for boys and 3.0% for girls in our study, which is comparable to the rates observed for snatch and clean and jerk based on (arithmetic)



**TABLE 2 |** Performance development, at the 50th and 90th percentiles, for different body weights: age at maximal annual rate of increase and age at peak performance.

Body mass intervals (kg)*	Women				Men			
	Age at max increase		Age at peak performance		Age at max increase		Age at peak performance	
	50th	90th	50th	90th	50th	90th	50th	90th
(50, 55]	14.0	16.8	23.5	24.9				
(55, 60]	15.0	17.2	23.7	24.9	17.0	16.0	24.5	26.6
(60, 65]	16.0	17.6	24.1	24.7	17.4	16.0	24.1	26.6
(65, 70]	17.6	18.5	24.7	24.9	17.8	16.0	24.3	26.6
(70, 75]	19.9	19.5	25.5	25.1	18.2	16.0	24.7	26.6
(75, 80]	21.1	20.5	26.3	25.3	18.9	17.4	25.3	26.2
(80, 85]	21.7	21.1	27.0	25.3	19.9	17.6	26.2	25.7
(85, 90]	21.9	21.7	27.8	25.5	20.7	18.1	27.0	25.5
(90, 95]					21.1	18.7	28.0	25.5
(95, 100]					21.3	19.7	29.0	25.5
(100, 105]					21.3	20.5	29.0	25.5
W (90, 174] M (105, 174]	22.3	22.1	29.0	25.5	21.3	21.1	28.0	25.5

\*Numeric intervals (a,b) include all values x, so that  $a < x \leq b$ .

mean performance (Solberg et al., 2019). This highlights the differences we have seen in our study (Table 2) between top athletes, e.g., 90th percentile compared to athletes competing at a high level at world championships but not at the top. Our analyses also distinguished results for athletes with different body mass. The largest annual rate of increase in the total weight lifted

was achieved between 16 and 17 years of age for both sexes with lower body mass. The age-associated increase was highest at ages 21–22 for athletes with higher body mass. After reaching the maximum annual change the performance levels continued to increase but in smaller increments each successive year until the peak performance was reached.

## Performance Development and Body Mass and Performance Level

The mean body mass for 18–30-year-old weightlifters at the IWF World Championships was  $83.4 \pm 23.9$  kg for men (median 76.8) and  $65.2 \pm 17.6$  kg (median 62.0) for women. In comparison, collegiate track and field athletes had a mean body mass of  $78.4 \pm 11.6$  kg for men and  $67.0 \pm 14.2$  kg for women. Throwers had the highest body mass of for men and women overall  $90.4 \pm 18.3$  kg (Hirsch et al., 2016). Thus, female weightlifters and female track and field athletes had similar average body mass, and male weightlifters were lighter than throwers. In track and field disciplines physique varies by discipline and higher body mass is critical for success in throwers (O'Connor et al., 2007; Hirsch et al., 2016). Athletes in Olympic weightlifting compete in different body weight classes and thus can be competitive within a range of morphological characteristics. The performance development curves showed that weightlifters with higher body mass were able to lift more weight across ages. The maximum annual rate of performance increase was reached at ages 15–22, younger for athletes with lower body mass and older for athletes with higher body mass. This was similar for athletes at the 50th and the 90th percentile. Top-level male and female weightlifters, in the 90th percentile, with higher body mass reached their peak performance at a younger age (25 years) than weightlifters with higher body mass in the 50th percentile (28–29 years). An older age of peak performance (30–31 years) was observed in second tier, and thus lower percentile, German weightlifters (Faber, 2012). Differences in ages of peak performance for different body weight classes was also observed for snatch and clean and jerk (Solberg et al., 2019).

## Limitations

There are limitations to our study. First, there may be a selection bias due to socio-economic factors leading adolescent and young adult weightlifters to discontinue training due to experiencing life events, such as transition to university, employment status, changes in personal relationships, or financial reasons. This could lead to an estimated age at peak performance that is younger than it would be if athletes were able to continue their careers until they reach their full potential. However, using an internationally diverse group as in our study may help balance cultural effects. Second, the athlete selection to participate in international competitions depend on numerous factors, and countries may handle this differently. While there could be higher performances at other competitions, the judging of performances at world championships is under uniform conditions that cannot be guaranteed in other events or circumstances. Third, anthropometric and training variables were not considered. Thus, body mass is the only indicator of the physique of the athlete. However, this enabled us to compare performance levels for athletes of different body mass which has not been distinguished within track and field disciplines to the best of our knowledge. Fourth, while undetected doping violations cannot be excluded from the data in our study, we excluded all results from

athletes who were found to violate anti-doping policies at any one time point.

## CONCLUSION

We quantified performance development in Olympic weightlifting for male and female adolescents and established an age range when maximum performance is reached. The age at peak performance differed for athletes from various geographical regions. Women and men reach their peak weightlifting performance at similar ages. Performance development differs for athletes with different body mass. The annual rate of performance increase was at its maximum in the mid-teens for athletes with lower body mass and in their early 1920s for athletes with higher body mass. This study provides new information related to age-associated performance increase for youth weightlifters stratified by body mass and sex. Such results may help to establish progression trajectories for young athletes. Further research is warranted to examine gender differences and socio-economic factors as they relate to age at peak performance.

## DATA AVAILABILITY

The datasets for world championships can be accessed from the website of the International Weightlifting Federation ([https://www.iwf.net/new\\_bw/results\\_by\\_events/](https://www.iwf.net/new_bw/results_by_events/)). Athletes with sanctions are listed for international athletes (<https://www.iwf.net/anti-doping/sanctions/>).

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the IRB, Michigan State University. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

## AUTHOR CONTRIBUTIONS

MH contributed to the concept. MH and AP performed the data analysis, interpreted the results, and wrote and approved the manuscript.

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# Field Tests of Performance and Their Relationship to Age and Anthropometric Parameters in Adolescent Handball Players

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Handball performance is influenced by age, anthropometric characteristics, technical skills, tactical understanding, and physical abilities. The aims of this study were (i) to determine differences in anthropometric characteristics and physical performance between adolescent handball players across age categories, and (ii) to determine which anthropometric and maturity variables have the greatest relative importance in fitness for this sport. Seventy-nine male handball players drawn from a team in the elite Tunisian Handball league [U18 ( $n = 10$ ); U17 ( $n = 12$ ); U16 ( $n = 17$ ); U15 ( $n = 18$ ); and U14 ( $n = 22$ )] volunteered for the investigation. Assessments included sprint performances; change in direction tests (T-half test and Illinois modified test); jumping tests (squat jump; counter movement jump; countermovement jump with aimed arms; five-jump test); medicine ball throwing; handgrip force; back extensor force and selected anthropometric measurements. The individual's age category affected all measurements, with U17 and U18 players showing larger body measurements and significantly better absolute results on all physical tests than U14, U15 and U16 contestants. Scores for the majority of physical performance tests were closely inter-correlated. We conclude that U17 and U18 players show significantly better absolute results than the younger players on all physical tests. Multiple linear regressions, using block-wise entry, indicate that age is the strongest predictor of jump and sprint performances. Several anthropometric characteristics, including body mass, standing height and lower limb length were closely correlated with performance test scores, but after allowing for age only body mass added to the prediction of jumping ability.

**Keywords:** sitting height, handgrip force, back extensor force, anthropometric characteristics, ball games

## INTRODUCTION

Handball performance is influenced not only by anthropometric characteristics, but also by technical skills, tactical understanding, and physical abilities that develop with a player's age (Chelly et al., 2010; Kruger et al., 2014; Schwesig et al., 2016). Contestants must undertake repeated periods of high intensity activity, sprinting, jumping, changing direction rapidly, making physical contacts,



and throwing they pass the ball, block opponents, and attempt to establish an optimal position for the throwing player, alternating with rapid recovery during periods of low intensity activity (Michalsik et al., 2013; Wagner et al., 2014, 2017, 2018, 2019; Michalsik et al., 2015; Hermassi et al., 2018a,b). The strength and power of both upper and lower limb muscles are important determinants of sprinting, jumping, throwing (Hermassi et al., 2017s) and changing direction rapidly (Hermassi et al., 2017b). It has thus been suggested that field assessments of handball players should include a broad range of measures of sprinting, jumping, ability to change direction and maximal strength (Matthys et al., 2013; Massuca et al., 2015; Haugen et al., 2016; Ortega-Becerra et al., 2018; Wagner et al., 2019). However, there may be considerable redundancy in typical assessments, since performance test scores are often quite closely correlated both with one another and with anthropometric data.

The only relevant previous study of adolescent players (Ortega-Becerra et al., 2018) focused upon a number of physical characteristics affecting throwing performance in 44 male players ranging from elite professionals to under-16 contestants. The present investigation examined widely used field measures (sprint times, change in direction tests, vertical jumping and upper and lower limbs strength) in adolescent handball players across various age categories, looking at the extent of correlations between individual test measures, and examining their relationships to age category and selected anthropometric characteristics (standing and sitting height, lower limb length and percentage body fat). Multiple linear regression analyses (MLR) examined how far measures of maturity and anthropometric characteristics added to the description of ability provided by age alone. Our initial hypotheses were (i) that anthropometric characteristics and physical performance would develop significantly over the age categories studied, and (ii) that a player's anthropometric characteristics would add to an age-related prediction of physical performance.

## MATERIALS AND METHODS

### Participants

The study was reviewed and approved by the Institute's Committee on Research for the Medical Sciences (Manouba University Ethics Committee), in accordance with current national laws and regulations and the Helsinki Declaration. Informed consent was gained from all participants and their parents or guardians after a verbal and a written explanation of the experimental protocol and its potential risks and benefits. Participants were assured that they could withdraw from the trial without penalty at any time.

Seventy-nine male U18 handball players with at least of 5 years playing experience, drawn from a team belonging to the first Tunisian Handball league volunteered to participate in the investigation; details of training experience, playing positions, handedness and maturity status are summarized in **Table 2**. All were in good health and had passed a medical

examination provided by the team physician before commencing the study. Their maturity status was calculated as a maturity offset (Mirwald et al., 2002):

$$\text{Maturity Offset} = -9.236 + 0.000278 \text{ leg length} \times \text{sitting height} - 0.001663 \text{ age} \times \text{leg length} + 0.007216 \text{ age} \times \text{sitting height} + 0.02292 \text{ weight} \times \text{height (years)}$$

Players were instructed to avoid any strenuous exercise on the day before testing, and no additional training was conducted on the 2 test days. The training routine comprised repeated ~90 min training sessions (8 per week for U18; 6 for U17 and U16; 5 per week for U15 and U14), together with a competitive game played on the weekend. Training consisted mainly of tactical skill development (60% of session time) and strength and conditioning routines (40% of session time).

### Experimental Design

We examined differences in anthropometric characteristics and physical performance of adolescent handball players across age categories, looked at test redundancy in terms of inter-correlations between the various performance measures, and finally examined the influence of age and anthropometric characteristics upon performance using both univariate and multi-variate regression equations.

When testing was undertaken, all players had been training for 5 months, and they were already 4 months into the competitive season (January 2017). Two weeks before definitive measurements, two test familiarization sessions were completed. The definitive protocol included anthropometric measures and assessments of sprint performance over 5-, 10-, 20-, and 30-m distances; change in direction tests [T-half test (T-half) and Illinois modified test (Illinois-MT)]; jumping tests [squat jump (SJ); counter movement jump (CMJ)]; countermovement jump with aimed arms (CMJA); five-jump test (5JT)]; a medicine ball throw; and determinations of handgrip force (HG) and back extensor strength. All test measurements were made at the same time of day, and under the same experimental conditions. Participants maintained their normal intake of food and fluids, but they abstained from physical exercise for 1 day, drank no caffeine-containing beverages for 4 h, and ate no food for 2 h before testing. A 15 min active warm-up comprising running, jumping, sprinting for short distances (10 and 15 m) and mobility exercises, as well as sport-specific drills with or without the ball) preceded each day's testing, and verbal encouragement ensured maximal effort throughout.

### Testing Schedule

Definitive tests were performed in a fixed order over 3-days. On the first day, anthropometric measurements were followed by vertical jump tests (SJ; CMJ; and CMJA). The second day was devoted to medicine ball testing, Illinois-MT, Back Extensor Strength measurements and 5JT. On the third day, 30 m sprint performance was evaluated, followed by the handgrip test and the T-half test.

## Anthropometry

Anthropometric measurements included: standing and sitting heights (Holtain stadiometer, Crosswell, Crymych, Pembrokeshire, United Kingdom, accuracy of 1 mm) and body mass (Tanita BF683W scale, Munich, Germany, accuracy of 0.1 kg). The overall percentage of body fat was estimated from the biceps, triceps, subscapular, and suprailiac skinfolds, using the equations of Durnin and Womersley (1974) for adolescent males aged 16.0–19.9 years:

$$\% \text{ Body fat} = [4.95 / (\text{Density} - 4.5)] \times 100$$

Where: Density = 1.1533–0.0643 (Log sum of 4 skinfolds) for participants < 17 years old, and

Density = 1.162–0.063 (Log sum of 4 skinfolds) for participants 17- and 19 years old

## Vertical Jumping

Jump height was assessed by the same investigator, using an infrared photocell mat connected to a digital computer (Optojump System, Microgate SARL, Bolzano, Italy). The optical acquisition system measured contact and flight times during a jump with a precision of 1/1000 s and calculated the jump height from this data. One minute of rest was allowed between the three trials of each test, the highest jump being used in subsequent analyses. Participants were instructed to land with the legs fully extended and then to flex the limbs on landing, to avoid artificially inflating flight-time. Participants began the SJ at a knee angle of 90 degrees, and avoiding any downward movement, they performed a vertical jump by pushing upward, keeping their legs straight throughout. The CMJ began from an upright position, with participants making a rapid downward movement to a knee angle of approximately 90°, arms akimbo and simultaneously beginning to push-off, after being instructed to jump as fast and high as possible. The hands were freely used during the CMJA.

## Medicine Ball Throw

Medicine ball throws were performed using 21.5 cm diameter 1 and 3 kg rubber medicine balls (Tigar, Pirot, Serbia). All subjects began with a familiarization session. A brief description of the optimal technique was given, suggesting a release angle to achieve a maximum distance of throw (Gillespie and Keenum, 1987). The medicine ball was lightly covered with chalk powder (magnesium carbonate) to absorb sweat and ensure a firm grip on the ball. The talc also marked the floor where the ball landed, allowing a precise measurement of the throwing distance. The sitting player grasped the medicine ball with both hands, and on the given signal forcefully pushed the ball from the chest. The score was measured from the front of the sitting line to the place where the ball landed.

## Modified Change in Direction Illinois Test

Modified Illinois test (Illinois-MT) outcomes were recorded using an electronic timing system (Microgate SARL, Bolzano, Italy). Two pairs of tripod-mounted timing sensors were set 1 m above the floor and facing each other 3 m apart on either side of the starting and finishing lines. The front foot was positioned on a line 0.20 m in front of the photocell beam. The change in direction area for the Illinois-MT was set-up with four cones.

On command, the player sprinted 5 m from a standing position, turns and came back to the starting line; then swerved in and out of the four markers, completing two 5 m sprints to finish the course (Hachana et al., 2014). Participants were told to complete the test as quickly as possible, but no advice is given on technique. They were also instructed not to cut over the markers, but to run around them. If they failed to do this, the trial was stopped and re-attempted after a standard recovery period.

## Back Extensor Strength

Maximal isometric back extensor strength was measured in kilograms, using back and leg dynamometers (Takei, Tokyo, Japan) as previously described (Hannibal et al., 2006). Participants stood on the dynamometer foot stand with their feet one shoulder-width apart and gripped the handle bar positioned across the thighs. The chain-length of the dynamometer was adjusted so that initially the legs were fully extended and the back was flexed at a 30° angle, positioning the bar at the level of the patella. Participants then stood upright without bending their knees and lifted the dynamometer chain, pulling upward as strongly as possible. Three trials were completed, and the highest score was recorded. A 30-s rest interval was allowed between each trial.

## Five-Jump Test (5JT)

The 5JT began from an upright standing position, with both feet flat on the ground. Participants tried to cover as much distance as possible with five forward jumps, alternating left- and right-leg ground contacts. The distance covered was measured to the nearest 1 cm using a tape measure (Meylan and Malatesta, 2009).

## 30 m Sprint Performance

Times over distances of 5-, 10-, 20-, and 30 m were recorded using a series of paired photocells (Microgate, Bolzano, Italy). Participants started from a standing position, with the front foot 0.2 m from the first photocell beam. Three trials were separated by 6–8 min of recovery, with the best result for each distance being noted.

## Handgrip Force

The subject held the hand dynamometer (Takei, Tokyo, Japan) with the arm at right angles and the elbow by the side of the body. The handle of the dynamometer was adjusted so that the base rested on first metacarpal and the handle rested on the middle of the four fingers. The dynamometer was squeezed maximally, and the contraction was maintained for 5 s. No ancillary body movements were allowed. Two trials were made with each hand, with 1 min of rest between trials. The highest readings were used in subsequent analyses.

## Modified Change in Direction *t*-Test

The *t*-test was used to determine speed with directional changes such as forward sprinting, left and right shuffling, and back-pedaling. Subjects began the test with both feet behind starting line A (Sassi et al., 2009). Participants sprinted forward to cone B and touched the base of it with their right hand. Facing forward and without crossing feet, they then shuffled to the left to cone C and touched its base with the left hand. They next shuffled

to the right to cone D, touching its base with the right hand. They then shuffled back to cone B, touching its base. Finally, they ran back as quickly as possible to line A. If they crossed one foot in front of the other, failed to touch the base of a cone, and/or failed to face forward throughout, they had to repeat the test. Two trials were conducted and the shortest time was recorded.

## Statistical Analyses

All statistical analyses were performed using SPSS version 22.0 for Windows (SPSS Inc., Chicago, IL, United States). The reliabilities of all dependent variables were assessed by calculating two-way mixed intra-class correlation coefficients (Vincent, 1995). Descriptive statistics [mean and standard deviation (SD)] were ascertained for all variables. Comparisons between age groups were performed using a series of one-way analyses of variance. If a significant *F* value was observed, Tukey's *post hoc* procedure was applied to locate pair-wise differences. Pearson's product moment correlation was calculated and used to determine relationships between all tests.

Multiple linear regressions (MLR) were calculated using a hierarchical block-wise entry method. Firstly, we tested how much variance our measure of maturity contributed to a simple age prediction of each variable. Then we analyzed how much each of a sequence of anthropometric variables supplemented this description, with the order of entry of predictors into the equation selected on the basis of univariate correlations with the performance variable in question and knowledge of past work. The number of physical performance variables was reduced for these analyses. Individual data for a characteristic such as sprinting were arbitrarily weighted, based on their correlations with anthropometric data (Table 5). Performance measures were then expressed as a percentage of the corresponding group mean (performance for individual – mean performance) × 100/mean performance, as shown in the following examples:

Composite Sprint score = (aS5m% + bS10m% + cS20m% + dS30m%).

Composite change in direction score = (aT-half% + bIllinois-MT%).

Composite jump score = (aSj% + bCMj% + cCMJA% + d5JT%).

Composite strength score = (aMedicine Ball% + bHandgrip right% + cHandgrip left% + dBack Extensor Strength%).

Normality of all data sets was checked using the Kolmogorov–Smirnov test. Multicollinearity was estimated by a variance inflation factor (VIF), with a VIF > 10 indicating excessive multicollinearity. Levene's test checked the homogeneity of variance, and scatter plots tested the linearity assumption.

## RESULTS

### Preliminary Analysis of the Data

Multicollinearity was tested, and height was excluded from the regression models because its VIF was > 10. Levene's test showed equal variance across samples, and the oval shape of scatter plots test showed linearity of the data. All performance measurements

**TABLE 1** | Intra-class correlation coefficients and coefficients of variation for measures of physical performance.

Performance test	ICC	95%CI of ICC	CV
5 m	0.847	0.760–0.902	4.8
10 m	0.983	0.973–0.989	5.2
20 m	0.996	0.993–0.997	6.8
30 m	0.967	0.948–0.979	7.3
T-half	0.987	0.980–0.992	4.3
Illinois-MT	0.952	0.926–0.970	2.7
SJ	0.921	0.876–0.949	15.5
CMJ	0.984	0.975–0.990	14.8
CMJA	0.926	0.884–0.953	13.9
5JT	0.990	0.984–0.993	17.1
Medicine ball throw	0.947	0.917–0.966	20
Handgrip force right	0.975	0.933–0.973	17.1
Hand grip force left	0.902	0.846–0.937	16
Back extensor strength	0.967	0.948–0.979	12.3

5JT, five-jump test; CI, confidence intervals; CMJ, counter-movement jump; CMJA, counter-movement jump aimed arms; CV, coefficient of variation; ICC, intra-class correlation coefficient; MT, modified test; SJ, squat jump.

reached an acceptable level of reliability (Table 1;  $r > 0.80$ ). All variables showed a normal distribution.

## Age Effects

There were significant main effects of age for all measurements of both physical characteristics (Table 2) and performance test scores (Table 3) and the majority of physical performance measures showed moderate to very large associations (Table 4). Chronological age had a consistently larger univariate effect on all variables than the age at peak height velocity (Table 5). The U17 and U18 age categories showed significantly larger anthropometric dimensions and larger absolute values for all physical test scores than the U14, U15, and U16 groups. The U16, U17 and U18 groups also performed significantly better than the U14 and U15 for all sprint COD times (Table 3). A consistent age trend was also seen in vertical and five-jump tests; although U17 and U18 players did not differ statistically from each other, significant inter-group differences were found for U 14, U 15, and U 16 players (Table 3).

## Test Redundancy

The correlation matrix showed that sprint-times over distances of 5–30 m were closely correlated with each other as were the standing jump, counter-movement jump score with and without use of the arms.

## Relationships Between Anthropometric Characteristics and Physical Performance

The majority of physical performance measures showed moderate to very large univariate associations with most anthropometric characteristics (Table 5), correlations being particularly strong for lower limb length, body mass, and standing height. However, back extensor strength did not

**TABLE 2** | Comparison of physical characteristics across age categories.

Player category	U14 (n = 22)	U15 (n = 18)	U16 (n = 17)	U17 (n = 12)	U18 (n = 10)
Age (years)	13.8 ± 0.3 a***b***c***d***	14.7 ± 0.3 a***b***c***	15.8 ± 0.3 a***b***	16.6 ± 0.3 a***	17.7 ± 0.3
APHV (years)	14.1 ± 0.4 a***b***c***	14.1 ± 0.3 a***b***c***	14.7 ± 0.34	15.0 ± 0.5	15.0 ± 0.4
Body mass (kg)	68.2 ± 4.4 a***b*	68.7 ± 3.8 a***	69.9 ± 5.8 a***	74.0 ± 8 a***	86.3 ± 5.9
Height (cm)	167.9 ± 5.9 a***b***c***d***	175.7 ± 5.3 a**	179.3 ± 2.8	180.1 ± 3.3	182.5 ± 2.4
Sitting height (cm)	79.1 ± 3.4 a***b*c*d*	82.0 ± 1.9 a**	81.8 ± 2.2 a**	82.2 ± 3.5 a**	86.2 ± 2.2
Lower limb length (cm)	88.8 ± 4.6 a***b***c***d***	93.7 ± 4.2 b*c*	97.4 ± 1.8	97.9 ± 2.2	96.3 ± 1.4
Body fat%	23.1 ± 7.2	21.6 ± 7.8	19.3 ± 6.2	21 ± 5.3	17.7 ± 7.6
Training experience (years)	5.4 ± 0.5	5.7 ± 0.5	6 ± 0.9	6.5 ± 1	7.8 ± 0.8
Right handed	16	13	11	10	8
Left handed	6	5	6	2	2
Back players	8	6	6	3	3
Wing players	7	5	5	4	3
Pivots players	4	4	3	3	2
Goal-keepers	3	3	3	2	2

APHV, age at peak height velocity; a, significantly less than U18; b, significantly less than U17; c, significantly less than U16; d, significantly less than U15; n, number of subjects; U, under. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .

influence sprinting or COD performance. Further, age, height, and lower limb length were significantly correlated with the results of all physical tests (Table 5). Body mass was also significantly correlated with the majority of physical performance measures except CMJ, CMJA, and 5JT. In contrast, body fat percentage (over the range of body fat values found in these players) was not correlated with any of the physical performance scores (Table 5).

## Multiple Regression Analyses

Some 59.3% of the variance in composite sprint score was attributable to age. After inclusion of this variable, no other potential terms in the prediction equation achieved statistical significance (Table 6). The equation for prediction of sprinting performance was thus:

$$\text{Composite sprint score (\%)} = -3.04 \text{ Age (year)} + 46.6$$

In terms of the composite jump score, 48.3% of the variance was explained by calendar age. Addition of the maturity variable (APHV) did not significantly change the prediction (Table 7). Body mass added a significant 4% to the description of variance, but after introduction of this variable, neither leg length nor body fat content added significantly to the regression. The jump score could thus be predicted using the equation:

$$\text{Composite jump score (\%)} = 8.43 \text{ Age (year)} - 0.48 \text{ Body mass (kg)} - 94.6$$

For the composite change in direction score, age, age at peak height velocity and leg length all contributed to the description of variance (Table 8), with the final equation contributing 59.3% of the variance in performance:

$$\text{Composite change in direction score (\%)} = -1.82 \text{ Age (year)} + 1.66 \text{ APHV (year)} - 1.36 \text{ Lower limb length (cm)} + 16.8$$

For the composite strength scores, 63.8% of the variance was described by age, with none of the other variables contributing to this description (Table 9). Thus, Composite strength score (%) = 8.23 Age (year) - 126.4

## DISCUSSION

Aspects of the present findings that merit specific comment include the issue of test redundancy, the impacts of age and maturity upon performance in handball, correlations of performance with anthropometric characteristics, the influence of playing position, and finally some strengths and limitations in the research to date.

### Test Redundancy

The close correlation observed between many of the performance measures used in this study highlights a substantial redundancy in the tests that are presently used in assessments of performance for team sports; inter-correlations are particularly close for the four sprint times and for the several measures of jumping performance (Table 4). Others, also, have commented on such inter-relationships and test redundancies (Chaouachi et al., 2009; Chelly et al., 2010; Schwesig et al., 2017; Ortega-Becerra et al., 2018). There is a need to use techniques such as factor analysis to discern underlying structures and measurements or measurement combinations that are aligned with specific components of actual game performance. This would simplify the task both of measuring laboratories and those practitioners who must interpret the resulting data.

### Age Effects

Age is an important variable for handball players (Lidor et al., 2005). Our age comparisons were admittedly cross-sectional in nature, but selection pressures were similar for each age category, and effects from social changes and secular trends to an increase

**TABLE 3** | Comparison of athletic performance of study participants across age categories.

Age category	U14 (n = 22)	U15 (n = 18)	U16 (n = 17)	U17 (n = 12)	U18 (n = 10)
<b>Sprint times</b>					
5 m (s)	1.22 ± 0.05 a***b***c***	1.21 ± 0.06 a**b**c**	1.15 ± 0.04	1.14 ± 0.02	1.14 ± 0.05
10 m (s)	2.10 ± 0.07 a***b***c***	2.08 ± 0.09 a***b***c***	1.91 ± 0.05	1.95 ± 0.05	1.91 ± 0.05
20 m (s)	3.68 ± 0.12 a***b***c***	3.73 ± 0.19 a***b***c***	3.30 ± 0.05	3.32 ± 0.05	3.34 ± 0.21
30 m (s)	5.28 ± 0.19 a***b***c***d*	5.03 ± 0.41 a***b***c***	4.68 ± 0.12	4.66 ± 0.08	4.58 ± 0.15
<b>Times for change in direction tests</b>					
T-half (s)	7.33 ± 0.27 a***c**	7.16 ± 0.39 a**	7.01 ± 0.16	7.10 ± 0.16 a*	6.78 ± 0.13
Illinois-MT (s)	13.41 ± 0.25 a***b***c***	13.21 ± 0.22 a***b***c**	12.93 ± 0.26 a**	12.84 ± 0.16	12.60 ± 0.17
<b>Vertical jump heights</b>					
SJ (cm)	25.7 ± 1.7 a***b***	26.1 ± 3.6 a***b**	28.2 ± 4.8 a**	30.7 ± 4.5	33.5 ± 2.0
CMJ (cm)	27.8 ± 2.2 a***b**	27.8 ± 3.5 a***b*	30.1 ± 5.1 a**	32.7 ± 4.2	35.2 ± 2.7
CMJA (cm)	31.3 ± 1.5 a***b**	32.1 ± 3.5 a***b*	33.9 ± 5.3 a*	36.9 ± 5.9	39.0 ± 2.5
<b>Horizontal jump</b>					
5JT (m)	8.1 ± 0.4 a***b***c***	8.4 ± 0.7 a*b***c***	10.5 ± 0.7	10.5 ± 0.6	10.0 ± 3
<b>Strength</b>					
Medicine Ball Throw (m)	3.3 ± 0.3 a***b***c***d**	3.7 ± 0.2 a***b***c***	4.9 ± 0.4	5.2 ± 0.3	5.0 ± 0.4
Hand grip force right (N)	356 ± 23 a***b***c**	386 ± 25 a***b**	416 ± 68 a***	463 ± 88	504 ± 32
Hand grip force left (N)	339 ± 18 a***b***c**	370 ± 30 a***b**	389 ± 62 a***	436 ± 70	467 ± 40
Back extensor force (N)	1154 ± 74 a**b**	1241 ± 84	1174 ± 180 a*b*	1342 ± 217	1340 ± 86

5JT, five jump test; a, significantly different from U18; b, significantly different from U17; c, significantly different from U16; CMJ, counter movement jump; CMJA, counter movement jump aimed arms; d, significantly different from U15; MT, modified test; n, number; SJ, squat jump; U, under. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .



**TABLE 5 |** Correlations between age, age at peak height velocity, anthropometric parameters and measures of physical performance.

	5 m	10 m	20 m	30 m	T-half	Illinois MT	SJ	CMJ	CMJA	5JT	Medicine ball throw	HG force right hand	HG force left hand	BEF
Age	-0.59***	-0.71***	-0.69***	-0.73***	-0.54***	-0.78***	0.63***	0.60***	0.59***	0.55***	0.84***	0.72***	0.69***	0.40**
APHV	-0.43***	-0.51***	-0.62***	-0.51***	-0.20	-0.52***	0.41***	0.44***	0.47***	0.42***	0.65***	0.41***	0.41***	0.29**
Body mass	-0.27	-0.38*	-0.31	-0.45***	-0.47***	-0.45***	0.31	0.28	0.21	0.12	0.44***	0.56***	0.50***	0.29
Standing height	-0.50***	-0.60***	-0.46***	-0.66***	-0.61***	-0.62***	0.44***	0.36*	0.35*	0.51***	0.66***	0.53***	0.51***	0.26
Sitting height	-0.33*	-0.42**	-0.23	-0.42**	-0.53***	-0.49***	0.38**	0.31	0.26	0.27	0.42**	0.52***	0.48***	0.21
Lower limb length	-0.48***	-0.56***	-0.49***	-0.56***	-0.50***	-0.54***	0.35*	0.30	0.32*	0.53***	0.64***	0.39**	0.39**	0.22
Body fat%	0.16	0.20	0.18	0.06	-0.01	0.18	-0.30	-0.28	-0.26	-0.26	-0.16	-0.10	-0.12	-0.08

APHV, age at peak height velocity; 5JT, five jump test; BEF, Back extensor force; CMJ, counter movement jump; CMJA, counter movement jump aimed arms; HG, handgrip; MT, modified t-test; SJ, squat jump; \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001. High correlations are highlighted.

### Influence of Anthropometric Factors

Several authors have discussed the importance of anthropometric variables to the performance of adult handball players (Lidor et al., 2005; Mohamed et al., 2009; Ziv and Lidor, 2009). However, research on adolescent players is limited. Using a stepwise multiple regression analysis, Visnapuu and Jurimae (2007) found that sitting height was associated with scores on basic motor tests in the 14- to 15-yr.-old group (16.5–52.4%;  $R^2 \times 100$ ) and with specific motor skills in 12- to 13-yr.-olds and 14- to 15-yr.-olds (13.4–41.6%;  $R^2 \times 100$ ). Chamari et al. (2008) previously noted that stride length and sprint performance were proportional to leg length. Aouadi et al. (2012) also reported significant relationships between stature, lower limb length, ratio of lower limb length/stature and sitting height/stature to the jump performance of volleyball players, and Kruger et al. (2014) demonstrated a close relationship between anthropometric data, sprinting, jumping, anaerobic and endurance performance.

Lucia et al. (2002) and Fowkes Godek et al. (2004) underlined the negative effects of excessive fat mass, although the International Handball Federation showed a trend toward the selection of heavier players among the best teams, presumably, the additional mass is here muscle rather than fat particularly in wing players (International Handball Federation, 2014). Handgrip strength is also important for catching and throwing the ball (Nag et al., 2003), and our results showed significant inter-group differences in handgrip performance.

Some studies have demonstrated that body composition influences actual game performance. Handgrip strength gives greater control of the ball, and a higher arm-span allows occupation of greater space in defensive and offensive actions (Fernández et al., 2004). Granados et al. (2007) also demonstrated that a greater fat-free mass was associated with a better performance, because of the increase in the muscular power and strength.

Our univariate data showed substantial correlations between several anthropometric parameters and physical performance, particularly standing height, lower limb length and body mass (Table 5), although the percentage of body fat percentage was not related to any performance measures except vertical and horizontal jumping. Body mass was significantly correlated with the score on all performance tests except vertical and horizontal jumping and 5 and 20 m sprint times. The lack of significant correlation with body mass for these items was surprising. This may possibly reflect differences in familiarity with the CMJA and 5JT. Coordination between the upper and lower limbs is vital to performance of these tests over the age groups studied, with poorer coordination in the younger and less experienced age categories. Further, in multivariate analyses where allowance was made for calendar age, the only statistically significant anthropometric variable was the influence of body mass on ability to change direction (Table 8).

### Playing Position

We did not have a sufficient number of players in any given age category to allow an analysis of our data by playing position. However, technical and physical on-court demands certainly vary

**TABLE 6** | Multiple regression analyses for composite jump scores.

Model	R	R square	Adjusted R square	SE of the estimate	Sig. F change
Model 1: Age	0.700	0.490	0.483	9.262	<0.001
Model 2: Age + APHV	0.701	0.491	0.477	9.314	0.718
Model 3: Age + body mass	0.739	0.546	0.528	8.854	0.003
Model 4: Age + body mass + LLL	0.739	0.546	0.522	8.913	0.915
Model 5: Age + body mass + body fat	0.743	0.553	0.535	8.790	0.204

LLL: lower limb length.

**TABLE 7** | Multiple regression analyses for composite sprint scores.

Model	R	R square	Adjusted R square	SE of the estimate	Sig. F change
Model 1: Age	0.770	0.593	0.588	3.451	<0.001
Model 2: Age + APHV	0.773	0.597	0.586	3.459	0.427
Model 3: Age + body mass	0.779	0.607	0.591	3.439	0.170
Model 4: Age + LLL	0.782	0.611	0.601	3.396	0.065
Model 5: Age + body fat	0.771	0.594	0.583	3.471	0.740

LLL: lower limb length.

**TABLE 8** | Multiple regression analyses for composite COD scores.

Model	R	R square	Adjusted R square	SE of the estimate	Sig. F change
Model 1: Age	0.732	0.535	0.529	2.094	<0.001
Model 2: Age + APHV	0.761	0.580	0.568	2.005	0.006
Model 3: Age + APHV + LLL	0.780	0.609	0.593	1.946	0.020
Model 4: Age + APHV + LLL + body fat	0.784	0.615	0.594	1.945	0.299

LLL: lower limb length.

**TABLE 9** | Multiple regression analyses for composite strength scores.

Model	R	R square	Adjusted R square	SE of the estimate	Sig. F change
Model 1: Age	0.799	0.639	0.633	8.508	<0.001
Model 2: Age + APHV	0.800	0.640	0.631	8.538	0.495
Model 3: Age + body mass	0.800	0.641	0.626	8.591	0.811
Model 4: Age + LLL	0.800	0.641	0.621	8.648	0.894
Model 5: Age + body fat.	0.801	0.642	0.617	8.893	0.823

LLL: lower limb length.

with respect to playing positions, and the literature contains data showing such effects in adult players. Wings undertake the greatest amounts of high-intensity running/sprinting, but are involved in fewer one-on-one duels than other players. Pivots cover less distance but are more involved in physical duels and contacts, while backs shoot and pass significantly more compared to the other playing positions (Milanese et al., 2011; Karcher and Buchheit, 2014). These differences lead to differences in anthropometric variables with playing position (Chaouachi et al., 2009; Vila et al., 2012). Chaouachi et al. (2009) demonstrated differences of heights between backs and wings, and in the percentage body fat between goalkeepers and backs in elite Tunisian national handball players. Others have reported

that relative to other playing positions wings were significantly lighter and shorter, with less lean body mass and fat mass (Srhoj et al., 2002; Sibila and Pori, 2009; Sporis et al., 2010; Milanese et al., 2011). Sporis et al. (2010) examined a sample of ninety-two elite Croatian handball players, finding that goalkeepers were the oldest, the wings were the shortest and the pivots were the tallest players in the team, while backcourt players had a low percentage of body fat. Ghobadi et al. (2013) also noted that line players (pivots) were the heaviest, backcourt and line players were the tallest, and goalkeepers were older than the center backcourt, backcourt and wing players ( $p < 0.05$ ).

Haugen et al. (2016) quantified differences in both anthropometric and physical characteristics according to



playing position and competitive level in elite male handball players. They showed that backs achieved higher throwing velocities than other positions, and wings sprinted faster and jumped higher than pivots and goalkeepers. However, back players and wings had greater squat strength than pivots, while pivots were 9% stronger than wing players in 1RM bench press. Massuca et al. (2015) also found significant effects of playing position on body size and fitness performance. Back left/right players had an advantage in handgrip strength, and central back and pivot players also scored better on handgrip strength than goalkeeper and wing players.

### **Practical Value of Data on Maturity Status and Anthropometric Characteristics**

Our univariate analyses suggest that age, maturity status and anthropometric characteristics all influence scores on performance-related physical tests. However, because a player's physical characteristics are closely related to age, multiple regression analyses using data that cover the adolescent age range attribute almost all of this variance to age alone. It remains to be demonstrated how far assessments of age, maturity and measurement of anthropometric characteristics can help in player selection, placement and training. In any sport, highly motivated individuals can succeed despite what seems a very unfavorable anthropometric profile, and trainers rely heavily on observing players during actual competition rather than on laboratory data. Nevertheless, these characteristics do seem to influence coaching decisions. Thus, Matthys et al. (2013) noted that youth players with the most advanced maturation status and the most favorable anthropometry and physical fitness scores were consistently positioned in the back position. In contrast, players with a less advanced maturity status and an overall smaller stature were placed on the wing or pivot positions.

### **Strengths and Limitations of Study**

The main strength of this research is the collection of data on a substantial sample of handball players across age groups that previously have not received great attention. The findings that we report are relevant to the current university population in Tunisia, but we recognize that the rate of attainment of maximal growth differs in other cultures and environments, limiting the generality of our results. Other important limitations include the overwhelming impact of age in the multiple regression analyses, the inability to examine the influence of playing position, and the absence of data on female adolescents. Future observations should focus on a large sample within a single age category, and should include information on performance during actual handball games. Further, we did not assess local muscle mass; this could be a much more interesting variable than total body mass to consider in future investigations. Also, the older and more experienced players had the advantage of having attempted many of the performance tests on previous occasions, and despite

familiarization sessions, this may have influenced the scores that they attained relative to the younger players. Other factors that merit consideration in future research include possible effects arising from an age-related displacement of the center of mass, and the development of player position-specific fatigue.

## **CONCLUSION**

The present findings underline the progressive age-related development of factors influencing performance throughout adolescence, indicating the importance of age-categorized competition in handball until at least the age of 19 years. The data also showed moderate to very large univariate relationships between the performance realized by both upper and lower limb muscles and the anthropometric characteristics of male handball players, particularly body mass, height and lower limb length. Future studies should focus on narrower age ranges, and should examine the impact of other anthropometric characteristics, such as chest circumference and the length and volume of the upper limbs.

## **ETHICS STATEMENT**

This study was reviewed and approved by the Institute's Committee on Research for the Medical Sciences (Manouba University Ethics Committee) and performed in accordance with the current national laws and regulations and the Declaration of Helsinki. Informed consent was gained from all participants and their parents or guardians after a verbal and a written explanation of the experimental protocol and its potential risks and benefits. Participants were assured that they could withdraw from the trial without penalty at any time.

## **AUTHOR CONTRIBUTIONS**

MC, MH, PC, SH, and RS carried out the formal analysis and supervised the study. HM, NG, and GA investigated the study. HM, NG, and SH developed the methodology. MC and HM administered the project. MH, NG, SH, and MC drafted the manuscript. SH, MH, MC, and RS reviewed and edited the manuscript.

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# Developmental Change in Motor Competence: A Latent Growth Curve Analysis

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**Background:** The development of childhood motor competence demonstrates a high degree of inter-individual variation. Some children's competence levels increase whilst others' competence levels remain unchanged or even decrease over time. However, few studies have examined this developmental change in motor competence across childhood and little is known on influencing factors.

**Aim:** Using latent growth curve modeling (LGCM), the present longitudinal study aimed to investigate children's change in motor competence across a 2-year timespan and to examine the potential influence of baseline weight status and physical fitness on their trajectory of change in motor competence.

**Methods:** 558 children (52.5% boys) aged between 6 and 9 years participated in this study. Baseline measurements included weight status, motor competence (i.e., Körperkoordinationstest für Kinder; KTK) and physical fitness (i.e., sit and reach, standing long jump and the 20 m shuttle run test). Motor competence assessment took place three times across a 2-year timespan. LGCM was conducted to examine change in motor competence over time.

**Results:** The analyses showed a positive linear change in motor competence across 2 years ( $\beta = 28.48$ ,  $p < 0.001$ ) with significant variability in children's individual trajectories ( $p < 0.001$ ). Girls made less progress than boys ( $\beta = -2.12$ ,  $p = 0.01$ ). Children who were older at baseline demonstrated less change in motor competence ( $\beta = -0.33$ ,  $p < 0.001$ ). Weight status at baseline was negatively associated with change in motor competence over time ( $\beta = -1.418$ ,  $p = 0.002$ ). None of the physical fitness components, measured at baseline, were significantly associated with change in motor competence over time.

**Conclusion and Implications:** This longitudinal study reveals that weight status significantly influences children's motor competence trajectories whilst physical fitness demonstrated no significant influence on motor competence trajectories. Future studies should further explore children's differential trajectories over time and potential factors influencing that change.

**Keywords:** latent growth curve analysis, motor competence, individual developmental change, children, weight status

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## INTRODUCTION

Motor competence, which reflects the degree of proficient performance in various motor skills as well as the underlying mechanisms (e.g., motor control and coordination; Utesch and Bardid, 2019), is considered a key component in developing a healthy and active lifestyle from early childhood onwards (Stodden et al., 2008; Robinson et al., 2015; Cattuzzo et al., 2016). Various terminologies have been used interchangeably in past literature to refer to this latent concept including “motor proficiency,” “motor performance,” “movement (skill) competence,” “motor ability,” “motor function,” “motor coordination,” and “fundamental movement/motor skills” (Robinson et al., 2015). In alignment with previous studies (e.g., D’Hondt et al., 2013; Robinson et al., 2015; Cattuzzo et al., 2016), this paper uses the term motor competence as a general construct encompassing all forms of goal-directed human movement involving gross body coordination and control.

The role of motor competence in children’s health is well described in the conceptual model of Stodden et al. (2008). This model denotes the relationship between motor competence and physical activity across childhood as well as their interrelations with perceived motor competence, weight status and physical fitness. Physical fitness can be defined as the capacity to perform physical activity and includes components such as cardiorespiratory fitness, musculoskeletal fitness (i.e., muscular endurance and strength) and flexibility (Caspersen and Christenson, 1985; Ortega et al., 2008). As noted in a review article by Robinson et al. (2015), a wealth of predominantly cross-sectional studies show that multiple health-related outcomes, including physical activity (Holfelder and Schott, 2014; Logan et al., 2015) and physical fitness (Hands, 2008; Cattuzzo et al., 2016; Utesch et al., 2019) are indeed positively associated with motor competence. Previous literature has also shown an inverse relationship between weight status and motor competence (D’Hondt et al., 2011; Cattuzzo et al., 2016). However, given the role of motor competence in the development of an active and healthy lifestyle, it is important to understand how motor competence develops across time during childhood. Therefore, we need more longitudinal research that examines the development of motor competence over time and its relationship with other health-related outcomes. For instance, de Souza et al. (2014) compared motor competence, physical activity and physical fitness of children at 6 years of age relative to their physical fitness and physical activity levels at 10 years. The authors found that children who were both fit and active at 10 years of age had a more favorable activity and fitness profile at 6 years and they were also more competent at 6 years compared to their unfit and sedentary peers. Similarly, Henrique et al. (2018) found a significant relationship between motor competence, physical fitness and weight status over time in children aged 6–9 years. Children with consistently better motor competence during the 4 years of follow-up had lower body weight, lower body mass index, lower subcutaneous fat, and higher physical fitness levels at age 6 compared to those with consistently low(er) levels of motor competence (Henrique et al., 2018). However, Henrique et al. (2018) focused on specific changes in motor

competence (i.e., stable and unstable trajectories of children scoring below or above a specific percentile) and not on how factors measured at baseline might influence the development of motor competence.

The development of motor competence during childhood is also noted by a high degree of inter-individual variation (Rodrigues et al., 2016). Some children’s competence levels increase whilst others’ competence levels remain unchanged or even decrease over time. However, few studies have taken into account individual change in motor competence development. To our knowledge, Rodrigues et al. (2016) were the first to highlight the importance of individual trajectories in motor competence and physical fitness measures over time. However, the study of Rodrigues et al. (2016) used a test battery that mainly focused on components of physical fitness. Therefore, further research using specific and standardized assessment tools is needed to explore change in actual motor competence over time.

Using latent growth curve modeling, the aim of the present longitudinal study was (1) to gain more insight into children’s individual change in motor competence across a 2-year timespan and (2) to investigate the potential influence of weight status and physical fitness at baseline on changes in motor competence trajectories over time. Based on previous studies (Stodden et al., 2008; Robinson et al., 2015; Rodrigues et al., 2016), it was hypothesized that there would be significant variability in children’s trajectory of motor competence at the individual level. It was also expected that children’s individual trajectory of motor competence would be influenced by age and sex as well as by their weight status and physical fitness level.

## MATERIALS AND METHODS

### Participants

The present study involved secondary data-analysis from a large-scale longitudinal research project (Vandorpe et al., 2011). These data were collected in primary school children between September 2007 and January 2009. Children were recruited from 13 randomly selected primary schools from all five Flemish provinces and the Brussels-capital region of Belgium. Motor assessments took place annually for three consecutive years (i.e., 2007, 2008, and 2009). Of the original sample of 712 children assessed at each time point, only those children who completed the motor assessments annually and the anthropometric measurements and physical fitness tests at baseline were retained for the purpose of this study. This resulted in a total sample of 558 children (i.e., 293 boys and 265 girls) aged between 6 and 9 years at baseline. Written informed consent was provided for each child by a parent or legal guardian. The study protocol was approved by the Ethics Committee of Ghent University Hospital.

### Procedures

All participants wore light sports clothing and were barefoot during testing, except for the 20 m shuttle run (for which they wore sports shoes). Assessments took place during the physical education classes in the gymnasium of the children’s

schools and were conducted three times on an annual basis (during the same season). Test sessions lasted approximately 85 min, with a group of trained examiners conducting the assessments using standardized instructions in accordance with the testing guidelines.

## Measurements

### Motor Competence

The Körperkoordinationstest für Kinder (KTK) was used to evaluate motor competence. It is a standardized normative product-oriented test battery for 5- to 15-year old children with typical and atypical motor development, which is widely used in Europe (Kiphard and Schilling, 1974, 2007, 2017). The test battery is considered a highly reliable instrument with excellent test–retest reliability for the total raw score ( $r = 0.97$ ), inter–rater reliability and intra–rater reliability for the subtest raw scores ( $r$  values  $> 0.85$  and  $r$  values = 0.80–0.96, respectively) (Kiphard and Schilling, 1974, 2007). Content and construct validity have been documented (Kiphard and Schilling, 1974, 2007), and its convergent validity has been established through moderately strong correlations with other standardized assessment tools such as the Bruininks-Oseretsky Test of Motor Proficiency – 2nd Edition (BOT-2; Bruininks and Bruininks, 2005; Fransen et al., 2014), the Motoriktest für Vier- bis Sechsjährige Kinder (MOT 4-6; Zimmer and Volkamer, 1987; Bardid et al., 2016), and the Movement Assessment Battery for Children (M-ABC; Henderson and Sugden, 1992; Smits-Engelsman et al., 1998). The KTK is also considered a very useful motor test battery for longitudinal research because each test item is identical at any age (D'Hondt et al., 2013). The test includes four subtests: (1) balancing backwards (BB) over three beams of decreasing width, (2) moving sideways (MS) with the aid of two wooden boards in 20 s (two attempts), (3) jumping sideways (JS) as often as possible over a bar in 15 s (two attempts), and (4) hopping for height (HH) on one leg over foam squares with consecutive steps of 5 cm per added foam square. For the purpose of the present analysis, the raw scores of each subtest were summed to compute an overall motor competence score. In addition, a standardized motor competence score (or motor quotient, MQ) was also computed using the manual's normative tables based on the performance of the reference sample (Kiphard and Schilling, 2017). To this end, the raw subtest scores were first transformed into standardized scores adjusted for age (all subtests) and sex (BB, JS, and HH). These standardized subtest scores were then summed and converted into the total KTK MQ.

### Physical Fitness

Different subtests of the European Test of Physical Fitness (EUROFIT) with adequate reliability were used to assess the health-related components of physical fitness (Council of Europe, 1988). The selection of these tests was based on practical considerations regarding age-appropriateness, user-friendliness and discriminative power among children aged 6–11 years. Cardiorespiratory fitness or endurance was assessed using the multistage fitness test, also known as the EUROFIT 20 m shuttle run test (20 m SR), with an accuracy of 0.5 min. This test involves continuous running between two lines (20 m apart) on time

in agreement with recorded beeps. The frequency of the sound signals is gradually increased during this test, requiring children to run faster with each increase in frequency of signals (plus 0.5 km/h each minute from a starting speed of 8.5 km/h). The test was stopped if the subject could no longer keep the pace and failed to reach the line (within 2 m) for two consecutive times and after a warning. The EUROFIT standing long jump test (SLJ) was used as an indicator of musculoskeletal fitness and explosive power (Pillsbury et al., 2013). In this test, participants have to jump as far as possible from standstill and land on both feet. The test is performed twice with the best result used for data analysis with an accuracy of 1.0 cm. Trunk flexibility and hamstrings length were assessed with the EUROFIT sit and reach test (SAR) with an accuracy of 0.1 cm. For this test, participants had to sit on the ground with straight legs, reaching as far as possible with the fingertips to a metal board, with the best score on two consecutive trials used for data analysis.

### Weight Status

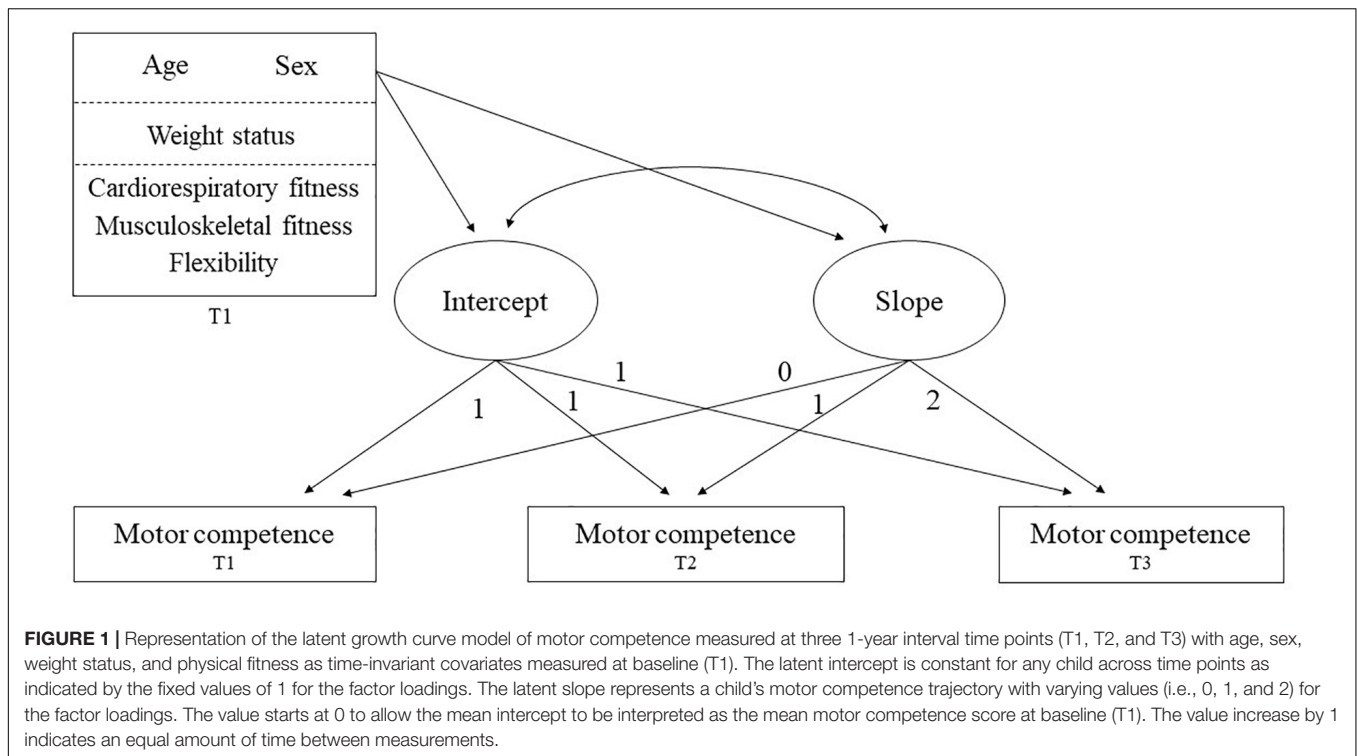
Participants' body height was measured by using a portable stadiometer with an accuracy of 0.1 cm (Harpenden, Holtain Ltd., Crymch, United Kingdom) and their body weight was determined using a digital scale with an accuracy of 0.1 kg (Tanita, BC-420 SMA, Weda BV, Naarden, Holland). These measures were then used to compute children's body mass index (BMI, kg/m<sup>2</sup>), which was used as an estimate of weight status.

### Statistical Analysis

Descriptive statistics were calculated for the motor competence scores (i.e., KTK total raw score) at each time point and for the different health-related components of physical fitness (i.e., 20 m SR, SLJ, and SAR) and weight status (i.e., BMI) at baseline using SPSS 25 for Windows.

Latent growth curve models (LGCMs; see **Figure 1**) were conducted to examine change in motor competence over time, summed into an overall motor competence score based on the raw scores on the KTK test items (at each time point). Effects of confounding factors such as sex and age were considered in the analysis. Additionally, effects of participants' baseline weight status (i.e., BMI) and physical fitness components (i.e., 20 m SR, SLJ, and SAR) on change in motor competence were examined. Maximum likelihood estimation was used for the LGCMs and significance level was set at  $p < 0.05$ . Different fit indices were used to assess model fit: (1) the chi-square test ( $\chi^2$ ), (2) the root mean square error of approximation (RMSEA), (3) the standardized root mean square residual (SRMR), and (4) the comparative fit index (CFI). Good model fit is indicated by values  $< 0.08$  (RMSEA),  $< 0.06$  (SRMR) and  $> 0.90$  (CFI; Hu and Bentler, 1999).

A series of LGCMs were run to investigate change in motor competence over time. First, an intercept-only model with the intercept mean and residual variance constrained across time points (Model 1) was run. The intercept variance was then estimated in Model 2. Next, the slope mean and variance were included in Model 3 to estimate change in motor competence over time. Subsequently, sex and age were added to the model (Model 4). Sex was inserted as a dummy variable (i.e., 0 = boy;



1 = girl), whereas age (months) was inserted as a continuous variable and mean centered in the LGCMs. Next, baseline weight status was included as a continuous variable in Model 5 to examine the potential influence on change in motor competence over time. Similarly, 20 m SR, SBJ, and SAR were entered as continuous variables in Model 6 to examine possible effects of baseline physical fitness on motor competence change over time. Both weight status and the three abovementioned physical fitness variables were z-transformed adjusting for age and sex. Finally, a model with only significant effects of baseline weight status and physical fitness components was run (Model 7). All latent growth curve analyses (LGCA) were conducted in R version 3.5.2 using the *lavaan* package (Rosseel, 2012).

Figures were also produced to illustrate individual trajectories of change in motor competence. To this end, children were divided into three groups based on their change in motor competence over time (i.e., difference in score between time point 1 and time point 3): low rate of change group (<P25), average rate of change group (P25–P75), and high rate of change group (>P75). **Figure 2** shows individual changes in motor competence over time based on the KTK total raw score, whereas **Figure 3** displays individual changes in motor competence development based on the total MQ of the KTK.

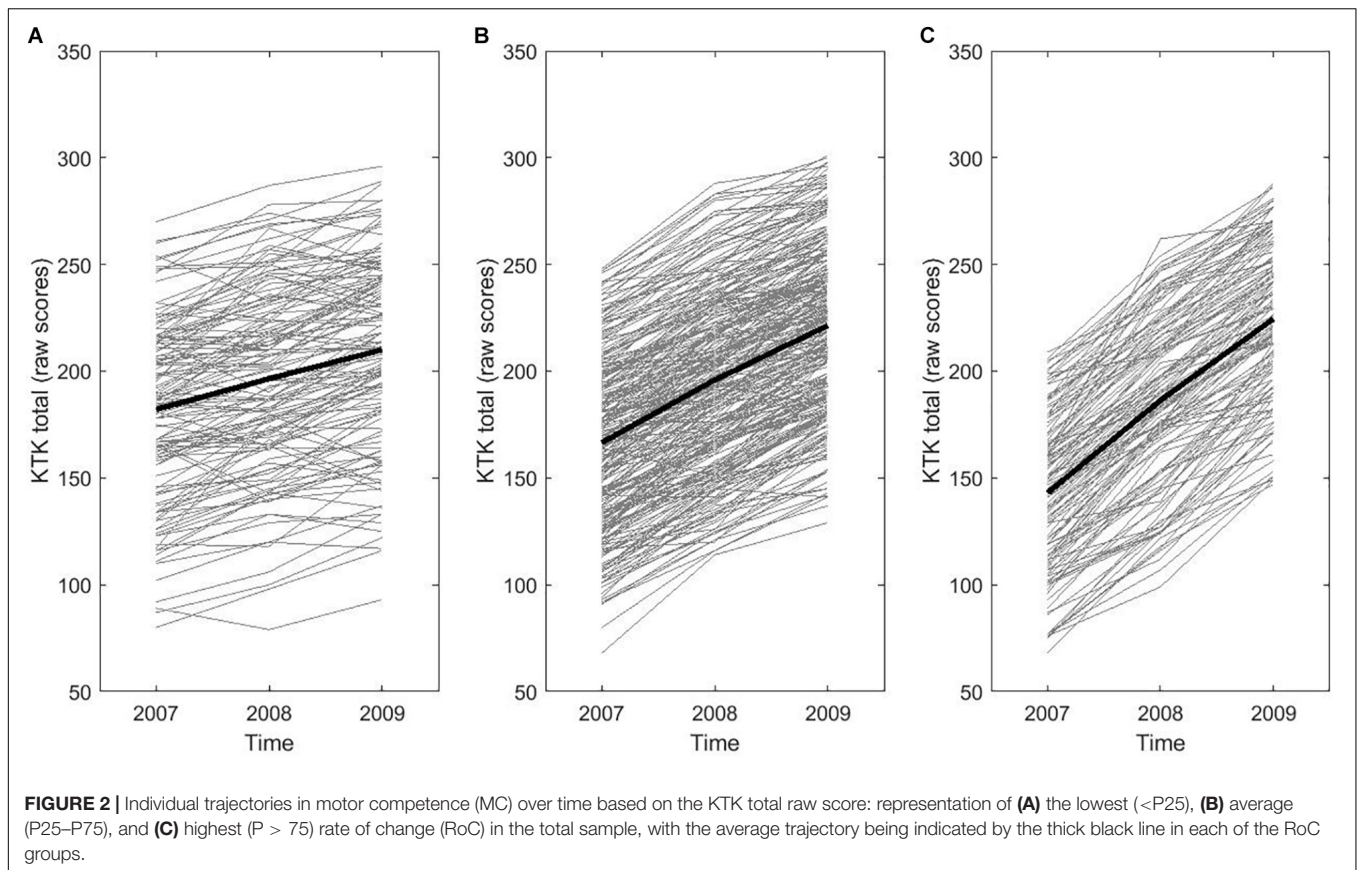
## RESULTS

**Table 1** shows the means and standard deviations of weight status at baseline (i.e., BMI), fitness scores at baseline (i.e., 20 m SR, SBJ, and SAR) and levels of motor competence (total raw scores on

the KTK) at each time point for boys and girls separately as well as for the total sample. Motor competence generally increased over time, which is also visualized by the thick black lines in **Figure 2** (change in total raw score) and **Figure 3** (change in total MQ), representing the average trajectory in the low, average and high rate of change group. Both figures show the variability in individual change of motor competence for the total sample visualized by the thin lines, representing the individual trajectory of motor competence across time.

The results of the LGCA are reported in **Table 2**. The LGCMs with random intercepts and slopes demonstrated good model fit (RMSEA  $\leq 0.073$ ; SRMR  $\leq 0.014$ ; CFI  $\geq 0.994$ ). Based on the total raw scores on the KTK, the analyses showed a positive linear change in motor competence over time ( $\beta = 28.48$ ,  $p < 0.001$ ) with significant variance in this change ( $p < 0.001$ ). There was no significant relationship between motor competence at baseline and change in motor competence over time, based on the overall total raw scores on the KTK ( $p = 0.33$ ).

Sex was not a predictor of differences in the KTK total raw score at baseline but was negatively associated with change in motor competence across 2 years; girls made less progress in motor competence than boys ( $\beta = -2.12$ ,  $p = 0.01$ ). Age was significantly related to the KTK total raw score at baseline, with older children demonstrating higher motor competence at baseline ( $\beta = 1.90$ ,  $p < 0.001$ ). Additionally, age at baseline was negatively associated with change in motor competence across time. Children who were older at baseline demonstrated less change in motor competence across 2 years ( $\beta = -0.33$ ,  $p < 0.001$ ). When considering the intercept and slope variance, 40.4 and 34.8% was explained by sex and age.



Children's weight status at baseline was negatively associated with the KTK total raw score at baseline as well as with the change in motor competence across 2 years. A higher BMI level at baseline was associated with decreased motor competence ( $\beta = -3.67$ ,  $p = 0.004$ ). Similarly, a higher BMI at baseline was inversely related to motor competence change over time ( $\beta = -1.418$ ,  $p = 0.002$ ). After accounting for sex and age, weight status explained 8.9 and 4.5% of the intercept and slope variance, respectively.

Of the physical fitness outcomes, baseline levels of 20 m SR ( $\beta = 8.08$ ,  $p < 0.001$ ) and SBJ ( $\beta = 15.51$ ,  $p < 0.001$ ) were directly related to motor competence levels at baseline. After accounting for sex, age, and BMI, both 20 m SR and SBJ explained 36.8% of the intercept variance. When considering change in motor competence over time, none of the physical fitness components measured at baseline was shown to be significantly associated.

## DISCUSSION

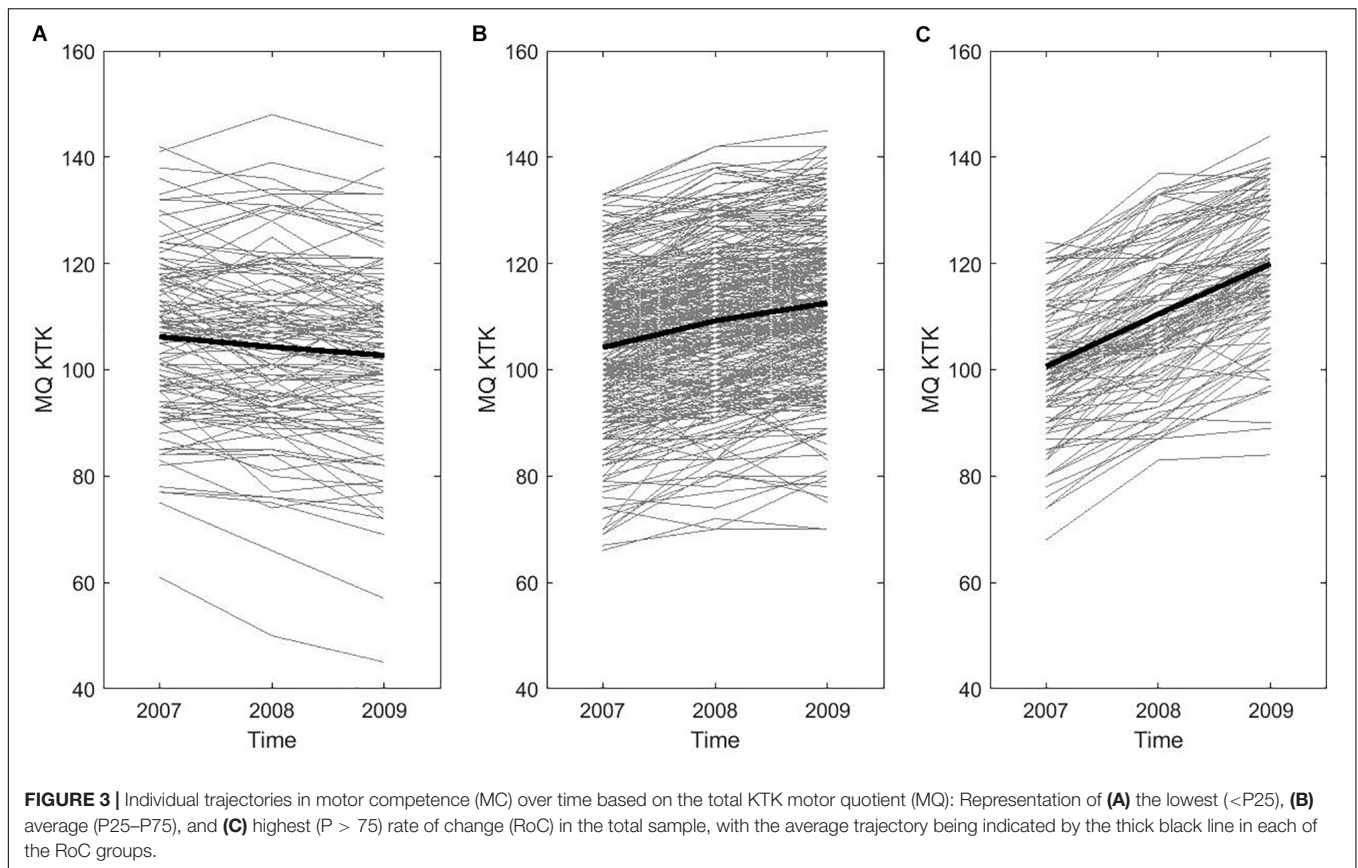
The purpose of this longitudinal study was to gain more insight into developmental change in children's motor competence over time and to investigate the potential influence of weight status and physical fitness on that change. Therefore, a LGCA was conducted to investigate the developmental change in motor competence. This approach is appropriate as it models each

child's trajectory of change in motor competence across time. It considers differences in developmental trajectories across children and the potential influence of baseline weight status and physical fitness on this individual change.

Consistent with previous research (e.g., Ahnert et al., 2009; Vandorpe et al., 2011; Dos Santos et al., 2018), an average positive change in motor competence over 2 years was found. Yet, the results of the LGCA revealed that there was significant variance in how children's motor competence develops over time, which is also consistent with Dos Santos et al. (2018), and clearly illustrated in both Figures 2, 3 of the present paper.

Visual inspection of Figure 2 shows a large variability in the rate of change across the current sample. In some children, the overall motor competence raw score demonstrates improvement in a linear fashion over the 2 years, whereas others show little change after 1 year followed by an improvement in year 2, and still others show an increase in year 1 that levels off in year 2. It is interesting to note that when these raw scores are converted to age- and sex-adjusted MQs as displayed in Figure 3, a number of children actually stagnated (1.1%) or demonstrated a delayed development (14.7%) of motor competence compared to the reference sample (Figure 3; Kiphard and Schilling, 2017). MQ is considered a relatively stable construct over time for the average child (Vandorpe et al., 2011), but results of the LGCA demonstrate there was statistically significant variability in trajectories of change in motor competence among individual





**TABLE 1 |** Descriptive statistics of age, weight status at baseline, physical fitness scores at baseline and motor competence raw scores at each time point, in boys, girls and the total sample.

	Boys ( $N = 293$ )	Girls ( $N = 265$ )	Total Sample ( $N = 558$ )
<b>Age (years)</b>	$8.2 \pm 1.09$	$8.1 \pm 1.15$	$8.2 \pm 1.1$
<b>Weight status (<math>\text{kg}/\text{m}^2</math>) at baseline</b>	$16.21 \pm 2.01$	$16.32 \pm 2.16$	$16.26 \pm 2.08$
<b>Physical fitness at baseline</b>			
Cardiorespiratory fitness: 20 m SR (min)	$4.85 \pm 2.22$	$3.55 \pm 1.73$	$4.23 \pm 2.10$
Musculoskeletal fitness: SBJ (cm)	$124.11 \pm 20.49$	$118.83 \pm 20.62$	$121.61 \pm 20.70$
Flexibility: SAR (cm)	$19.51 \pm 5.18$	$22.57 \pm 4.93$	$20.96 \pm 5.28$
<b>Motor competence at each time point</b>			
KTKTOTAL T1 (RAW SCORE)	$166.10 \pm 39.84$	$162.89 \pm 43.01$	$164.57 \pm 41.37$
KTKTOTAL T2 (RAW SCORE)	$196.24 \pm 40.17$	$191.52 \pm 41.00$	$194.00 \pm 40.60$
KTKTOTAL T3 (RAW SCORE)	$224.85 \pm 40.70$	$216.99 \pm 42.23$	$221.12 \pm 41.59$

SR, shuttle run; SBJ, standing broad jump; SAR, sit and reach; KTK, Körperkoordinationstest für Kinder.

children. Whilst an improvement in raw scores on the KTK was present in virtually every child in the sample (99.6%; see **Figure 2**), this improvement may be considered insufficient to keep up with the expected motor development, which is evident from **Figure 3**, where only 39.0% makes progress, with respect to age- and sex-related norms.

Regarding the level of motor competence at baseline, the LGCA showed that there was no significant relationship between motor competence at baseline and the change in motor competence over time. Indeed, inspection of **Figures 2** and **3**

shows that each rate of change group included children with a high(er) or low(er) level of motor competence at baseline. Our results thus suggest that each child can improve his/her level of motor competence over a period of 2 years, regardless of his/her initial level of motor competence. The finding of inter-individual variation in motor competence development is in agreement with the study of Rodrigues et al. (2016), where children also demonstrated divergent developmental pathways in fitness across childhood. Interestingly, the study by Rodrigues et al. (2016) reported that many children in the low rate of change group

**TABLE 2** | Results of the latent growth curve analyses.

	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6	Model 7
<b>Intercept mean</b>	192.53***	192.53***	165.16***	165.33***	165.19***	165.20***	165.20***
Sex				-0.36n.s.	-0.33n.s.	-0.25n.s.	-0.25n.s.
Age				1.89***	1.90***	1.90***	1.90***
Weight status					-9.97***	-3.66**	-3.67**
Cardiorespiratory fitness						8.40***	8.08***
Musculoskeletal fitness						15.38***	15.51***
Flexibility						1.98n.s.	
Intercept variance		1191.05***	1583.02***	943.15***	859.66***	543.65***	546.97***
Residual variance	2116.59	925.55	131.13	131.13	130.84	130.63	130.63
<b>Slope mean</b>			27.37***	28.41***	28.47***	28.48***	28.48***
Sex				-2.19**	-2.22***	-2.12*	-2.12*
Age				-0.29***	-0.0***	-0.30***	-0.3***
Weight status					-1.38***	-1.76***	-1.42**
Cardiorespiratory fitness						-0.49n.s.	
Musculoskeletal fitness						-0.72n.s.	
Flexibility						0.34n.s.	
Slope variance			45.30***	29.52***	28.21***	26.76***	27.50***
Covariance			-86.23***	10.17n.s.	-1.75n.s.	10.96n.s.	11.10n.s.
$\chi^2$	2407.75	1905.34	11.88	15.80	16.11	22.87	23.01
Df	7	6	3	5	6	9	10
RMSEA	0.784	0.753	0.073	0.062	0.055	0.053	0.049
SRMR	0.634	0.380	0.014	0.010	0.009	0.007	0.011
CFI	0.000	0.027	0.995	0.995	0.996	0.994	0.995

\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; n.s., not significant.

did not change in raw performance or actually decreased in raw performance over time, whereas the present study demonstrated a general positive change in motor competence over time irrespective of the level of motor competence at baseline. In contrast, Dos Santos et al. (2018) found that higher levels of motor competence at 6 years of age demonstrated lower rate of change over time. It should be noted that both studies included samples of one age group or grade (i.e., 6 years/grade 1) followed over time, whilst the present study sample covered a larger age range at baseline (i.e., 6–9 years). Additionally, Rodrigues et al. (2016) mainly focused on components of fitness rather than motor competence (i.e., SLJ, 50 m dash, 10 m SR, 60 s sit-ups, flexed arm hang, SAR, 20 m SR). The extent to which children can improve their motor competence level and redirect their trajectories later on, remains a pertinent question and should be further explored.

The present findings showed that there was no significant difference in motor competence between boys and girls at baseline. Most previous studies have reported sex differences in favor of boys in this age range although different results have been found across specific motor domains. In their systematic review, Barnett et al. (2016) found strong evidence for boys scoring better on motor coordination compared to girls whilst reporting inconclusive evidence for girls outperforming boys on stability measures. As the KTK covers both aspects of motor coordination and dynamic balance, this might explain the divergent finding in the present study. Interestingly, boys

in our sample made more progress in motor competence over time compared to girls. Prior research has generally not specifically investigated how sex influences motor competence development. The study of Dos Santos et al. (2018), however, found differences in the trajectory of change favoring girls whilst the study of Rodrigues et al. (2016) found no differences. In light of these contrasting findings, there is clearly a need for more research into how boys and girls develop their motor competence levels over time and how this might be (differently) affected by factors such as physical activity participation and sports preferences.

In alignment with previous literature (e.g., Barnett et al., 2016), the present study results showed a positive relationship between age and the level of motor competence at baseline. However, our data indicate that as age increases, change in motor competence decreases. Although data on this topic in literature is limited, it is generally assumed that early childhood is marked by major changes in physical and motor development (Gallahue et al., 2012). However, as noted by Gallahue and Ozmunn (2005), middle childhood is characterized by “slow but steady increases in height and weight and progress toward greater organization of the sensory and motor systems” (p. 178). Although older children still make progress in their motor competence, these findings do seem to support early interventions focused on developing motor competence at a younger age. This, in turn, will help children to successfully participate in sports, games and other types of physical activity as they grow older.

Another purpose of this study was to examine if weight status and physical fitness influenced children's individual trajectory of change in motor competence over time. Results revealed a significant inverse relationship between weight status and motor competence at baseline, which is in agreement with previous research (Martins et al., 2010; Lopes et al., 2012; D'Hondt et al., 2013, 2014; Cattuzzo et al., 2016; Rodrigues et al., 2016; Lima et al., 2018). Moreover, children's baseline weight status was inversely associated with change in motor competence. Specifically, a higher weight status at baseline was associated with less progress in motor competence. This partly supports Stodden et al. (2008) notion of a negative spiral of disengagement where children with a less optimal weight status are at greater risk to end up in becoming less motor competent over time, which may lead to reduced physical activity participation and lower physical fitness.

With respect to physical fitness, it was indeed shown that baseline levels of cardiorespiratory and musculoskeletal fitness were significantly related to motor competence at baseline, which is consistent with findings from earlier studies (Lubans et al., 2010; Cattuzzo et al., 2016; Utesch et al., 2019). However, no significant relationship between trunk flexibility and motor competence at baseline was found. Contrary to these findings, Lopes et al. (2017) found a positive association between flexibility and motor competence in children. It should be noted that there is an age difference between the sample of the present study (6–9 years) and that of the study of Lopes et al. (2017; 9–12 years). More research is warranted to further understand the association between motor competence and flexibility as there is currently limited evidence available on this relationship (Cattuzzo et al., 2016; Utesch et al., 2019). Although physical fitness is considered an important marker of current and future health in both children and adults, none of the components of physical fitness (i.e., cardiorespiratory fitness, musculoskeletal fitness and flexibility) were significantly associated with change in motor competence over time. This is in contrast with the study by Dos Santos et al. (2018), who found that children in the age range of 6–9 years with higher levels of physical fitness demonstrated higher scores on motor competence across a 4-year timespan. It should be noted that our study particularly focused on how physical fitness at baseline influenced change in motor competence over time. In light of the limited longitudinal evidence on this topic, there is a need for more research investigating the role that different physical fitness components may have on motor competence development across childhood.

The present study investigated children's trajectories of change in motor competence across 2 years and explored the influence of baseline weight status and physical fitness on these trajectories. The longitudinal design and the use of LGCA are major strengths of the current study. Using this statistical approach allows for the estimation of inter-individual variability in intra-individual trajectories of change over time, whereas more traditional methods for analyzing repeated measures data are more limited in this respect. However, some limitations need to be addressed. First, the present study only investigated linear change in motor competence as data were only collected across three time points. However, considering

the variability in individual trajectories (see **Figure 2**) across a longer time frame should be investigated and may demonstrate non-linear change in motor competence across time (i.e., including  $\geq 4$  time points). This type of analysis will provide a more comprehensive understanding of childhood developmental pathways of motor competence. Second, children's BMI was used as the sole indicator of weight status. As BMI is an indirect estimate of adiposity, further investigations should include additional anthropometric measures, such as waist circumference and skinfolds or more advanced techniques (such as Bioelectrical Impedance Analysis (BIA) or Dual-energy X-ray Absorptiometry (DXA) to better estimate weight status and/or fat percentage. Third, the present study focused solely on gross motor coordination and did not examine other behavioral attributes such as physical activity and sport participation. For this reason, it is impossible to determine if the variability in intra-individual trajectories of change over time is related to sports practice or physical activity participation. As motor competence is associated with many health-related outcomes, more research is recommended to explore how other components of motor competence change over time and how this variation is linked with both physiological and psychological factors (e.g., physical fitness, weight status, perceived competence, and motivation) as well as behavioral and environmental factors (e.g., physical activity, and socioeconomic background). As noted by Robinson et al. (2015), children's development is "a complex and multifaceted process that synergistically evolves across time" (p. 1273). Barnett et al. (2016) determined that child-level variables such as age, sex, weight status, physical activity, fitness, and socioeconomic background are all important individual correlates of motor competence. Therefore, future longitudinal studies are recommended to further explore the potential role of such (additional) correlates in order to gain more insight in the mechanisms underlying children's individual trajectories of motor competence across time.

## CONCLUSION

In summary, this 2-year longitudinal follow-up study demonstrates a general positive linear change in children's motor competence over time, although there is significant variance in trajectories among individuals. Moreover, the level of motor competence at baseline was not found to be associated with change in motor competence over time. Our findings call for a shift toward a person-centered developmental approach for understanding change in motor competence development. This study further showed that weight status is not only negatively associated with motor competence at baseline, but it also negatively influences change in motor competence across childhood. This suggests that overweight children are at higher risk of making less positive change in motor competence over time. Additionally, whilst both cardiorespiratory and musculoskeletal fitness were positively related to motor competence at baseline, they did not significantly affect change in motor competence over time. Our findings highlight the importance of exploring

individual change in motor competence across childhood in order to develop more effective movement programs and to better support children's motor development.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

## ETHICS STATEMENT

This study was approved and carried out in accordance with the recommendations of the Ethics Committee of Ghent University Hospital. All participants and their parents/legal

guardians gave written informed consent in accordance with the Declaration of Helsinki.

## AUTHOR CONTRIBUTIONS

EC, FB, DS, and ML were involved in the conceptualization of the study. EC, FB, and ML analyzed data of the manuscript. All authors wrote the manuscript and approved the final version of the manuscript.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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# Using a Dance Mat to Assess Inhibitory Control of Foot in Young Children

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The development of motor response inhibition is critical during preschool years and has been associated with an improvement in gross motor coordination in this population. However, the assessment of inhibitory abilities in young children is challenging in terms of task selection and subject engagement, especially when investigating foot responses. Thus, the aim of this study was to describe a child-friendly Go/No-go paradigm to assess inhibitory control of foot based on a dance mat protocol. In this method, Go and No-go stimuli are modeled in the context of a fishing game, and behavioral responses are assessed by recording the latency to touch the mat and the accuracy of the touches. In this protocol article, we (1) describe the stages of the experimental set-up, (2) provide an illustrative data collection example in a sample of children aged 3–4 years, and (3) describe how to process the data generated. The utilization of the dance mat provides a feasible tool for researchers interested in studying the development of motor inhibitory control of foot in preschoolers. Potential applications of this protocol may include studies on developmental differences between hand and foot specialization, sports-related performance and neuroimaging.

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## INTRODUCTION

Inhibitory control is important for numerous aspects of cognitive maturation during infancy. It is defined as: the ability to regulate focus on selective stimuli, suppressing irrelevant information (interference control or inhibitory control of attention); the ability to resist prepotent mental representations, such as unwanted memories and thoughts (cognitive inhibition); and the ability to prevent planned or ongoing movement from interfering in the performance of certain tasks or behaviors (response inhibition or motor response inhibition) (Carlson and Moses, 2001; Diamond, 2013; Brevers et al., 2018). Inhibitory ability enables children to solve complex problems, such as mathematical questions (e.g., switch the addition operation to subtraction) (Bull and Scerif, 2001; Crova et al., 2014), supports learning (e.g., suppressing distractive and attending to specific information) (Lee et al., 2015) and emotion (e.g., inhibiting negative expression in unpleasant situations) (Johnstone et al., 2005; Carlson and Wang, 2007). This ability is also important for the development of children's motor activities. Impairment, such as developmental coordination disorder (DCD), and delays in motor skills have been associated with immature motor response

inhibition, which may affect motor activities, including those that are automatic, such as walking (Ruddock et al., 2016; Bernardi et al., 2018). Studies suggest that children with an impairment to their motor skills present failures in cerebellar mechanisms responsible for motor control (Rigoli et al., 2012), resulting in a deficit of central control and sensory organization (Ruddock et al., 2016; Speedtsberg et al., 2017). Moreover, an abnormal connection between the frontal cortex (the predominant region for inhibitory ability) and the cerebellum is related to poor integrity of motor response inhibition (Rigoli et al., 2012; Bernardi et al., 2018; He et al., 2018). Children in these conditions may present poor postural control, smaller step length, slower velocity during the task and continuous imbalances, increasing the incidences of falling (Deconinck et al., 2010). Furthermore, this atypical activity may affect motor planning and execution which may slow the motor response or cancellation of ongoing action (Schachar et al., 2007; Speedtsberg et al., 2017). Motor inhibition abilities have also been positively correlated with motor prediction, which is characterized as a re-organization of movement according to environmental conditions (Ruddock et al., 2015, 2016). Problems in predictive control of movements are associated with disturbances in fine and gross motor skills and poor performance of executive function, including inhibitory response (Wilson et al., 2013; Adams et al., 2017). An evaluation protocol could also be used to identify disorders related to a weak inhibitory control, such as DCD and Attention Deficit and Hyperactivity Disorder (ADHD) (Berryessa, 2016). Therefore, there is a need for assessment procedures of motor inhibition abilities suitable for young children, particularly to investigate foot responses at behavioral and brain levels.

However, assessing motor inhibitory function in preschool children is challenging. For instance, common inhibitory tasks use abstract stimuli that can be difficult for young children to understand. Moreover, adult-like paradigms fail to engage the interest of young children. Therefore, an increasing number of developmental cognitive neuroscience studies have developed child-friendly versions of common executive function tasks (for example see Perlman et al., 2016). Among the adaptations made by these studies are the provision of a coherent story line and the use of engaging graphics. The development of tasks that are more child-centered is thus crucial for a valid and reliable evaluation of motor inhibition in the first stages of the lifespan.

Paradigms suitable for a child may need to consider that the first years of life are a critical period for inhibitory control processes. Studies suggest that inhibition may present developmental signals at about 12 months of life, however, at around 3 years of age children show important gains in inhibitory ability (Booth et al., 2003; Wiebe et al., 2011). The development of inhibition seems to be related with frontal lobe maturation which is also marked during infancy and continues to develop through adolescence and adulthood (Carlson and Moses, 2001; Luna et al., 2004). Furthermore, evidence indicates that the cerebellum and the frontal lobe have a parallel development, which is associated with improvement of motor and cognitive skills, including motor response inhibition, in first infancy (Diamond, 2000; Booth et al., 2003; Gilbert et al., 2011; Wu et al., 2017). Therefore,

appropriate child tasks may offer evidence of the development of the inhibition ability in behavioral and neuroimaging studies.

The assessment of inhibitory control abilities has been widely carried out using Go/No-go tasks. The classical version of the Go/No-go task requires participants to make a motor response to one stimulus category (the Go condition), and to withhold the response to another class of stimulus (the No-go condition) (Liu et al., 2013). In the context of evaluating preschool children, Wiebe et al. (2012) adapted the Go/No-go task using a fishing game scenario. The authors instructed the children to catch the fish (pressing the button with their hand) and avoid catching the shark (not pressing the button). They found that children were capable of responding to the task, and also showed that with age, the young children become more strategic and responsible for their responses in inhibitory tasks as they progressively improved in accuracy and speed between conditions (Go and No-go) and ages (Wiebe et al., 2012). Also using the fishing game format, Howard and Okely (2015) examined differences between a standard button press version and a touchscreen version in a sample of preschoolers. However, no studies have yet proposed an adaptation to the Go/No-go task which examines foot responses during first infancy.

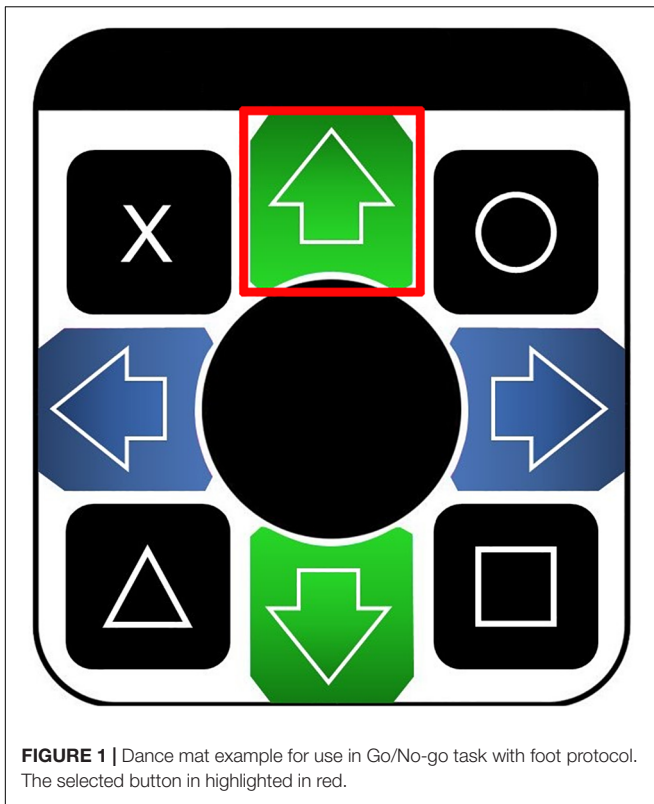
Foot structure is essential for regulating balance and locomotion and enables young children to develop motor and social skills (Price et al., 2018). Compared with the hand, the foot is less stimulated by the environment, which means that it develops later. As a result, investigating foot development may be a better indicator of maturational processes (e.g., myelination and dendritization) and hemispheric specialization (limb lateral dominance) (Bushnell and Boudreau, 1993; Gabbard, 1993, 1996). Moreover, Tabu et al. (2012) reported that hand and foot responses activated the same brain areas in an inhibitory task with adults, suggesting that the foot could be an appropriate alternative limb for evaluating inhibitory control. Therefore, assessing foot responses in preschoolers could offer more precise indications of the development of the brain mechanism of inhibitory control than an assessment using hand responses.

The purpose of the current study was to describe a protocol with a child-friendly modified version of the Go/No-go task proposed by Wiebe et al. (2012) to assess motor inhibitory control of foot in young children using a dance mat. Here we describe the development of the paradigm and its implementation, as well as results of an illustrative assessment of a sample of 3–4-year-old preschoolers.

## MATERIALS AND METHODS

### Equipment and Setup

- (1) Dance mat: to be used as a button-press response device for foot, suitable for preschooler populations (example, Dance Mat of DDR Game). The dance mat consists of equipment with the dimensions  $36 \times 32 \times 1/4$  inches and a 6-foot long cable (**Figure 1**). The dance mat may need a USB 2.0 adaptor and should have its configuration installed in the computer. The computer



will recognize the mat as a peripheral joystick with buttons and axis. Select one of the buttons to be coded by the stimuli presentation software, preferably one that is easily accessible by the child. The task requires only one active button, so the remaining sensors should be covered to reduce potential distractors.

- (2) Computer hardware and software to generate stimuli: stimuli can be presented via any option of hardware/software configuration that can smoothly display visual stimuli and record responses (with millisecond accuracy), for example DMDX<sup>1</sup> or Presentation (Neurobehavioral Systems)<sup>2</sup>. Stimuli can be displayed on a computer screen or projected. Pictures should be big enough to be easily discriminated, for example 1280 × 720 pixels, and positioned in the center of the screen.

## Visual Stimuli

The fishing game protocol using a dance mat was programmed as a functional near-infrared spectroscopy (fNIRS) paradigm. The fish and shark stimuli times were based on the Wiebe et al. (2012) study. They found that children between 3 and 4 years of age were able to respond to 1500 ms stimuli using their hands. However, our protocol proposed a foot version of the task. Tabu et al. (2012) showed that feet respond more slowly than hands in adults. Wiebe et al. (2012) also verified that more than 2000 ms would

be too long for children of this age, so we proposed 2000 ms of stimuli as appropriate for a foot response. The duration of the intervals during the task (interstimulus interval and resting block) were based on the attention level of the children and on the brain's hemodynamic responses (Wiebe et al., 2012; Zamorano et al., 2014; Walsh et al., 2017; Herold et al., 2018). The whole experiment lasted around 5 min, which is the length of time recommended by Aslin et al. (2015). The number of stimuli (Go and No-go) presentations was established based on the studies by Wiebe et al. (2012) and Wilcox and Biondi (2015).

## Stimuli Programming

- (1) Select a child-friendly drawn picture of a fish and a shark to comprise, respectively, the Go and No-go stimuli. Also select picture drawings for feedback stimuli (for example, a fishing net) (Figure 2).
- (2) Design a paradigm for stimuli presentation. Stimuli are presented in a blocked fashion. In the Go block, the picture of the fish should be presented 7 times (duration 2000 ms). In the No-go block, also present the fish 7 times and randomly present the shark 3 times within the block (shark duration 2000 ms). Alternate the presentation of three Go and No-go blocks, interleaved with a 15 s-resting block (Figure 3). The presentation of task conditions is fixed and always starts with a Go block followed successively by a No-go block.
- (3) Show different feedback screens (duration 1000 ms) in response to corrected responses to the fish (Figure 2C), misses to the fish (Figure 2D), corrected responses to the shark (Figure 2E) and false alarms to the shark (Figure 2D). Add a fixation cross after the feedback screen as an interstimulus interval (random duration 1000–2285 ms).
- (4) Configure the fish and shark screens to record responses (i.e., touches to the dance mat).
- (5) The duration of the task should be around 5 min.

## Procedure

Ensure that the study protocol is approved for use by the appropriate Human Subjects Committee. The protocol described here was approved by the Ethical Research Committee of the Universidade de Mogi das Cruzes, from Mogi das Cruzes, Brazil (approval number 2.626.590).

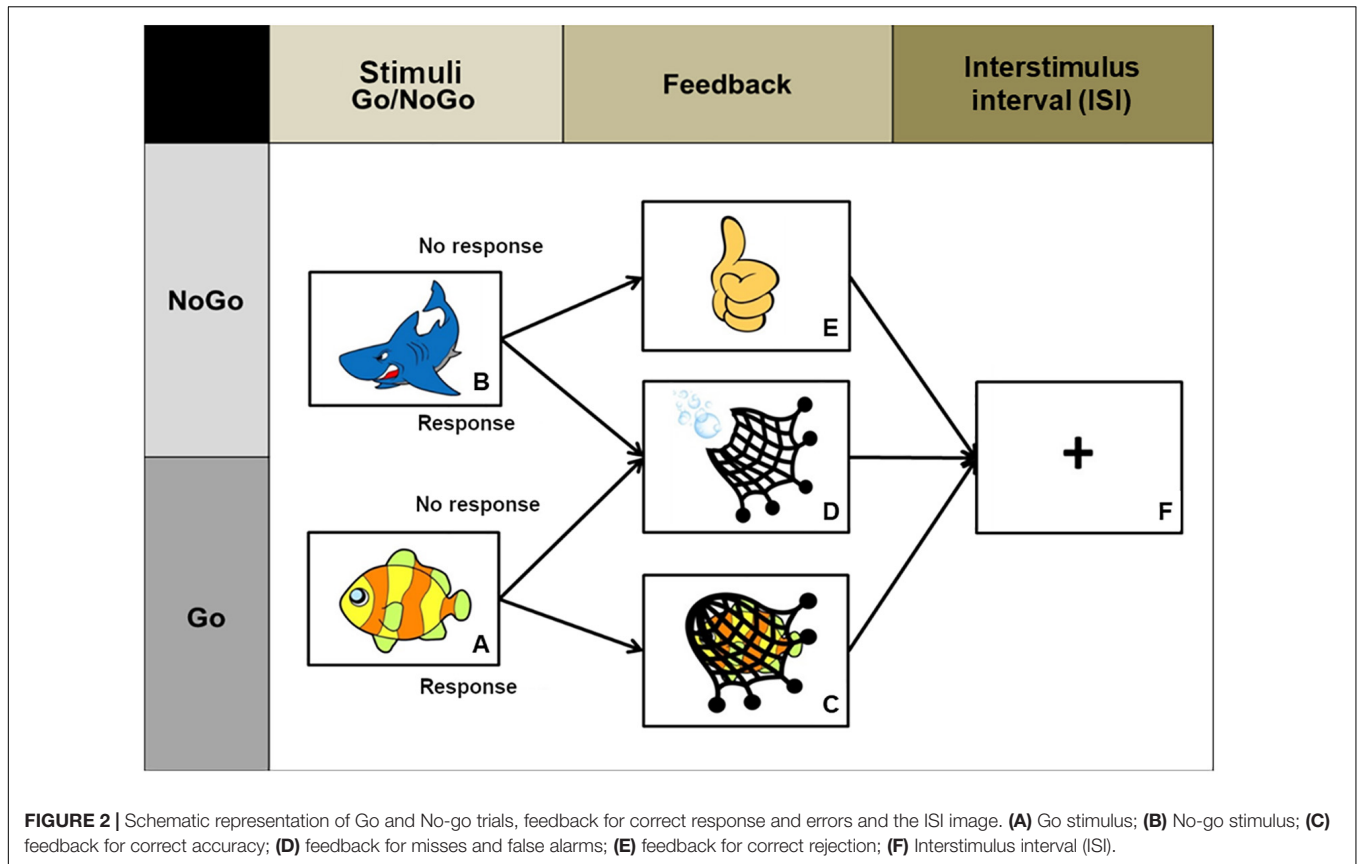
## Task Description

- (1) Obtain written informed consent from parents or legal guardians and the child's assent.
- (2) Tell children that they will take part in a fishing game. Position the child in the center of the mat, in a place without sensors, then explain the aims of the task: tell the children that they must catch the fish every time it appears on-screen by stepping on a particular button on the mat, the experimenter should show which button this is, and that they should not catch the shark, telling them "let the shark go home" to facilitate understanding of game's goals. The instructor also tells them that the fish swim fast and that they should make sure not to let the

<sup>1</sup>[http://www.u.arizona.edu/~sim\\$forster/dmdx/dmdx.htm](http://www.u.arizona.edu/~sim$forster/dmdx/dmdx.htm)

<sup>2</sup><http://www.neurobs.com>

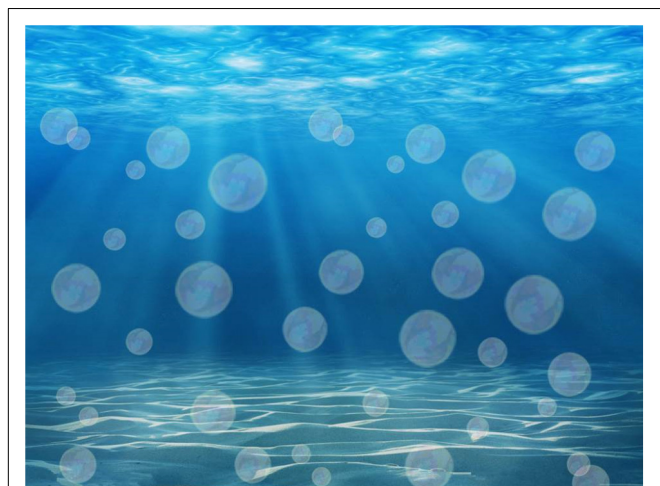




fish escape. To differentiate the conditions (Go and No-go) use familiar and easily distinguishable images (e.g., fish and shark) for the preschool phase and present the goals in a transparent way, “catch the fish, but do not catch the sharks” (Wiebe et al., 2012). The experimenter should model the instructions for the child by stepping

on the answer button on dance mat, and should stay with the child throughout the whole experiment, giving them voice feedback for correct answers and errors.

- (3) Adjust the monitor location to the height of the children’s eyes, providing a target that facilitates their balance (Wittenberg et al., 2017). Instruct the children to keep standing in the center of the mat (i.e., a place without sensors). The child should perform a training session, based on a No-go block, to ensure task comprehension. Depending on the specificities of the study’s objectives, the child can use a predefined foot (right or left) to perform the task.



**FIGURE 3 |** Static representation of the video interval between the Go and No-go blocks.

### Outcome Measures

The computer hardware and software to generate the stimuli may be programmed to record outcome variables. In the foot version of Go/No-go tasks, measurements included Go accuracy (responses to the fish), false alarms (responses to the shark), correct rejections (no responses to the shark) and misses (no responses to the fish). The dance mat protocol was also programmed to record the reaction time of Go accuracy, related to the fish stimulus, and the reaction time of false alarms, related to the shark stimulus, i.e., the time that children took to respond to the specific stimuli after they appeared on the screen. The correct responses (Go accuracy and correct rejections), omission (misses) and commission (false alarms) errors may be computed singly and be compared in order to

verify the difficulty level of the task. As reported by Wiebe et al. (2012), it is expected that children present around 75% Go accuracy and about 25% errors. It is also expected that the reaction time of Go accuracy is slower than reaction time of false alarms.

## ILLUSTRATIVE DATA COLLECTION

We evaluated a sample of 31 children (14 boys, 17 girls) from a public preschool in Mogi das Cruzes, São Paulo state, Brazil. The children were between 3 and 4 years old, with a mean of 3 years and 6 months, and had no history or evidence of neurological disorders. Written consent was obtained from all of the parents (or legal guardians), and verbal assent was obtained from all of the participants. The Affordances in the home environment for motor development (AHEMD) questionnaire was used to assess the influence of domestic environment on motor development (Rodrigues et al., 2005). To evaluate the level of motor development, we used the Test of gross motor development second edition (Ulrich, 2000) that assesses 12 motor abilities related to locomotion and object manipulation. The participants' dominant foot was determined according to their performance on the kick-a-ball ability task from TGMD-2. In our sample, 9.7% were classified as left-foot dominant and 90.3% as right-foot dominant. **Table 1** describes the demographic characteristics of the sample and the AHEMD and TGMD-2 results. **Table 2** describes the results for the

main outcome measures of the Go/No-go procedure. Prior to calculating the measures, trials in which RT were <300 ms were removed from analysis as they were considered too fast to be a valid response to the stimuli (Howard and Okely, 2015; Magnus et al., 2017). Regarding task compliance, all children completed the task and one child committed a great number of errors.

The proportion of correct Go responses was high for both Go and No-go blocks, suggesting the same level of performance in response selection (as demonstrated in **Table 2**). The task was also sensitive enough to prompt an average number of commission errors (i.e., false alarms - falsely pressing the button in No-go trials), which is commonly used as an interference measure to assess behavioral performance.

The results of this illustrative data collection suggest that the dance mat provides a feasible tool for researchers interested in studying the development of motor inhibitory control of foot in preschoolers. The format of the Go/No-go protocol presented here is particularly suitable for block-designed neuroimaging studies using fNIRS. The procedure is appropriate for use with very young children (3–4 years). Additional pilot testing may be required to adjust the rate of stimuli presentation when investigating samples with different age ranges and/or neuropsychiatric disorders.

## ETHICS STATEMENT

This study was carried out in accordance with the recommendations of Ethical Research Committee of the Universidade de Mogi das Cruzes with written informed consent from all subjects. All subject's parents gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the same local ethical committee (approval number 2.626.590).

## AUTHOR CONTRIBUTIONS

NP, SG, and JB conceived and designed the study and acquired, analyzed or interpreted the data. NP, SG, TS, and JB drafted the manuscript. GG, AS and TS contributed administrative, technical, or material support.

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**TABLE 1** | Description of the sample according to the ranking in AHEMD and TGMD-2.

Subjects	AHEMD (%)			TGMD-2 (%)			
	Low	Moderate	High	Low	Moderate	High	
Participants	31	22.60	70.95	6.45	22.60	64.50	12.90

**TABLE 2** | Descriptive data for the foot version of the Go/No-go task. Results are expressed as (mean  $\pm$  standard deviation and median and min-max interval) percentage of Go accuracy, correct rejections, misses, false alarms, reaction time of Go accuracy and reaction time of false alarms of foot in the blocks Go and No-go.

	Go Block	No-go Block
Go Accuracy	86.64 $\pm$ 8.90% 85.71% (61.90–100)	84.49 $\pm$ 11.40% 85.71% (47.62–100)
Reaction Time of Go Accuracy	1099 $\pm$ 200.4 ms 1114.42 ms (627.75–1386.99)	1140 $\pm$ 230 ms 1183.23 ms (369.94–1440.43)
Correct Rejections	–	71.33 $\pm$ 25.46% 77.78% (0–100)
Misses	13.36 $\pm$ 8.90% 14.29% (0–38.10)	15.51 $\pm$ 11.40% 14.29% (0–52.38)
False Alarms	–	28.67 $\pm$ 25.46% 22.22% (0–100)
Reaction Time of False Alarms	–	884.2 $\pm$ 382.2 ms 869.8 ms (352.9–1549)

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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# Exercise Physiology Across the Lifespan in Cystic Fibrosis

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Cystic fibrosis (CF), a severe life-limiting disease, is associated with multi-organ pathologies that contribute to a reduced exercise capacity. At present, the impact of, and interaction between, disease progression and other age-related physiological changes in CF on exercise capacity from child- to adult-hood is poorly understood. Indeed, the influences of disease progression and aging are inherently linked, leading to increasingly complex interactions. Thus, when interpreting age-related differences in exercise tolerance and devising exercise-based therapies for those with CF, it is critical to consider age-specific factors. Specifically, changes in lung function, chronic airway colonization by increasingly pathogenic and drug-resistant bacteria, the frequency and severity of pulmonary exacerbations, endocrine comorbidities, nutrition-related factors, and CFTR (cystic fibrosis transmembrane conductance regulator protein) modulator therapy, duration, and age of onset are important to consider. Accounting for how these factors ultimately influence the ability to exercise is central to understanding exercise impairments in individuals with CF, especially as the expected lifespan with CF continues to increase with advancements in therapies. Further studies are required that account for these factors and the changing landscape of CF in order to better understand *how* the evolution of CF disease impacts exercise (in)tolerance across the lifespan and thereby identify appropriate intervention targets and strategies.

**Keywords:** cystic fibrosis, pediatric, exercise capacity, aging, exercise prescription

## INTRODUCTION AND OVERVIEW OF CYSTIC FIBROSIS

Cystic fibrosis (CF) is the most common genetic disease in the Caucasian population, caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene (Rowe et al., 2005, 2016; Cutting, 2015; Ratjen et al., 2015; Elborn, 2016; Farrell et al., 2017). CF is a multisystem disease affecting the pulmonary, gastrointestinal (GI), and reproductive systems, thereby resulting in increased morbidity and mortality (Rowe et al., 2005, 2016; Ratjen et al., 2015; Stoltz et al., 2015; Elborn, 2016). Defects in *CFTR* result in airway dehydration and the production of hyper-viscous and acidic mucus, which contributes to defective mucociliary clearance (Fahy and Dickey, 2010; Peabody et al., 2018; Shei et al., 2018). As a consequence, the airways are prone to chronic inflammation and recurrent infection, leading to a vicious cycle that causes progressive, irreversible

lung damage and airway obstruction. The resulting pulmonary disease, in combination with a host of other factors including malnutrition (due to exocrine and endocrine pancreatic insufficiency), physical inactivity, and intrinsic muscle abnormalities, contribute to exercise intolerance in people with CF (Marcotte et al., 1986b; Cox and Holland, 2017; Gruet et al., 2017; Urquhart and Saynor, 2018).

Regular airway clearance and inhaled antibiotic therapy, in combination with the recent development of highly effective CFTR modulator therapies, have greatly extended the life expectancy of people living with CF, allowing these patients to live into, or indeed beyond, their fifth or sixth decade (Solomon et al., 2015; De Boeck and Amaral, 2016; Quon and Rowe, 2016; De Boeck and Davies, 2017; Burgener and Moss, 2018; McElvaney et al., 2018; Rubin, 2018; West and Flume, 2018). This increased expected lifespan has highlighted the need to better understand the evolution of people with CF as they reach ages that were previously impossible or, at best, improbable. Indeed, with age, secondary co-morbidities become more prominent and prevalent. Specifically, co-morbidities such as chronic infections from an ever-changing spectrum of pathogens, some of which may become multi-drug resistant [i.e., *Pseudomonas aeruginosa* (PsA), *Burkholderia cepacia* (*B. cepacia*), and atypical mycobacteria, **Figure 1A**], and more frequent and severe pulmonary exacerbations lead to a progressive lung function decline and may, ultimately, increase mortality (Rowe et al., 2005, 2016; Ratjen et al., 2015; Elborn, 2016). Furthermore, this “aging CF population” has shown an increased incidence of CF-related diabetes (CFRD), low bone mineral density, and endothelial dysfunction due to chronic inflammation. In addition to their system-specific effects, each of these co-morbidities have deleterious consequences for quality of life (QoL) and aerobic fitness. Given the high prognostic value of aerobic fitness for mortality and QoL (Nixon et al., 1992; Moorcroft et al., 1997; Pianosi et al., 2005; Ward et al., 2013; Vendrusculo et al., 2018), understanding *how* exercise capacity changes, as well as the impact of disease progression, as people with CF age, may aid in developing appropriate, individually tailored exercise recommendations, in improving adherence, and, ultimately, in engendering better health outcomes in this population.

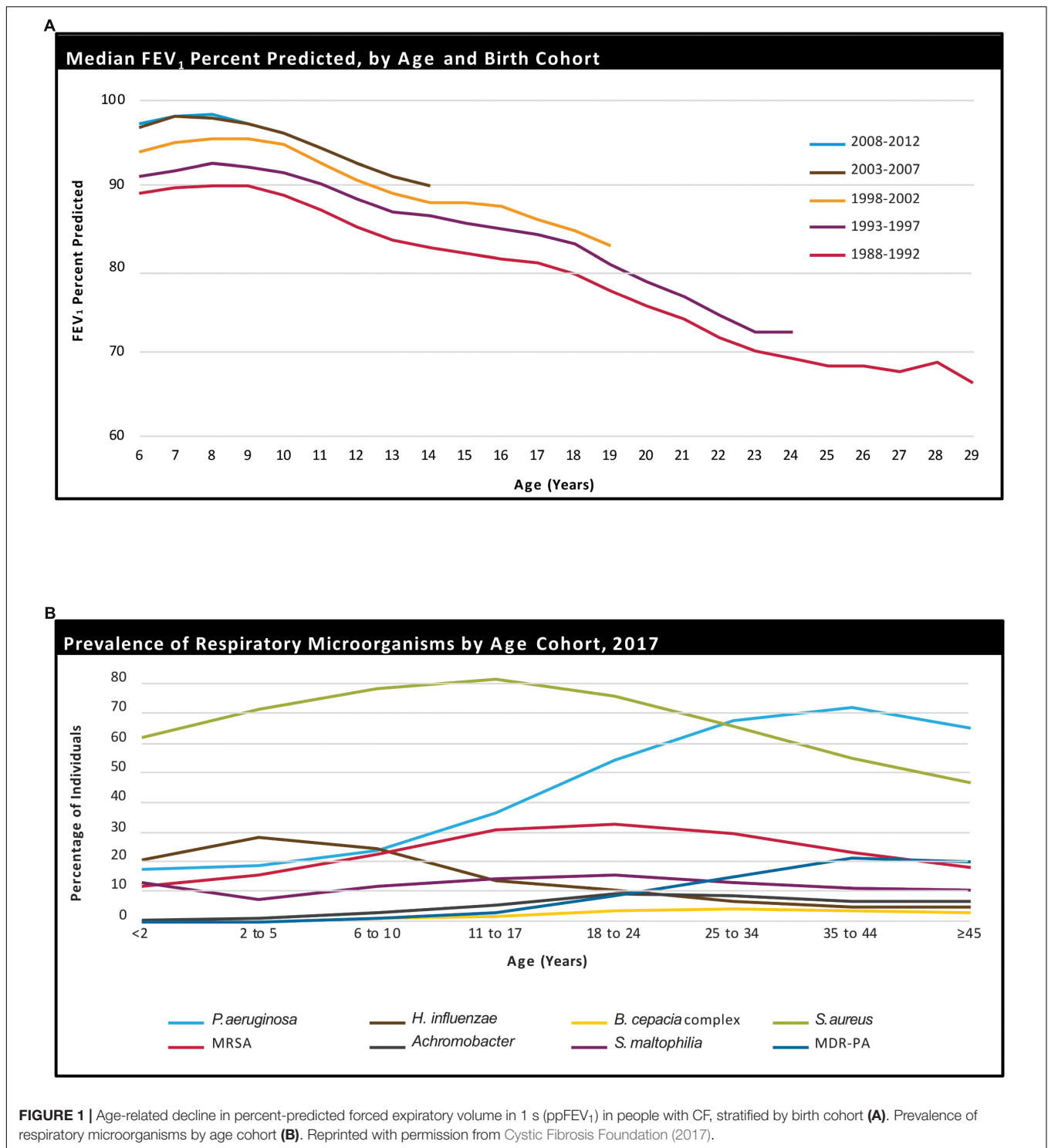
While it has long been established that children are not simply “mini-adults,” this is particularly pertinent in clinical populations in which aging occurs concomitantly, and potentially interactively, with, but nonetheless distinctly from, disease progression. Despite the importance of this distinction, the majority of research has failed to account for the influence of age, as well as the process and rate of aging, leading to potentially misleading conclusions regarding the disease progression itself. Indeed, comparisons between children and adults with CF are further compounded by the very different treatment strategies used in each age group – the treatment currently received by children is likely to significantly alter the course of their disease progression, and their experience of it, compared to that of those who are now adults. Therefore, changes in disease pathology, co-morbidities, disease complications, and treatment strategies as patients with CF age must be carefully assessed when designing, conducting and interpreting exercise studies in CF.

## EXERCISE CAPACITY IN CF

Deficits in exercise capacity (i.e., the maximum amount of physical exertion that a patient can sustain; (Goldstein, 1990) in those with CF result from a combination of factors, including, but not limited to, ventilatory dysfunction, changes in nutritional status, abnormalities in peripheral muscles (i.e., muscle weakness and putative metabolic abnormalities), cardiac constraint, and disease-related deconditioning (**Figure 2A**). The mechanisms of exercise limitation in CF have been reviewed elsewhere (Hulzebos et al., 2015). Briefly, ventilatory dysfunction in CF may contribute to exercise intolerance through deleterious changes in lung function, dead space ventilation, respiratory muscle function, ventilatory reserve, and ventilatory control. Together, these ventilatory constraints may limit exercise tolerance, particularly in more severe disease states. Specific to ventilatory dysfunction, exercise-induced hypoxemia may be more prevalent in CF, at least in part due to ventilation-perfusion mismatching secondary to increases in physiologic dead space and intrapulmonary arterio-venous shunting (Coffey et al., 1991). Nutritional status also plays an important role in determining exercise limitation in CF, particularly in those who are malnourished. Indeed, malnutrition predisposes individuals with CF to loss of both muscle mass and body fat, impaired diaphragmatic performance, and negatively affects cardiac function (Marcotte et al., 1986a; Lands et al., 1992a,b). Finally, muscle abnormalities including muscle weakness, mitochondrial dysfunction, and altered muscle metabolism may also contribute to exercise intolerance in CF (de Meer et al., 1995; Meer et al., 1999; Divangahi et al., 2009; Lamhonwah et al., 2010; Wells et al., 2011; Gruet et al., 2016; Werkman et al., 2016; Gruet et al., 2017). Whilst the mechanisms underpinning exercise limitation in those with CF are complex and interdependent (Schöni and Casaulta-Aebischer, 2000; Divangahi et al., 2009; Lamhonwah et al., 2010; Pastré et al., 2014; Hulzebos et al., 2015; Jiang et al., 2016; Gruet et al., 2017), it has been postulated that age-related progressions in CF disease severity may be integral to the annual decrements typically observed.

## EFFECTS OF AGING ON EXERCISE IN CF

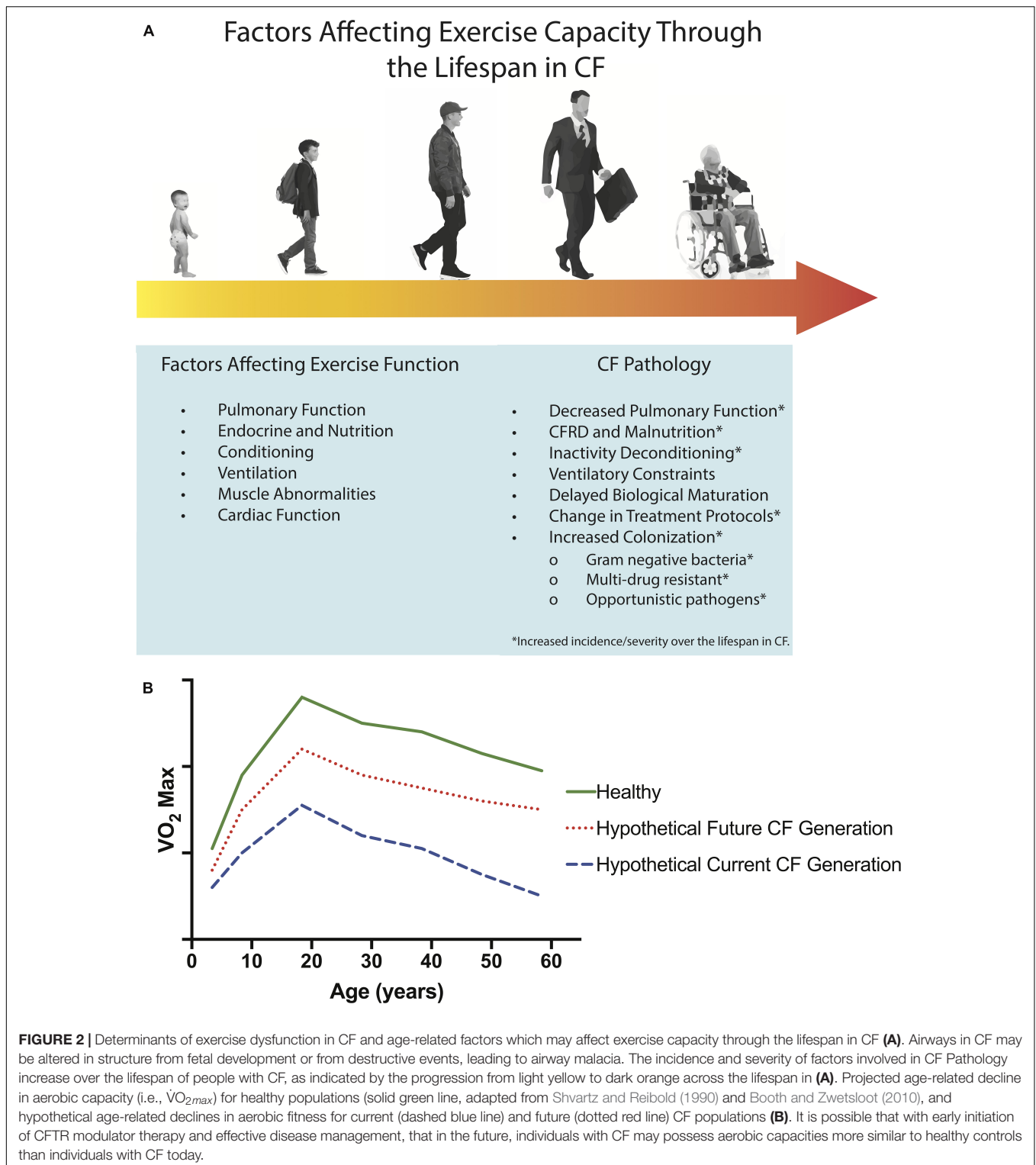
Despite evidence that exercise in people with CF improves aerobic capacity and thereby reduces mortality (Radtke et al., 2017), one of the most significant challenges is ensuring engagement in habitual physical activity (White et al., 2007; Myers, 2009). Indeed, engagement from a young age is imperative as not only is this likely to attenuate the decline in fitness and function and to promote the level from which this decline occurs, but importantly, behaviors established during childhood track into adulthood (Dishman et al., 1985). This therefore highlights the need to instill healthy behaviors at an early age. Recent case series reports regarding the influence of Orkambi®, one of the CFTR modulator combination therapies, on daily physical activity and exercise tolerance over a 2 year period are highly encouraging (Savi et al., 2019), especially when considered in conjunction with reports of improvements in peak oxygen



consumption ( $\dot{V}O_{2peak}$ ) and percent predicted forced expiratory volume in 1 s (ppFEV<sub>1</sub>; Hatziagorou et al., 2018; Philipsen and Pressler, 2018). Thus, as these therapies become more common and are able to be initiated at younger ages, exercise capacity may be preserved and/or improved in individuals with CF across their lifespan. The question remains, nonetheless, whether these

individuals will be able to exercise normally or will still need individually targeted exercise prescriptions due to a sub-normal exercise capacity.

Children and adults with CF have been shown to have a significantly lower  $\dot{V}O_{2peak}$ , lower gas-exchange threshold, reduced work capacity, and reduced oxygen uptake efficiency



compared to their healthy peers (Pouliou et al., 2001; Perpati et al., 2010; Tomlinson et al., 2018). Similarly, a reduced time to exhaustion during ramp testing (Saynor et al., 2016), impaired blood flow regulation, and an exaggerated oxidative stress and slower oxygen uptake response to submaximal exercise have been

shown in children with CF (Tucker et al., 2018). While these impairments in exercise capacity are evident from a young age in CF and persist into adulthood, it is not yet known whether the differences between CF and healthy controls may become more pronounced with age. Indeed, no studies have sought to



directly compare the exercise capacity of children and adults with CF. However, it is pertinent to note that such comparisons may be complicated by changes in pharmacological and non-pharmacological treatment strategies over the last few decades. Therefore, longitudinal studies that account for patient variations in treatment strategies are required; the increasing availability and utilization of cardiopulmonary exercise testing during annual reviews will be instrumental in capturing this information. In healthy populations, maximal oxygen consumption  $\dot{V}O_{2max}$  is known to decline with age, although regular exercise can slow this decline (**Figure 2B** – including hypothetical projected age-related declines for CF populations; Hagberg, 1987; Hawkins and Wiswell, 2003). The magnitude and rate of decline in  $\dot{V}O_{2max}$  in those with CF due to an accelerated ageing process still remains to be disentangled from the influence of CF *per se*.

## Bacterial Colonization

One of the most prominent differences between children and adults with CF is the degree and consistency of pulmonary colonization. Patient Registry data from the US Cystic Fibrosis Foundation shows that children under the age of 17 years tend to be colonized predominantly with *Staphylococcus aureus* (*S. aureus*), methicillin-resistant *S. aureus*, and *Haemophilus influenzae*, whilst adults have a larger percentage of *PsA* and an increase in the proportion of *Stenotrophomonas maltophilia* and *B. cepacia* (**Figure 1B**; Parkins and Floto, 2015; Cystic Fibrosis Foundation, 2017). Recurrent and increasingly persistent respiratory infections in youth, along with viral and fungal infections, contribute to repeated pulmonary damage, and as the disease progresses, these people with CF become more susceptible to colonization and infection with gram-negative bacteria, including multidrug resistant *PsA* (Elborn, 2016; Winstanley et al., 2016).

Recurrent and difficult to treat pulmonary infections are major determinants of progressive pulmonary decline over the lifespan, which coincides with progressive loss of exercise capacity (Rowe et al., 2005, 2016; Ratjen et al., 2015; Elborn, 2016; Radtke et al., 2017). Deteriorating lung function itself can contribute to airflow limitation, ventilatory-perfusion mismatch, predisposition to desaturation during exercise, and respiratory muscle weakness, all of which have profound impacts on exercise capacity. However, despite impaired exercise capacity being a common characteristic of CF, the underpinning physiological mechanisms are generally unknown. It is therefore unsurprising that studies and reviews incorporating children, adolescents, and adults with CF have failed to consider how age mediates, or impacts, exercise capacity (Radtke et al., 2017; Abdelbasset et al., 2018). Nonetheless, recent research has shown that even young children, with fewer exacerbations, had a lower exercise capacity in comparison to healthy counterparts. Indeed, Abdelbasset et al. (2018) found that CF children's  $\dot{V}O_{2peak}$  was significantly correlated with quadriceps strength and endurance. Therefore, the function of the peripheral muscles may play a significant role in the decreased exercise capacity in children with CF (Ferrari et al., 2015; Gruet et al., 2017). In adults with CF, the strength of the quadriceps has been associated with aerobic capacity and lung function, with those with airway obstruction unable to

undertake continuous exercise due to lower extremity fatigue (Moorcroft et al., 2005). Across the age spectrum, Troosters et al. (2009) reported reduced skeletal muscle strength and endurance of those with CF, which was associated with decreased exercise capacity and subsequent clinical complications. This has been postulated to be associated with early neuromuscular activity deteriorations in the quadriceps, as observed following high-intensity aerobic exercise in chronic obstructive pulmonary disease (COPD) (Mador et al., 2000), although it should be noted that the pathophysiologic basis for this may differ between COPD and CF. Therefore, when designing, conducting, and evaluating exercise physiology studies in CF, it is important to not only account for age, but the associated changes in colonization and systemic inflammation, which can be markedly different across age groups in CF.

## Frequency of Pulmonary Exacerbations and Hospitalization

Progression of CF lung disease with age contributes substantially to the more frequent occurrence of pulmonary exacerbations and consequent hospitalizations. Importantly, during hospitalizations for acute pulmonary exacerbations, physical activity is reduced or even absent (Ward et al., 2013). This may be due, at least in part, to a lack of access to exercise facilities (due to infection control measures; Saiman et al., 2014), in addition to the pulmonary symptoms and associated treatments resulting from the exacerbation itself. When aerobic exercise is conducted during hospital admissions in children with CF, a substantially improved  $\dot{V}O_{2max}$  has been reported at discharge (Selvadurai et al., 2002), highlighting the importance of maintaining physical activity, irrespective of form (i.e., exercise), during hospitalization. The increased frequency of hospitalizations in adulthood likely contributes to declining physical activity during, and immediately following, these episodes. Any decline in physical activity levels could impact on the ability to complete activities of daily living, accelerate their decline in pulmonary function, and deteriorate QoL in those with CF (Wilkes et al., 2009; Dwyer et al., 2011).

## Nutrition

Nutrition is increasingly recognized as a key determinant of physical, mental and social health across the lifespan, especially for people with CF. Because CFTR is abundantly expressed in the exocrine pancreatic and biliary secretory system, mutations in *CFTR* can result in mucus obstruction in these organs and consequent exocrine pancreatic insufficiency (Rowe et al., 2005, 2016; Ratjen et al., 2015; Elborn, 2016). Thus, prominent GI manifestations of CF disease are evident, resulting in malabsorption of fat, secondary nutritional loss, malabsorption and deficiency of fat-soluble vitamins, chronic gastro-esophageal reflux, and susceptibility to recurrent small bowel obstruction. Progressive damage to the endocrine pancreas, resulting from protein accumulation (consequent to CFTR dysfunction) and precipitation within the pancreatic ducts that causes ductal destruction and ischemic damage (Laguna et al., 2010; Gibson-Corley et al., 2016), leads to the occurrence of CFRD in a majority

of individuals with CF, which is clinically distinct from traditional Type 1 and Type 2 diabetes mellitus in non-CF populations. The prevalence of CFRD increases across the lifespan, from 2% in children to 19% in adolescents and 50% in adults ( $\geq 18$  years) living with CF (Moran et al., 2010; Lewis et al., 2015); over 90% of pancreatic insufficient CF patients have CFRD by approximately 50 years old. Thus, CFRD is an important comorbidity, which becomes more prevalent with age and may contribute to the loss of exercise capacity. Exercise training has been shown to improve glycemic control in CF (Beaudoin et al., 2017), highlighting nutrition not only as a determinant of exercise capacity but also a potential area of benefit from regular exercise training.

CF-related diabetes is highly relevant given that malnutrition, weight loss, and lean muscle mass loss all contribute to exercise intolerance. Moreover, CFRD also negatively impacts pulmonary function and, consequently, morbidity and mortality (Chase et al., 1979; Milla et al., 2000; Pencharz and Durie, 2000; Borowitz et al., 2002; Sinaasappel et al., 2002; Milla, 2007; Moran et al., 2010; Lewis et al., 2015; Wolfe and Collins, 2017; Collins, 2018; Rozga and Handu, 2019). Lung bacterial clearance is negatively affected by hyperglycemia with hyperglycemia contributing to increases in inflammation and infection (Brennan et al., 2007; Hunt et al., 2014), which may partially account for these CFRD sequelae. CFRD and malnutrition may affect exercise capacity most directly by predisposing patients toward lean muscle mass loss, atrophy, and cachexia, particularly when nutritional needs are not adequately met. Furthermore, secondary morbidities associated with uncontrolled CFRD, such as neuropathy, nephropathy and retinopathy, can have additional deleterious effects on exercise capacity and QoL (Rosenecker et al., 2001; Laguna et al., 2010). Specifically, these deleterious effects are mediated by factors including, but not limited to: loss of balance, coordination, reflexes, and muscle weakness resulting from diabetic neuropathy; impaired blood pressure control, nausea, and vomiting, fatigue, peripheral edema and potentially progression to renal failure resulting from diabetic nephropathy; and blurred vision, glaucoma, cataracts, and macular edema resulting from diabetic retinopathy. Furthermore, underweight patients ( $\text{BMI} < 18 \text{ kg m}^{-2}$ ) can experience a sustained catabolic state resulting, in part, from prolonged malnutrition, ultimately resulting in chronic weight loss and difficulty maintaining or gaining weight, which negatively affects lung function. This clinical presentation can adversely affect lean muscle mass, contribute to muscular atrophy and cachexia, and inadequate bioenergetic stores to support exercise. Indeed, malnutrition is increasingly observed throughout maturity, with growing recognition that a low-fat free mass may be hidden by a normal, or elevated, BMI. Therefore, proper management of nutrition in CF (and of CFRD in individuals who present with it) is an important method to preserve or even enhance, exercise capacity. At present, however, exercise nutrition guidelines specific to the CF population are not available, and the most recent clinical nutrition guidelines (Turck et al., 2016; Rozga and Handu, 2019) do not address exercise nutrition in CF. We therefore suggest that future research seek to address the unique nutritional needs of individuals with CF during exercise.

While it is still important to consider the nutritional status in pediatric individuals with CF, the acute and chronic effects of malnutrition become more evident with age partially due to increasing chronic inflammation and infections, and therefore require more interventions to manage. Such interventions may include the use of percutaneous endoscopic gastric feeding tubes, peripherally inserted central catheters and/or ports, all of which may represent barriers to exercise. For example, anecdotal evidence suggests that patients with feeding tubes experience pain in the abdominal muscles during workouts, which may prevent them from engaging in core-strengthening exercises, or dynamic exercises which require significant activation of the core muscles. It is also important to consider the socioecological factors associated with such interventions, especially in young pre-pubertal and pubertal CF populations, which further predispose them to avoid physical activity.

## Biological Maturation

There is contradicting evidence regarding whether biological maturation, most commonly assessed through age at peak height velocity (PHV) and age at menarche, is delayed in youth with CF (Johannesson et al., 1997; Aswani et al., 2003; Bournez et al., 2012; Scaparrotta et al., 2012; Sands et al., 2015). Specifically, while the rate of PHV and final height are suggested to be lower in people with CF compared to their healthy peers, it is still equivocal whether the age of onset of puberty is also different in CF. Alterations in biological maturation could be the result of nutritional factors, the use of corticosteroids, and differences in sex hormone secretion (e.g., androgen secretion, which is known to be ergogenic), each of which have been suggested to be abnormal in CF. Regardless of etiology, in CF, delayed biological maturation may influence the evolution of exercise capacity as patients age, especially with respect to sex hormone secretion, which is an important determinant of exercise capacity (Kindermann et al., 1982; Ogawa et al., 1992; Sheel et al., 2004; Molgat-Seon et al., 2018), and should thus be considered in exercise studies of people with CF. In particular, sex hormone secretion may influence the onset of puberty, rate of maturity, and in non-CF children, sex differences in exercise capacity exist even in pre-pubescent children. Whether the CF population exhibits more or less pronounced sex differences in exercise capacity is, however, presently unknown. Limited evidence suggests that adult and adolescent females with CF have lower physical fitness compared with males when matched for disease severity, however, it is presently unknown whether this is related to pathophysiology, behavior, or both, and whether these findings may extend to youth (Eisenstadt et al., 2016). Differences in the onset and rate of maturity are also likely to have social implications with regards to peer perceptions that influence engagement in physical activity and exercise.

## Treatment Strategies for Children and Adults With CF

The advent of CFTR modulator therapies has revolutionized the treatment of CF. These therapies can be broadly divided into CFTR potentiators, which improve ion and fluid conductance

through the CFTR channel, and CFTR correctors, which aid in chaperoning mutated CFTR proteins during protein folding, thereby preventing endoplasmic reticulum (ER)-mediated degradation (Burgener and Moss, 2018; McElvaney et al., 2018; Habib et al., 2019). Currently approved therapies cover roughly 40% of individuals with CF, depending on age and mutation class (Cystic Fibrosis Foundation, 2017), and ongoing Phase 3 clinical trials of triple-combination therapies have the potential to extend that coverage to roughly 90% of people with CF (Davies et al., 2018; Holguin, 2018; Keating et al., 2018; Vertex Pharmaceuticals Incorporated, 2018a,b). Moreover, several ongoing trials are evaluating the use of CFTR modulator therapies in infants and toddlers (ranging from 0 to 24 months) with CF (Cystic Fibrosis Foundation, 2017). If successful, these trials will enable early initiation of highly effective CFTR modulator therapy, which has the potential to preserve pulmonary function at near-normal levels, and to arrest the annual decline in lung function that is currently characteristic of CF. This may, in turn, create a new “generation” of individuals with CF whose disease course is markedly different from the current CF population who may only have initiated CFTR modulator therapy after significant lung damage had already occurred. It is therefore postulated that such early intervention and preservation of lung function would substantially improve individuals’ exercise capacity by abrogating ventilatory dysfunction.

The age at which people with CF begin CFTR modulator therapy can have a profound effect on the disease course, and consequently, both morbidity and mortality. Most prominently, improvements in pulmonary function, as well as arresting the rate of decline in pulmonary function, greatly improve clinical outcomes, QoL, and the ability to exercise. The effects of CFTR modulator therapies on extra-pulmonary CF disease manifestations are less clear, and, in particular, it is still unclear whether CFTR modulators can improve diabetic status (Bellin et al., 2013; Tsabari et al., 2016; Thomassen et al., 2018; Kelly et al., 2019; Li et al., 2019). Nonetheless, CFTR modulator therapies have been shown to have a positive effect on weight gain and body mass index (Gelfond et al., 2017; Houwen et al., 2017; Gifford et al., 2018; Stallings et al., 2018), which might be beneficial for exercise capacity in malnourished or undernourished individuals with CF. Other therapies that help manage symptoms and sequelae of CF, such as inhaled hypertonic saline, airway clearance therapies, inhaled mucolytics, anti-inflammatories, and anti-microbials, may also vary in their use across the CF lifespan. For example, a recent investigation found that inhaled hypertonic saline could safely be used in infants with CF (Stahl et al., 2019), therefore enabling early intervention, which may aid in preserving lung function and slowing disease progression. It is clear, however, that when appropriate, the younger individuals with CF are when the treatment is initiated and the better these treatments are maintained, the better the clinical outcomes, morbidity and QoL (Davies et al., 2016; Rosenfeld et al., 2018). It also remains to be elucidated how the use of these therapies, and the age at which they are initiated, affects exercise capacity, physical activity levels and how to account for differences between individuals with CF in these regards when designing and analyzing exercise and physical

activity studies. However, based on the clinical efficacy of these therapies, it is likely that early initiation of CFTR modulator therapy, effective symptom management and infection control, and regular airway clearance therapy all enhance the ability of individuals with CF to exercise. Thus, future studies should aim to determine how and to what extent these therapies are beneficial for exercise, and also control for the use of these therapies when classifying the CF population.

## LIMITATIONS IN COMPARING CHILDREN AND ADULTS WITH CF

Overall, the progression of disease throughout the lifespan produces marked differences between children and adults with CF. It is therefore important to consider age-specific differences when interpreting exercise studies in these populations. Importantly, as those with CF age, they experience progressive declines in pulmonary function and increases in the incidence and prevalence of co-morbidities, both of which ultimately contribute to reduced exercise capacity. That is not to say that children with CF do not also possess reduced exercise capacity, however, the etiology of exercise impairment appears to be more complex in adults. Thus, when designing and conducting exercise studies in CF, it is important to ensure that appropriate descriptive measures which encompass these factors are employed, and that analyses control for, or account for, these factors. These types of analyses may require multivariate or mixed-model designs, thus it becomes important for researchers to include methodologists and biostatisticians in order to ensure both the design and analysis of exercise studies are appropriate and account for potential modifiers of exercise capacity that are unique to CF.

In addition to careful consideration of study design and analysis, the changes in pathophysiology across the lifespan in CF also highlights the need to conduct studies in both pediatric and adult populations including eras before and after initiation of CFTR modulator therapies, and not to simply use one population to draw conclusions about the overall CF population. It is clear that these populations are physiologically unique beyond simply being different in age. Therefore, understanding the differences in exercise physiology and pathophysiology in children vs. adults in CF may provide insight into how disease progression affects exercise intolerance. This information may aid in designing appropriate interventions and exercise prescription to reduce the decline in exercise capacity and improve patient health.

Presently, exercise training and testing are recommended for individuals with CF (Dwyer et al., 2011; Hebestreit et al., 2015; Radtke et al., 2017; Hebestreit et al., 2018; Urquhart and Saynor, 2018; Cox and Holland, 2019). However, specific recommendations for exercise prescription need to be developed further, and a recent systematic review on physical exercise training for CF concluded that the moderate quality of current evidence and small size, duration, and incomplete reporting of studies limits conclusions about the efficacy of physical exercise training for CF (Radtke et al., 2017). Future large, high-quality studies, including randomized controlled studies,

are necessary to determine the optimal training components (including exercise modality, frequency, intensity, and duration) for individuals with CF (Radtke et al., 2017). In addition, we recommend future studies account for the factors discussed in this review, in order to better control for differences in age, disease progression, nutritional status, and other factors which may impact exercise capacity.

## CONCLUSION

In summary, disease progression in CF from childhood through adolescence to adulthood leads to progressively more complex exercise intolerance and provides a unique model to study differences in exercise capacity across the lifespan of individuals with CF. When undertaking exercise studies in CF, it is critically important to consider factors such as declining pulmonary function, increased chronic colonization by increasingly pathogenic and drug-resistant bacteria, increased frequency and severity of pulmonary exacerbations, endocrine comorbidities, nutritionally related factors, and modulator therapy. In particular, accounting for how these factors ultimately influence the ability to exercise is important to better understand exercise impairments in individuals with CF. As the expected lifespan with CF continues to increase with advancements in therapies, it is also important to better understand how these factors evolve over the lifespan as individuals with CF age, and to clarify how adult and pediatric populations differ. It is therefore important to conduct studies in both pediatric and adult populations to account for age-related differences and

better understand *how* the evolution of CF disease impacts exercise (in)tolerance across the lifespan. Moreover, longitudinal studies of CF patients who begin CFTR modulator therapy early in life may also aid in understanding how arresting disease progression early in life affects exercise capacity over the lifespan of these patients.

## AUTHOR CONTRIBUTIONS

All authors contributed to the manuscript conceptions, preparation, critical revision, and final editing, and approved the final manuscript before submission.

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# Cardiopulmonary Capacity in Children During Exercise Testing: The Differences Between Treadmill and Upright and Supine Cycle Ergometry

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**Background/Hypothesis:** Cardiopulmonary exercise testing (CPET) is used in the assessment of function and prognosis of cardiopulmonary health in children with cardiac and pulmonary diseases. Techniques, such as cardiac MRI, and PET-scan, can be performed simultaneously with exercise testing. Thus, it is desirable to have a broader knowledge about children's normal cardiopulmonary function in different body postures and exercise modalities. The aim of this study was to investigate the effect of different body positions on cardiopulmonary function in healthy subjects performing CPETs.

**Materials and Methods:** Thirty-one healthy children aged 9, 12, and 15 years did four CPETs: one treadmill test with a modified Bruce protocol and three different bicycle tests with different body postures, sitting, tilted 45°, and lying flat (0°). For the bicycle tests, a 20-watt ramp protocol with a pedal frequency of 60 ± 5 rotations per minute was used. Continuous ECG and breath-by-breath  $\dot{V}O_2$  measurements was done throughout the tests. Cardiac structure and function including aortic diameter were evaluated by transthoracic echocardiography prior to the tests. Doppler measurements of the blood velocity in the ascending aorta were measured prior to and during the test. Prior to every test, the participants performed pulmonary function tests with maximum voluntary ventilation test.

**Results:** There is a significantly ( $p < 0.05$ ) lower peak  $\dot{V}O_2$  in all bicycle tests compared with the treadmill test. There is lower corrected peak  $\dot{V}O_2$  (ml kg<sup>-0.67</sup> min<sup>-1</sup>), but not relative peak  $\dot{V}O_2$  (ml kg<sup>-1</sup> min<sup>-1</sup>), in the supine compared with the upright bicycle test. There are no differences in peak stroke volume or cardiac output between the bicycle modalities when calculated from aortic blood flow. Peak heart rate decreases from both treadmill to upright bicycle and from upright bicycle to the supine test (0°).

**Conclusion:** There are no differences in peak cardiac output between the upright bicycle test and supine bicycle tests. Heart rate and corrected peak  $\dot{V}O_2$  are lower in the supine test (0°) than the upright bicycle test. In the treadmill test, it is a higher absolute and relative peak  $\dot{V}O_2$ . Despite the latter differences, we are convinced that both upright and supine bicycle tests are apt in the clinical setting when needed.

**Keywords:** cardiopulmonary exercise testing, children's physiology, spirometry, cardiac output, peak  $\dot{V}O_2$ , cardiopulmonary capacity, exercise testing



## INTRODUCTION

Cardiopulmonary exercise testing (CPET) is important in the assessment of function and prognosis of children's cardiopulmonary health in a clinical setting (Albouaini et al., 2007; Leclerc, 2017; Stephens, 2017). CPET is important in diagnostics of diseases such as exercise-induced laryngeal obstruction (EILO) (Johansson et al., 2015) and exercise-induced asthma or bronchial obstruction (EIA/EIB) (Anderson and Kippelen, 2012). In addition, CPET is used for monitoring function in children with congenital and acquired heart diseases (Carano et al., 1999; Hebert et al., 2014) as well as in children and adolescents with cerebral palsy (Verschuren and Balemans, 2015). A treadmill or an upright bicycle test is the most used exercise modality for CPET (Cooper, 1995).

There is a growing interest in conduction of simultaneous MRI-scanning, PET-scanning, or echocardiography during a CPET (Gusso et al., 2012). By conducting these supplementary investigations, one may achieve improved overview of cardiopulmonary health during the CPET (Chesler and Stein, 2004; Cullen and Pellikka, 2011; Barber et al., 2016). This is possible if the test subject is fixed and in a supine body position during the tests. In addition, a supine bicycle test will accommodate a better way to perform CPETs for patients who are unable to perform the test in an upright body position due to physical disabilities. Thus, advantages and disadvantages of different body positions during CPETs have been investigated in later years.

In the literature, there is no generally accepted definition of exercise capacity. However, peak oxygen consumption (peak  $\dot{V}O_2$ ) is commonly used as an indicator of physical fitness and exercise capacity (Shuleva et al., 1990; Armstrong et al., 1991; Figueroa-Colon et al., 2000; Fletcher et al., 2001; LeMura et al., 2001; American Thoracic Society, 2003; Johnston et al., 2005; Armstrong and Welsman, 2007).

Due to a relatively smaller cross section area of leg muscles (Bar-Or, 1983; Lexell et al., 1992), most children will have a lower peak  $\dot{V}O_2$  on a bicycle than on a treadmill. Previous studies have found a significant lower peak  $\dot{V}O_2$  when using the upright bicycle for CPET in young children (Armstrong et al., 1991; LeMura et al., 2001). These results correlate with similar research done in adults (Quinn et al., 1995; Egaña et al., 2006).

There is some knowledge of children and adolescent's normal cardiopulmonary response to CPET in the supine bicycle positions (Moller et al., 2009), but there is still a need to further investigate the cardiopulmonary response in different body positions.

This study anticipates that both the gravitation and the changes of hemodynamic conditions will affect the cardiopulmonary performance of the children in a negative direction in CPETs in the supine body posture. This study aimed to assess cardiopulmonary responses in CPETs in four different body postures, performed by healthy children. In addition, the differences in cardiopulmonary capacity in CPETs between age groups, ranging from children to adolescents was studied. This was to study differences in cardiopulmonary capacity before and during puberty, as it is known that both blood pressure and muscle mass change during puberty.

## METHOD

### Subjects

Three cohorts of children, born in 1999, 2002, and 2005, were included in the study. They were tested in 2014 and hence, at ages of 9, 12, and 15 years, respectively. Ten children in the two oldest age groups and 11 children in the youngest age group were included. The children were recruited from schools in Bergen, Norway. The subjects were excluded if they had history of smoking, cardiovascular or lung disease, family history of cardio-pulmonary diseases, and physical difficulties performing the tests. The test subjects served as their own controls in the comparison of the different test positions. Lean body mass was calculated with the Peters equation (Peters et al., 2011) for the 9- and 12-year-olds and the Boer equation for the 15-year-olds (Table 1; Boer, 1984).

Prior to the start of the study, the participants' parents signed informed written consents. The study was approved by the Regional Committee for Medical and Health Research in Western Norway (REK Vest 2014/1056).

### Exercise Test

The tests were performed in a randomized order and conducted at the Heart and Lung Test Laboratory at The Department of Child and Adolescents Medicine at Haukeland University Hospital, Bergen, Norway.

### Exercise Protocol

All participants performed the following tests: (1) treadmill CPET, (2) ergometer upright bicycle (sitting) CPET, (3) CPET in a supine bike tilted at 45° to the floor, and (4) CPET in a supine bike tilted at 0° to the floor (illustrated in Figure 1). The tests were conducted with at least 24 h between them. The test subjects were not instructed to refrain from their normal day activities or diet.

For the upright bicycle CPET, an electromagnetic resistance seeking ergometer bicycle (Corival, Lode B.V., Groningen, The Netherlands) was used. An electromagnetic resistance seeking tilt bicycle (Ergoselect 1,200, ergoline, Bitz, Germany) was used in the 0° and 45° bicycle tests. For all three bicycle tests, a 20-watt (W) ramp protocol (Buys et al., 2012; Armstrong and Welsman, 2019) was utilized. In the 20-W protocol, the resistance starts with a resistance of 20 W and increases with 2 W every fifth second, i.e., 20 W every minute. It was of importance to keep the length of the test within a timeframe so that the children did not get impatient. The participants were instructed to keep a speed of 60 rotations per minute (rpm) with a range between 55 and 65 rpm (Blanchard et al., 2018), which in our experience is the range that suits most children well.

The incremental peak treadmill (ELG 70, Woodway, Weil am Rhein, Germany) exercise test was executed with a modified and computerized Bruce protocol identical for all subjects (Duff et al., 2017). Speed and elevation were gradually increased every 60 s (see Supplementary Table S1), starting

**TABLE 1** | Calculated anthropometric values for the test groups.

	Height $\pm$ SD (cm)	Weight $\pm$ SD (kg)	BMI $\pm$ SD (kg/m <sup>2</sup> )	LBM $\pm$ SD (kg)	Number of participants
9-year-olds	139.5 $\pm$ 3.9	32.7 $\pm$ 4.6	16.8 $\pm$ 2.5	27.7 $\pm$ 2.5	11
12-year-olds	158.0 $\pm$ 6.6*	47.6 $\pm$ 8.8*	18.9 $\pm$ 2.2	38.7 $\pm$ 1.8*	10
15-year-olds	169.6 $\pm$ 6.9* <sup>†</sup>	55.7 $\pm$ 7.8* <sup>†</sup>	19.3 $\pm$ 1.8*	47.4 $\pm$ 5.7*	10

BMI, body mass index; LBM, lean body mass; SD, standard deviation. \*Significantly different from the 9-year-old calculated mean. <sup>†</sup>Significantly different from the 12-year-old calculated mean.



**FIGURE 1** | A 0° supine bicycle set-up illustrated, here with an adult volunteer. Informed, written consent for publication was obtained from the individuals in this photograph.

from an initial slow-walking phase (Cumming et al., 1978; Paridon et al., 2006; Clemm et al., 2012).

The exercise test was considered to have reached peak level when the participant indicated subjective exhaustion, preferably supported by a plateau in  $\dot{V}O_2$  or heart rate (HR) response (Paridon et al., 2006), or a respiratory exchange ratio (RER) higher than 1.1 (Rowland et al., 2017). Thus, the tests were not considered to be max tests, but rather peak tests. Direct breath-by-breath measurements were continuously monitored on the computer screen throughout the tests, to supervise the physiological response to exercise.

### Cardiopulmonary Exercise Testing Measurements

Prior to the start of each test, the participants did a forced spirometry test (Vmax 29, SensorMedics, Yorba Linda, CA, USA) and a maximum voluntary ventilation (MVV) test. Variables of gas exchange and airflow were measured breath-by-breath with a facemask (Hans Rudolph Inc., Kansas City, MO, USA) connected to Oxycon pro<sup>®</sup>JLAB 5.x. version 1.0 (Jaeger<sup>®</sup>, Care Fusion, San Diego, CA, USA) set up with standard layout Vmax29 cardiopulmonary exercise unit CPET computer program (SensorMedics, Yorba Linda, CA, USA).

The participants wore a mask with a digital TripleV-Volume sensor. Cardiopulmonary measurements were averaged per 10 s. The highest value determined during the last 60 s was used as peak value. Peak  $\dot{V}O_2$  was reported as ml min<sup>-1</sup>, ml kg<sup>-1</sup> min<sup>-1</sup>, or as a corrected value, which has been used by some groups (ml kg<sup>-0.67</sup> min<sup>-1</sup>) (Zapletal, 1987; Pettersen and Fredriksen, 2003; Wasserman et al., 2005).

During the CPET, a 12-lead ECG (GE CardioSoft V6.51, General electric company, Fairfield, CT, USA) recorded heart activity simultaneously, and blood pressure was measured every 2 min with SunTech Tango+ (SunTech Medical, Morrisville, NC, USA).

### Echocardiography

Echocardiography was performed using an ultrasound system with a 2.5-MHz transducer (Vivid E9, GE Vingmed, Canada). These measurements were performed by the same operator at every test. Prior to exercise testing, normal cardiac structure and function were confirmed. The internal aortic diameter was measured in the parasternal long- and short-axis at the valvular level. The aortic diameter was assumed to be constant throughout a cycle and during exercise as there is only a

small increase in aortic root size at the aortic valve annulus (Iskandar and Thompson, 2013).

Thereafter, Doppler measurements were obtained from the ascending aorta at every minute during the bicycle tests, including the post-exercise period (5 min). In the supine tests, this was done by continuous-wave (CW) Doppler measurements at the aortic valve visualized in the four-chamber view. In the upright bicycle test, the velocity of ascending blood was measured by a two-dimensional continuous-wave transducer positioned in the suprasternal notch pointing toward the origin of the aortic root. The measurement from the suprasternal notch has proved to give an accurate measurement of aortic blood flow (Lima et al., 1983). Stroke volume (SV) was assessed by standard Doppler echocardiographic methods and estimated as the product of the mean velocity-time integral (VTI). VTI was calculated tracing the velocity curve contour across the aortic valve, and the end-point of each contour was marked by aortic valve closure. The best-defined spectral curves out of three were averaged every minute (Quiñones et al., 2002; Vignati and Cattadori, 2017). Cardiac output (CO) was calculated by multiplying SV with heart rate (HR) (Leyk et al., 1994).

## Statistics

SPSS 25 (IBM Corporation, Armonk, NY, USA) was used for all statistical analyses. Groups were checked for normality. Group means, standard deviation, and ranges were calculated as appropriate.  $p < 0.05$  was considered significant.

A repeated-measures one-way ANOVA with Greenhouse-Geisser correction was used to investigate the mean differences in ventilatory and respiratory variables. *Post hoc* tests using the Bonferroni correction was used to further explore the differences in mean in the different body positions for main outcome variables.

For investigation of mean differences between age groups, a one-way ANOVA for independent measures was performed for each ventilatory and respiratory variable.

Using a paired sample *t*-test, the difference between rest values and peak values for the variables SV, HR, and CO for each test person in each age group was investigated.

## New Equipment

New and updated equipment, as well as software, had to be used for five subjects in the 9-year-olds group. This was due to renovation of the Children's Hospital during the test period. Replacements of equipment included the treadmill (bari-mill, Woodway, Weil am Rhein, Germany), Jaeger® Vyntus CPX Canopy metabolic cart and SentrySuite® respiratory software platform (Jaeger®, CareFusion, San Diego, CA, USA), custo cardio 100 12-lead ECG recorder (Custo Med, GmbH, Ottobrunn, Germany), and Tango M2 blood pressure system (SunTech Medical, Morrisville, NC, USA). According to international recommendations, our exercise facilities are set up with biological controls. In biological controls, no systematical or significant alterations in variables between old and new equipment have been detected. Similarly, there are no differences (Student's

*t*-test) in values between age-matched children tests on the new equipment compared with tests on the old equipment.

## RESULTS

Main anthropometric variables are shown in **Table 1**. It is not segregated based on gender in the groups as the participants function as their own control. Analysis shows significant mean differences in height between all age groups ( $139.5 \pm 3.9$ ,  $158.0 \pm 6.6$ ,  $169.6 \pm 6.9$  cm). The weight does not differ significantly between the 9- and 12-year-olds, but it does between the other groups ( $32.7 \pm 4.6$ ,  $47.6 \pm 8.8$ ,  $55.7 \pm 7.8$  kg). Also, only the mean difference in body mass index (BMI) between 9- and 15-year-olds is significant ( $16.8 \pm 2.5$ ,  $19.3 \pm 1.8$ ).

### Pulmonary Function Test

Forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV1) are higher in standing and sitting positions compared with the lying position ( $0^\circ$  supine) (FVC;  $3.2 \pm 1.2$  vs.  $3.0 \pm 1.2$  L), (FEV1;  $2.6 \pm 0.9$  vs.  $2.4 \pm 0.9$  L), and lower maximal voluntary ventilation (MVV) in  $45^\circ$  supine position than in the sitting and  $45^\circ$  tilted position ( $85 \pm 31$  vs.  $92 \pm 35$  L). All values are presented in **Table 2**.

### Breath-by-Breath Measurements

Peak  $\dot{V}O_2$ , reported in  $\text{ml min}^{-1}$  (absolute),  $\text{ml kg}^{-0.67} \text{min}^{-1}$  (corrected), and  $\text{ml kg}^{-1} \text{min}^{-1}$  (relative), are significantly higher during the treadmill exercise test compared with all three bicycle modalities ( $52.9 \pm 7.6$  vs.  $43.0 \pm 6.2$   $\text{ml kg}^{-1} \text{min}^{-1}$ ). It is not a difference between the bicycle modalities for relative peak  $\dot{V}O_2$ , but for absolute and corrected peak  $\dot{V}O_2$  there is a decrease from upright bicycle to the  $0^\circ$  supine test (**Table 3**). When accounted for lean body mass (LBM) it is a difference between the upright bicycle and the supine bicycle ( $53.7 \pm 7.9$  vs.  $51.0 \pm 6.6$ ) as well. There is a significantly higher mean minute ventilation (VE) and tidal volume (VT) during the treadmill test than in the other test modalities ( $85.5 \pm 27.7$  vs.  $69.8 \pm 26.4$ ) (**Table 4**).

Both when comparing the peak  $\dot{V}O_2$  given in  $\text{ml kg}^{-1} \text{min}^{-1}$  and peak  $\dot{V}O_2$  given in  $\text{ml kg}^{-0.67} \text{min}^{-1}$ , between the age groups, there is a significant increase between the

**TABLE 2** | Calculated means for spirometry variables obtained prior to the exercise tests.

	FEV1 (L) mean $\pm$ SD	FVC (L) mean $\pm$ SD	MVV (L/min) mean $\pm$ SD
Treadmill/standing	2.6 $\pm$ 0.9	3.2 $\pm$ 1.2	92 $\pm$ 35
UB/sitting	2.7 $\pm$ 0.9	3.4 $\pm$ 1.3	92 $\pm$ 33
45° SB	2.4 $\pm$ 0.8*†	3.1 $\pm$ 1.2†	85 $\pm$ 31*†
0° SB	2.4 $\pm$ 0.9*†	3.0 $\pm$ 1.2*†	81 $\pm$ 37*†

UB, upright bicycle; SB, supine bicycle; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 s; MVV, maximum voluntary ventilation.  $N = 30$ . \*Significantly different to treadmill test. †Significantly different to upright bicycle test.

**TABLE 3** | Calculated means for peak oxygen uptake in all test groups.

	Peak $\dot{V}O_2$ (ml min <sup>-1</sup> ) mean $\pm$ SD	Peak $\dot{V}O_2$ (ml kg <sup>-1</sup> min <sup>-1</sup> ) mean $\pm$ SD	Peak $\dot{V}O_2$ (ml kg <sup>-0.67</sup> min <sup>-1</sup> ) mean $\pm$ SD	Peak $\dot{V}O_2$ adjusted for LBM (ml kg <sup>-1</sup> min <sup>-1</sup> ) mean $\pm$ SD
Treadmill	2,373 $\pm$ 710	52.9 $\pm$ 7.6	184.0 $\pm$ 30.5	62.6 $\pm$ 7.8
Upright bicycle	2050 $\pm$ 683*	45.3 $\pm$ 7.3*	158.1 $\pm$ 31.2*	53.7 $\pm$ 7.9*
45° supine	2014 $\pm$ 714*	44.1 $\pm$ 7.6*	154.5 $\pm$ 33.7*	52.4 $\pm$ 8.5*
0° supine	1945 $\pm$ 622* <sup>†</sup>	43.0 $\pm$ 6.2*	150.2 $\pm$ 27.3* <sup>†</sup>	51.0 $\pm$ 6.6* <sup>†</sup>

peak  $\dot{V}O_2$ , peak oxygen consumption; LBM, lean body mass. N = 31. \*Significantly different to treadmill test. <sup>†</sup>Significantly different to upright bicycle test.

**TABLE 4** | Calculated means for ventilatory variables in all test groups.

	HR <sub>peak</sub> (beats/ min) $\pm$ SD	VE (L/min) mean $\pm$ SD	VT (L) mean $\pm$ SD	RER (CO <sub>2</sub> production/ O <sub>2</sub> uptake) mean $\pm$ SD
Treadmill	199 $\pm$ 8	85.5 $\pm$ 27.7	1.5 $\pm$ 0.1	1.18 $\pm$ 0.1
Upright bicycle	182 $\pm$ 10*	77.2 $\pm$ 30.9*	1.4 $\pm$ 0.1*	1.14 $\pm$ 0.1
45° supine	176 $\pm$ 15*	71.7 $\pm$ 28.0*	1.3 $\pm$ 0.1* <sup>†</sup>	1.13 $\pm$ 0.1
0° supine	173 $\pm$ 16* <sup>†</sup>	69.8 $\pm$ 26.4*	1.3 $\pm$ 0.1* <sup>†</sup>	1.12 $\pm$ 0.1*

HR, heart rate; VE, ventilation; VT, tidal volume. N = 31. \*Significantly different to treadmill test. <sup>†</sup>Significantly different to upright bicycle test.

9-year-olds and 12-year-olds in all four different test positions. However, there is no significant increase in corrected peak  $\dot{V}O_2$  from 12- to 15-year-olds. The different peak  $\dot{V}O_2$  means for all tests and age groups are shown in **Figure 2**. Results of breath-by-breath measurements are presented in **Tables 3, 4**.

## Cardiac Measurements

HR<sub>peak</sub> is higher in the treadmill tests compared with the bicycle tests (199  $\pm$  8 vs. 173  $\pm$  16 beats/min) (**Table 4**). Comparison of the three bicycle tests gives no differences in HR<sub>peak</sub>. There are small differences between the age group means for HR<sub>peak</sub>. Comparison of age groups shows significant changes in CO between 9- and 12-year-olds, as well as 9- and 15-year-olds in the three bicycle tests. No difference in HR between age groups.

There are no significant differences in SV at CO<sub>peak</sub> or in CO<sub>peak</sub> when comparing the different modalities (**Table 5**). There is a significantly higher mean HR at CO<sub>peak</sub> in the upright bicycle test than in the 0° supine bicycle test (180  $\pm$  10 vs. 171  $\pm$  17 beats/min).

Investigation of change in HR, CO, and SV from rest to CO<sub>peak</sub> shows significant increase of all values, except for SV from rest to peak in the upright bicycle for the youngest group (**Supplementary Figure S6**).

## DISCUSSION

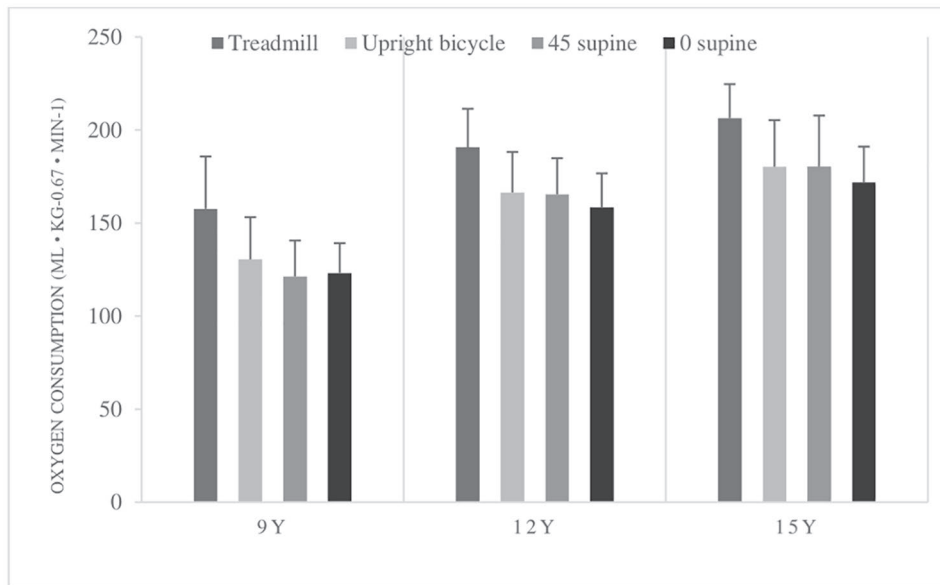
In this study, there is a lower absolute, corrected, and relative peak  $\dot{V}O_2$  in all bicycle tests compared with the treadmill

test as well as lower absolute and corrected peak  $\dot{V}O_2$  in the supine compared with the upright bicycle test. There are no differences in peak stroke volume or cardiac output between the bicycle modalities. Peak heart rate decreased from both treadmill to upright bicycle and from upright bicycle to the supine tests.

Other studies have previously reported that the differences in children's peak  $\dot{V}O_2$  with altered body position to be smaller when adjusted for lean body mass (LBM) (Vinet et al., 2003; Eiberg et al., 2005). On the contrary, this study shows an additional change in peak  $\dot{V}O_2$  when adjusted for LBM, where the results from the 0° supine bicycle is significantly lower than the upright bicycle test. The same result is present in the absolute and corrected peak  $\dot{V}O_2$  results, but not the relative peak  $\dot{V}O_2$ . Correction with exponential factor of 0.67 has been claimed to express the peak  $\dot{V}O_2$  of adolescents more correctly. This has been discussed to be due to that the increase in peak  $\dot{V}O_2$  in children and adolescents is masked by the increase in body mass with age (Pettersen and Fredriksen, 2003). With the exponential correction factor, there is a significant difference in mean peak  $\dot{V}O_2$  when comparing the upright bicycle and 0° supine bicycle, which was not present when only expressing the peak  $\dot{V}O_2$  relative to body weight. This may strengthen the argument that a correction factor is needed in evaluation of peak  $\dot{V}O_2$  in children and adolescents.

## Pulmonary Function Tests

As previously reported in adults, this study also found lower spirometry values in the supine position than in the sitting and standing positions (Vilke et al., 2000; Naitoh et al., 2014). However, there is no decrease in FEV1 and FVC from the standing to the sitting posture (Pierson et al., 1976). A systematic review of pulmonary function tests with different body positions is showing somewhat conflicting results in the literature regarding the effect of different body positions (Katz et al., 2018). But in general, FEV1, FVC, FRC, maximal expiratory pressure (PE<sub>max</sub>), maximal inspiratory pressure (PI<sub>max</sub>), and peak expiratory flow (PEF) values were higher in more erect positions. For subjects with tetraplegic SCI, FVC and FEV1 were higher in supine vs. sitting position. In our study, MVV is lower in the supine than in the sitting position in accordance with results shown in adults by Vilke et al. (2000).



**FIGURE 2 |** Peak oxygen consumption (peak  $\dot{V}O_2$ ) ( $\text{ml kg}^{-1} \text{min}^{-1}$ ) means with standard deviations in all body postures in all age groups. 9Y = 9-year-olds, 12Y = 12-year-olds, 15Y = 15-year-olds. There is a significant higher peak  $\dot{V}O_2$  in the treadmill test compared with the bicycle tests, and higher in the upright bicycle test compared to the 0° supine test. Peak  $\dot{V}O_2$  was higher in the 12- and 15-year-olds in all tests compared to the 9-year-olds. No differences in peak  $\dot{V}O_2$  between 12-year-olds and 15-year-olds.

**TABLE 5 |** Group means of cardiac output (CO), heart rate (HR), and stroke volume (SV) with standard deviations.

	SV at $CO_{\text{peak}}$ (ml/beat) ± SD	HR at $CO_{\text{peak}}$ (beats/min) ± SD	$CO_{\text{peak}}$ (L/min) ± SD
Upright bicycle	95.5 ± 30	180 ± 10	17.1 ± 5.6
45° supine	98.0 ± 36	174 ± 15	17.2 ± 6.8
0° supine	97.9 ± 30	171 ± 17*	16.8 ± 5.6

\*Significantly different from upright bicycle value.

### Ventilation During Exercise

Studies of ventilatory function during exercise have shown conflicting results. LeMura et al. reported a trend but no significant differences in VE in their 5- and 6-year-olds when comparing treadmill and upright bicycle (LeMura et al., 2001). Boileau et al. studied a group of 11- to 14-year-old boys, and did not find any differences in VE when achieved on a treadmill or an ergometer bicycle (Boileau et al., 1977).

### Age-Group Differences in Cardiopulmonary Capacity

When comparing the test modalities, there were significant differences for most of the ventilatory and respiratory variables. There is also a higher CO in the 12- and 15-year-olds compared with the 9-year-olds. This appears to be due to higher SV in the oldest groups, as the HR was without any significant changes throughout the age groups. Stroke

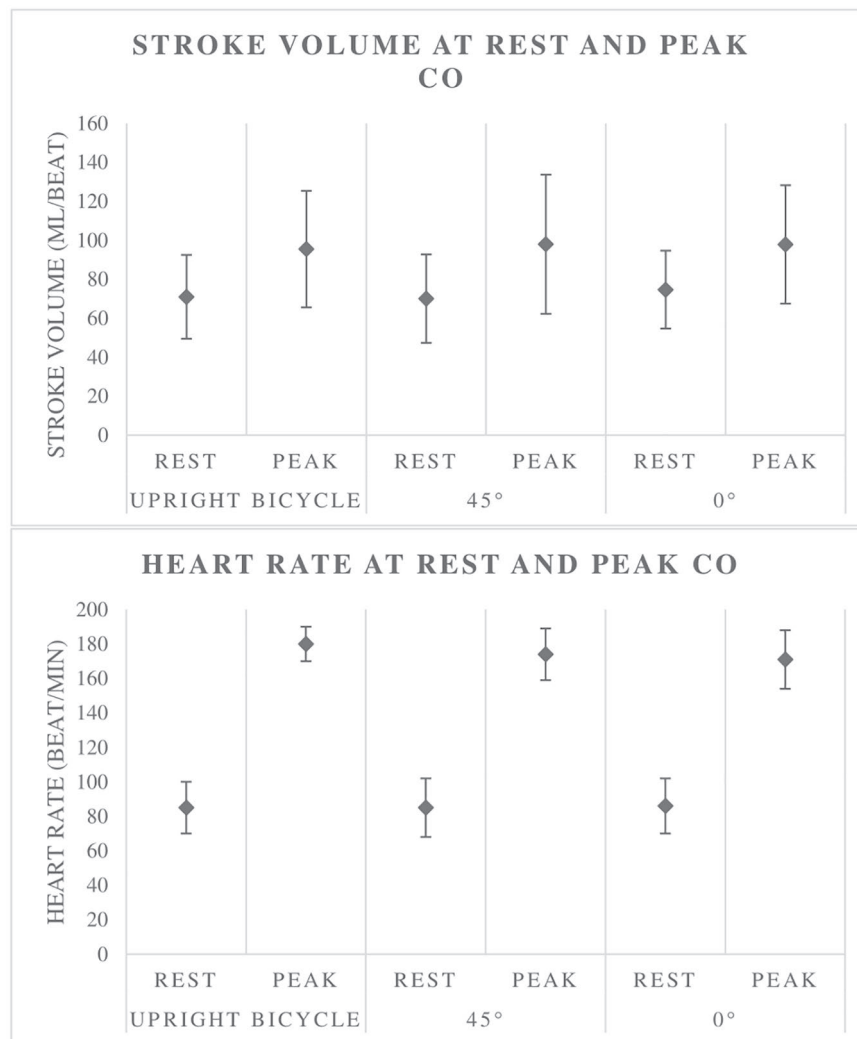
volume is related to body surface area, which explains our results well.

An interesting aspect of the between-group analysis is that the differences in peak  $\dot{V}O_2$ , when corrected for weight, is less than the absolute value and when using the correction factor of 0.67. However, when correcting with a power of  $-0.67$  instead of  $-1$  as used by some groups, there is still no increase in peak  $\dot{V}O_2$  from 12- to 15-year-olds. A possible explanation is that the relative increase in weight compared with the increase in peak  $\dot{V}O_2$  is greater, and thus gives a smaller increase in weight-adjusted peak  $\dot{V}O_2$ . This has been suggested in earlier studies as well (Krahenbuhl et al., 1985; Pettersen and Fredriksen, 2003).

As there is a lack of increase in relative and corrected peak  $\dot{V}O_2$ , as well as CO, between 12- and 15-year-olds, our results indicate that most changes in peak  $\dot{V}O_2$  and CO occur between 9 and 12 years of age.

### Cardiopulmonary Measurements

In adults, linearity between CO and  $\dot{V}O_2$  has been shown throughout exercise tests (Thadani and Parker, 1978; De Cort et al., 1991). Similar linearity is visualized in **Supplementary Figures S1–S3**. It was previously suggested that the linearity might be affected by fitness level (Beck et al., 2006; Trinity et al., 2012), and a study has shown that this linearity ceases at a certain point in the exercise, where CO starts decreasing as peak  $\dot{V}O_2$  is approached (Stringer et al., 2005). Figures in the supplementary show (**Supplementary Figures S1–S3**) a similar tendency of a flat, or decreasing, CO toward the end is observed. This is however not present in all tests.



**FIGURE 3 |** Stroke volume and heart rate at rest and at peak cardiac output (CO). Illustrates the increase in CO with exercise as well as little difference between the different tests.

There is an absence of differences in relative peak  $\dot{V}O_2$  between bicycle CPETs in three different body positions. This is supported by the findings on CO, which is unchanged between the three different bicycle tests. However, there are some results suggesting a change in hemodynamics in the supine test. HR at  $CO_{peak}$ , as well as absolute peak  $\dot{V}O_2$ , is significantly lower in the supine tests than in the upright bicycle test (Tables 3–5). SV<sub>peak</sub> also shows a somewhat higher mean value in the supine position, but this is not significant (Table 5, Figure 3). These findings may be explained by a higher preload in the supine position, a body posture which will give less work toward gravitational forces, thus improving venous backflow. Thus, the same CO was obtained at a lower HR.

Studies have investigated the hemodynamics with change of body position. Higginbotham and coworkers have reported individual differences with increase of end-diastolic volume

in a supine position, which partly can explain the variation of SV changes (Higginbotham et al., 1986). It is also subjected that the decrease in intrathoracic pressure in an upright position might cause a shift of blood to the legs, and thus a reduced heart volume. Other hemodynamic considerations include a higher ventricular filling in a supine position, and a rise in HR and vascular resistance in an upright posture (Bevegard et al., 1960; Ray and Cureton, 1991).

Thadani et al. discussed that normal adult subjects have individual variation in stroke volume response to exercise in supine body positions, as studies have found conflicting results on this (Bevegard et al., 1960; Ross et al., 1965; Thadani and Parker, 1978). Also, CO has shown different results in studies, which underlines the variation in the normal population (Bevegard et al., 1960). Nevertheless, due to a lack of agreement of changes in SV and CO with altered body position, one can argue that the hemodynamics during exercise is not completely understood.

## Strength and Weaknesses

This study has mapped a small number of individuals and their performance in four different CPET modalities. Every participant conducted all four tests, which gave a strong basis for intra-individual comparisons. No segregation was made based on gender in the test groups. This might mask differences in peak values as sex differences have been reported in other studies. However, the main purpose of the test subjects was to serve as their own control when changing body posture and not to define absolute reference values. Conducting echocardiographic measurements, including VTI measurements, are challenging because of movement artifacts, especially at the end of the tests. The test personnel were the same throughout the project, providing less room for methodical human errors.

## CONCLUSION

This study does not find any differences in relative peak  $\dot{V}O_2$  or peak cardiac output between the upright bicycle test and the supine bicycle tests. When correcting relative peak  $\dot{V}O_2$  with an exponential factor of 0.67, we find a lower peak  $\dot{V}O_2$  in the supine test ( $0^\circ$ ) compared to the upright bicycle test. Heart rate and absolute peak  $\dot{V}O_2$  are lower in a supine than the upright bicycle test. In the treadmill test, higher absolute, corrected, and relative peak  $\dot{V}O_2$  values are found compared to all bicycle tests. Our study supports the view that both upright and supine bicycle tests are apt in the clinical setting when needed.

## DATA AVAILABILITY STATEMENT

Datasets are available on request. The anonymous raw data supporting the conclusions of this manuscript will be made available by the authors, depending on individual approval from the Data Protection Officer at Haukeland University Hospital.

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## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Regional Etisk Komité (REK Vest 2014/1056). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the participants for the publication of any identifiable information or images.

## AUTHOR CONTRIBUTIONS

AB and GG conceived of the presented idea, developed the theory, and supervised the findings of this work. TF and MA carried out the experiment. TF performed the analysis and interpretation and wrote the manuscript with support from MA, AB, and GG.

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# Multidirectional Plyometric Training: Very Efficient Way to Improve Vertical Jump Performance, Change of Direction Performance and Dynamic Postural Control in Young Soccer Players

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The aim of the study was to assess the effects of multidirectional plyometric training (MPT) on vertical jump height, change of direction performance (CODP), and dynamic postural control (DPC) in young soccer players. Twenty-eight young male soccer players were randomly assigned to an experimental group (EG,  $n = 14$ ; age:  $11.8 \pm 0.4$  years) and a control group (CG,  $n = 14$ ; age:  $11.6 \pm 0.5$  years). The EG introduced 8-week MPT, two days per week into their in-season training, while CG continued training without change. Measurements of vertical jump height, CODP, and DPC were completed at the beginning and end of the 8-week MPT. A significant group  $\times$  time interaction was observed for Squat-Jump ( $p < 0.05$ ), for Counter-Movement Jump ( $p < 0.05$ ), and for CODP test ( $p < 0.05$ ). In addition, a significant group  $\times$  time interaction was observed for DPC in seven axes for the dominant- (anterior, lateral, postero-lateral, posterior, postero-medial, medial, and antero-medial;  $p < 0.05$  for all) and in seven axes for the non-dominant- (anterior, antero-lateral, lateral, posterior, postero-medial, medial and antero-medial;  $p < 0.05$  for all) legs. The rest of the axes of both legs did not show any significant group  $\times$  time interaction ( $p > 0.05$ ). In conclusion, incorporating MPT into the in-season regimen of young male soccer players improved performance of various indices related to soccer activity (i.e., vertical jump height, CODP, and DPC). MPT has the potential to be appealing to coaches, as it requires little time while yielding valuable results in the physical preparation of young soccer players.

**Keywords:** muscle power, stretch-shortening-cycle, agility, balance, adolescents

## INTRODUCTION

Soccer is an intermittent sport in which the capacity of the player to perform actions such as sprinting, jumping, kicking and changing direction have a major influence on match performance (Stolen et al., 2005). Research has characterized the intermittent activity pattern of youth soccer players. Rebelo et al. (2014) reported a match total distance covered of  $6311 \pm 948$  m, of which 12%

were performed at high-intensity activities. Likewise, Castagna et al. (2003) showed that 9% of the total time played ( $3789 \pm 109$  s) were performed at high-intensity activity with similar duration (11%) spent standing still.

Many soccer-specific movements are characterized by high-velocity concentric and eccentric muscular contractions, involving muscular stretch-shortening cycle (SSC). In that regard, plyometric training (PT) is known to improve the ability of soccer players to cope with the game demands. More specifically, PT can develop the ability of soccer players improving their neuromuscular control by promoting anticipatory postural adjustments (Gantchev and Dimitrova, 1996; Asadi et al., 2015). Indeed, balance and stability challenges during PT can result in proactive and/or feed-forward adjustments that would adjust appropriate muscles contractions before pitch-contact/landing (Marigold and Patla, 2002; Paillard et al., 2005). Furthermore, PT seems to result in improved sensitivity of afferent feedback pathway during exercise (Borghuis et al., 2008). Bedoya et al. (2015) recently suggested that the observed gains in performance could reflect various neuromuscular adaptations, such as an increased neural drive, improved inter-muscular coordination, changes in muscle size and architecture, and/or changes in single-fiber mechanics, as well as changes in muscle-tendon mechanical-stiffness (Markovic and Mikulic, 2010). Therefore, all these improvements could increase the performance and also potentially minimize the risk of injuries in soccer players (Chimera et al., 2004). Moreover, PT is attractive to soccer coaches, because it requires little space or equipment, and uses short periods from the training sessions' time (Ramirez-Campillo et al., 2014).

PT can take the form of vertical or horizontal exercises, or a combination of both. The SSC contributes less to horizontal than to vertical jumping performance, because a vertical loading of the musculo-tendinous unit accumulates greater elastic energy during movement the excentric phase (Kawamori et al., 2013). Ramirez-Campillo et al. (2015) showed that a combination of vertical and horizontal jumping yielded greater gains for both strength and balance of the players than vertical or horizontal jumping performed separately.

The soccer is multidirectional sport (Taylor et al., 2017) and consequently the adequate physical preparation must meet such a characteristic. The study of Geoff et al. (2016) in Badminton has shown that resistance and multidirectional PT among badminton players has improved their specific physical qualities in this multi-directional sport. Consequently, as soccer is also a multi-directional sport, one may hypothesize that the optimal preparation of soccer players should also include multidirectional exercises.

Although some authors have argued that PT is detrimental to young players, increasing the risk of injury and stunting growth, such issues are easily avoidable if an age-appropriate regimen is followed (Behm et al., 2008; Faigenbaum et al., 2009). Current guidelines for youth (Behm et al., 2008; Faigenbaum et al., 2009) require that PT be carried out on 2–3 non-consecutive days per week for 8–10 weeks, and that the volume of training should be relatively low (generally approximately 60 foot contacts per session, and increasing to no more than 120 foot

contacts per session) (Ramirez-Campillo et al., 2013). In terms of efficacy, a recent study in young male soccer players (Chaabene and Negra, 2017) indicated that over 8 weeks of training, a high-volume program had no greater effects than a low-volume program on sprint time, change of direction performance (CODP), or jumping performances. The effectiveness of low-volume training, resulting as effective a high-volume plyometric program in young soccer players could be explained by the intermittent nature of young soccer players' activity during the matches. Indeed, the total match time is characterized by 11% of standing time and 9% of high-intensity activity (Castagna et al., 2003). The relatively low amount of high-intensity activity and the capacity in young players to recover faster from intense exercise than adults (Hebestreit et al., 1993) could potentially explain the effectiveness of low-volume polymeric program in young soccer players.

An optimal training regimen for young soccer players should also enhance dynamic postural control (DPC) (Paillard et al., 2006), minimizing the risk of lower-extremity injuries (Zech et al., 2010) through an increased contraction force in the lower extremity muscles (Myer et al., 2006), and/or enhancement of proprioception and neuromuscular control (Hewett et al., 2002). The effects of multidirectional plyometric training (MPT) on the DPC of young soccer players are as yet unknown, but a favorable adaptation might be expected given that the increases in the lower-extremity muscle power can be associated with improvements in postural performance since there would be a relationship between very early rapid torque of the leg extensor muscles and performance postural in young subjects (Paillard, 2017b).

The vertical jump performance, CODP, and DPC are soccer specific physical qualities and their improvement might improve soccer performance in young soccer players (Meylan and Malatesta, 2009; Ramirez-Campillo et al., 2015; Negra et al., 2017). Therefore, the main objective of this research was to study the effects of 8-week in-season MPT on the vertical jump performance, CODP, and DPC of young soccer players. We hypothesized that MPT would enhance these indices of physical performance abilities in young soccer players.

## MATERIALS AND METHODS

### Participants

All procedures were approved by the Manouba University Institutional Review Committee for the ethical use of human participants and were conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants and their parent after they had received both verbal and written explanations of the experimental protocol and its potential risks and benefits. All participants were ensured that they could withdraw from the trial at any time without any penalty.

The participants were 28 male soccer players from a soccer academy (all playing positions except goalkeeper) (**Table 1**). They were randomly assigned to an experimental group (EG;  $n = 14$ ) and a control group (CG;  $n = 14$ ). None reported any

recent history of hip, knee or ankle injury, or other pathological conditions affecting their lower limbs.

## Experimental Design

The study was performed over an 8-week period during March and April. The PT program was started at the 18th week of the training season starting in September and finishing in June. Two sessions for familiarization with the testing were held 2 weeks before the beginning of experimentation. Data were collected before modification of the regimen and after the EG had completed the 8-week period of MPT. Initial and final measurements were made at the same time of day (4 to 6 pm) (Chtourou et al., 2012) and under the same experimental conditions at least 3 days after the most recent competition, and 5 days after the last MPT session.

Testing was integrated into the weekly training schedule. A standardized warm-up, including progressive running and dynamic stretching exercises (Haddad et al., 2014), was performed before each testing session. Experimental tests were performed by the same investigator in a fixed order over 3 consecutive days. On day 1, anthropometric measurements were completed, followed by vertical jumping height tests (i.e., squat jump: SJ and countermovement jump: CMJ). Day 2 was devoted to assess the agility from COD obtained during T-test, and on day 3 participants undertook the DPC test to evaluate balance.

## Anthropometric Measurements

Height was measured using a graduated measuring rod (version 216, Seca® Hamburg, Germany), and players were weighed with light clothes and barefoot, using an EKS® Focus 9800 scale (Gislaved, Sweden). Body mass index (BMI) ( $\text{kg}/\text{m}^2$ ) was calculated as body mass (kg) divided by the square of height ( $\text{m}^2$ ). Moreover, an individual maturity index was calculated from peak height velocity. The method of predicting years from peak height velocity (PHV) was as follows:  $\text{PHV} = -7.999994 + [0.0036124 \times \text{age} \times \text{height}]$  (Moore et al., 2015). Finally, the dominant leg was determined according to the method of van Melick et al. (2017).

## Vertical Jump Height Assessments

Jump heights during SJ and CMJ were assessed using an infrared photocell connected to a digital computer (Optojump System, Microgate®, Bolzano, Italy). This allowed the measurement of contact time and flight time ( $t_f$ ) with a precision of 1/1000 s. The jump height (h) in meter was calculated as  $h = g \times (t_f)^2/8$  as indicated by Lehance et al. (2005). For the SJ, participants placed their hands on their hips in a semi-squat position, with the knees flexed to  $\sim 90^\circ$  (Ghoul et al., 2019). CMJ started from a standing position with the hands on the hips; at the verbal signal, a downward movement was made to a knee angle of  $\sim 90^\circ$ , and the participant then pushed upward as rapidly as possible (Ghoul et al., 2019). For both jumps, three trials were made, and the average used for analysis. For the SJ and CMJ, intraclass coefficients (ICCs) for 3 repeated trials before and after intervention period were 0.95 and 0.94; the 95% confidence interval (CI) were of 0.92–0.96 and also 0.92–0.96, respectively.

## Time to Complete Change of Direction Test

On the second test day, to assess the CODP, we have used a valid, reliable and sensitive test in soccer (Sporis et al., 2010): the T-test was administered as described by Semenick (1990). One cone was placed 9.14 m ahead of the starting cone and 2 further cones were placed 4.57 m on either side of the second cone. Times were recorded using an electronic timing gate (Photocells, Microgate®, Bolzano, Italy). The photoelectric cells were placed at 0.7 m height from the floor. Participants sprinted forward 9.14 m to the first cone, touching its tip with their right hand, next shuffled 4.57 m left to the second cone, touching it with their left hand, then shuffled 9.14 m right to the third cone, touching it with their right hand, next shuffled 4.57 m left to the middle cone, touching it with their left hand before finally running backward to the starting line of 2 m wide. Trials were deemed unsuccessful if participants failed to touch a designated cone, crossed their legs while shuffling or failed to face forward at all times. All participants performed familiarization trials before undertaking three definitive trials separated by one minute recovery intervals. The average of the three trials has been used for analysis. The ICC for 3 repeated trials before and after intervention period were 0.96 and with 95% CI ranging between 0.94 and 0.97, respectively.

## Dynamic Postural Control

During the third test day, the star excursion balance test assessed DPC. This unilateral balance test integrates a single-leg stance with maximum reach of the opposite leg (Hertel et al., 2000). Participants stood in the center of a grid, with 8 lines radiating at  $45^\circ$  increments from the center of the grid [antero-lateral (AL), anterior (A), antero-medial (AM), medial (M), postero-medial (PM), posterior (P), postero-lateral (PL), and lateral (L) (Figure 1)]. Reach distances were normalized by dividing each excursion distance by the participant's leg length and multiplying the value obtained by 100. The average of the three trials has been used for analysis. ICCs for 3 repeated trials before and after intervention period of the 8 directions using dominant and non-dominant legs ranged from 0.90 to 0.94, with 95% CI ranging between 0.88 and 0.97, respectively.

## Multidirectional Plyometrics Training (MPT)

The EG integrated 20–25 min MPT sessions into their regular training sessions on every Tuesday and Thursday throughout the 8-week intervention period, whereas the controls continued with their standard soccer training in season (Tables 2, 3).

The MPT was always supervised by the same coach, and there were no injuries resulting from the training sessions during the intervention.

## Statistical Analysis

Statistical analyses were carried out using the SPSS 20 program for Windows (SPSS®, Chicago, IL, United States). Before using parametric statistics, the normality of data was confirmed, using the Shapiro-Wilk test. The sphericity was checked by the Mauchly test and, when it was not met, the significance of F-ratios was

**TABLE 1** | Participants characteristics before the intervention program (mean  $\pm$  SD).

Variable	Control group	Experimental group
Age (yr)	11.6 $\pm$ 0.5	11.8 $\pm$ 0.4
Body height (m)	1.42 $\pm$ 0.04	1.43 $\pm$ 0.10
Body mass (kg)	34.2 $\pm$ 3.6	36.5 $\pm$ 5.1
Leg length (m)	0.85 $\pm$ 0.03	0.86 $\pm$ 0.10
Body mass index (kg/m <sup>2</sup> )	16.8 $\pm$ 1.2	17.8 $\pm$ 1.8
Time to predicted PHV (yr)	-2.0 $\pm$ 0.4	-1.9 $\pm$ 0.3
Soccer experience (yr)	3.8 $\pm$ 0.4	3.6 $\pm$ 0.5

No inter-group differences are statistically significant ( $p > 0.05$ ). PHV, peak height velocity.

**TABLE 2** | Soccer training program during the week.

Session 1 and Session 2	Session 3
Warm up	Warm up
General	General
Specific	Specific
Training	Training
Training of fast footwork	Training of fast footwork
Technical skills and moves (easy/difficult)	Position games with ball (small/big)
Technical skills and moves (easy/difficult)	Tactical games with various objectives
Cool down exercises	Cool down exercises

adjusted according to the Greenhouse-Geisser procedure or the Huynh-Feldt procedure. The test-retest reliability of measures was assessed using intra-class correlation coefficients (ICCs) (Vincent, 1995). Descriptive values are presented as means and standard deviations. The effects of the intervention were assessed using a 2-way analysis of variance with repeated measures: (EG vs. CG)  $\times$  (before vs. after). To evaluate within-group pre-to-post performance changes, paired sample *t*-tests were applied. Effect sizes (ES) were determined by converting partial eta-squared values to Cohen's *d* with the Excel spreadsheets. According to Cohen (1988) ES can be classified as small ( $0.00 \leq d \leq 0.49$ ), medium ( $0.50 \leq d \leq 0.79$ ), or large ( $d \geq 0.80$ ). The alpha level of significance was set at  $p < 0.05$ .

## RESULTS

During our experiment, control group participants were trained for  $23.64 \pm 0.85$  soccer training session and the experimental group for  $23.57 \pm 0.75$  soccer training session and  $15.71 \pm 0.61$  PT session. Furthermore, no significant inter-group difference was noted for anthropometric data at baseline (Table 1).

### SJ, CMJ, and T-Test Performance

Descriptive values of Pre and Post tests for SJ, CMJ and T-Test are presented in Table 4. ANOVA demonstrated significant group  $\times$  time interaction for SJ, CMJ, and T-Test ( $p < 0.05$  for all). For SJ, paired *t*-test demonstrated significant progress for EG however, no significant progress for CG (EG:  $p < 0.05$ ,

$\Delta = 11.14\%$ ; CG:  $p = 0.33$ ,  $\Delta = 0.42\%$ ). For CMJ, paired *t*-test demonstrated significant progress for EG however, no significant progress for CG (EG:  $p < 0.05$ ,  $\Delta = 9.91\%$ ; CG:  $p = 0.10$ ,  $\Delta = 0.42\%$ ). For T-test, paired *t*-test demonstrated significant progress for EG however, no significant progress for CG ( $p < 0.05$ ,  $\Delta = -3.07\%$ ; CG:  $p = 0.19$ ,  $\Delta = 0.42\%$ ).

### DPC on the Dominant Leg Performance

Descriptive values of Pre and Post tests of DPC on the dominant leg performance are presented in Table 5. ANOVA demonstrated significant group  $\times$  time interaction for seven axes (anterior, lateral, postero-lateral, posterior, postero-medial, medial and antero-medial;  $p < 0.05$  for all). However, no significant group  $\times$  time interaction for antero-lateral axis ( $p = 0.50$ ). Paired *t*-test demonstrated significant progress in EG, however, no significant change in CG: anterior (EG:  $p < 0.05$ ,  $\Delta = 3.79\%$ ; CG:  $p = 0.43$ ,  $\Delta = -0.26\%$ ); lateral (EG:  $p < 0.05$ ,  $\Delta = 2.18\%$ ; CG:  $p < 0.05$ ,  $\Delta = -0.92\%$ ); postero-lateral (EG:  $p < 0.05$ ,  $\Delta = 4.93\%$ ; CG:  $p = 0.08$ ,  $\Delta = -0.59\%$ ); posterior (EG:  $p < 0.05$ ,  $\Delta = 10.19\%$ ; CG:  $p = 0.76$ ,  $\Delta = -0.11\%$ ); postero-medial (EG:  $p < 0.05$ ,  $\Delta = 6.39\%$ ; CG:  $p = 0.85$ ,  $\Delta = 0.07\%$ ); medial (EG:  $p < 0.05$ ,  $\Delta = 5.93\%$ ; CG:  $p = 0.11$ ,  $\Delta = -0.51\%$ ); antero-medial (EG:  $p < 0.05$ ,  $\Delta = -3.79\%$ ; CG:  $p = 0.43$ ,  $\Delta = -0.26\%$ ).

### DPC on the Non-dominant Leg Performance

Descriptive values of Pre and Post tests of DPC on the non-dominant leg performance are presented in Table 6. ANOVA demonstrated significant group  $\times$  time interaction for seven axes (anterior, antero-lateral, lateral, posterior, postero-medial, medial and antero-medial;  $p < 0.05$  for all). However, no significant group  $\times$  time interaction for postero-lateral axis ( $p = 0.54$ ). Paired *t*-test demonstrated significant progress in EG, however, no significant change in CG: anterior (EG:  $p < 0.05$ ,  $\Delta = 7.25\%$ ; CG:  $p = -0.12$ ,  $\Delta = -0.96\%$ ); antero-lateral (EG:  $p < 0.05$ ,  $\Delta = 2.80\%$ ; CG:  $p = 0.07$ ,  $\Delta = -0.58\%$ ); lateral (EG:  $p < 0.05$ ,  $\Delta = 1.17\%$ ; CG:  $p = 0.08$ ,  $\Delta = 0.76\%$ ); posterior (EG:  $p < 0.05$ ,  $\Delta = 4.96\%$ ; CG:  $p = 0.85$ ,  $\Delta = -0.04\%$ ); postero-medial (EG:  $p < 0.05$ ,  $\Delta = 3.89\%$ ; CG:  $p < 0.05$ ,  $\Delta = -0.98\%$ ); medial (EG:  $p < 0.05$ ,  $\Delta = 5.89\%$ ; CG:  $p < 0.05$ ,  $\Delta = -0.05\%$ ); antero-medial (EG:  $p < 0.05$ ,  $\Delta = 4.86\%$ ; CG:  $p = 0.43$ ,  $\Delta = -1.21\%$ ).

## DISCUSSION

The main objective of the present study was to investigate the effects of low volume MPT on the vertical jump performance, CODP, and DPC in young soccer players. The result shows that the MPT enhanced three important qualities that are relevant to the performance of young male soccer players (i.e., vertical jump height, CODP, and DPC).

Previously to the present work, several studies have examined the effects of unidimensional PT in young male soccer players; these have used high volume programs lasting from 8 to 16 weeks (Diallo et al., 2001; Meylan and Malatesta, 2009; Sohnlein et al., 2014) with a potential risk of overuse injuries in the growing athletes who could potentially undergo growth related injuries.

**TABLE 3 |** Multi-directional plyometric training program.

Week	Exercises	Directions	Sets x repetitions per-session	Foot contacts per-session
1	• Alternating jumps (right-left leg) forward throughout hoops	V-A	3 x 6	54
	• Alternating jumps lateral (right-left leg) throughout hoops	V-L	3 x 6	
	• Jumps with feet together and then separated throughout hoops	V-A-L	3 x 6	
2	• Alternating jumps (right-left leg) forward throughout hoops	V-A	4 x 6	72
	• Alternating jumps lateral (right-left leg) throughout hoops	V-L	4 x 6	
	• Jumps with feet together and then separated throughout hoops	V-A-L	4 x 6	
3	• Jumping, feet together throughout hoops	V-A	4 x 8	96
	• Alternating jumps lateral (right-left leg) throughout hoops	V-L	4 x 8	
	• Jumps with feet together and then separated throughout hoops	V-A-L	4 x 8	
4	• Jumping, feet together throughout hoops	V-A	4 x 8	104
	• Alternating jumps lateral (right-left leg) throughout hoops	V-L	4 x 9	
	• Jumps with feet together and then separated in hoops	V-A-L	4 x 9	
5	• Jumps forward between barriers (30 cm)	V-A	4 x 10	112
	• Lateral jumps over a bench (20 cm)	V-L	4 x 9	
	• Jumps with feet together and then separated throughout hoops	V-A-L	4 x 9	
6	• Jumps forward between barriers (30 cm)	V-H	4 x 10	116
	• Lateral jumps over a bench (20 cm)	V-L	4 x 10	
	• Jumps with feet together and then separated throughout hoops	V-A-L	4 x 9	
7	• Jumps forward between barriers (30 cm)	V-H	4 x 12	120
	• Lateral jumps over a bench (20 cm)	V-L	4 x 9	
	• Jumps with feet together and then separated throughout hoops	V-A-L	4 x 9	
8	• Jumps forward between barriers (30 cm)	V-A	4 x 12	124
	• Lateral jumps over a bench (20 cm)	V-L	4 x 10	
	• Jumps with feet together and then separated throughout hoops	V-A-L	4 x 9	

V-A, vertical-anterior-posterior; V-L, vertical-lateral; V-A-L, vertical-anterior-posterior-lateral.

**TABLE 4 |** Vertical jump and T-Test performance before and after the intervention program.

Group	Control			Paired t-test	Experimental			Paired t-test	ANOVA Group x Time	
	Pre	Post	% Δ	p value	Pre	Post	% Δ	p value	p value	Cohen's d
SJ (m)	0.19 ± 0.01	0.19 ± 0.01	0.42 ± 1.3	0.33	0.19 ± 0.02	0.21 ± 0.02	11.14 ± 2.9	0.00*	0.00*	5.77
CMJ (m)	0.21 ± 0.01	0.21 ± 0.02	-0.79 ± 1.8	0.10	0.21 ± 0.02	0.23 ± 0.02	9.91 ± 2.8	0.00*	0.00*	5.15
T-Test (s)	13.6 ± 0.7	13.6 ± 0.6	0.3 ± 0.7	0.19	13.7 ± 0.8	13.3 ± 0.8	-3.07 ± 1.1	0.00*	0.00*	3.53

SJ, squat jump; CMJ, counter movement jump; \* < 0.05.

**TABLE 5 |** Dynamic postural control (SEBT) of the dominant leg performance before and after the intervention program.

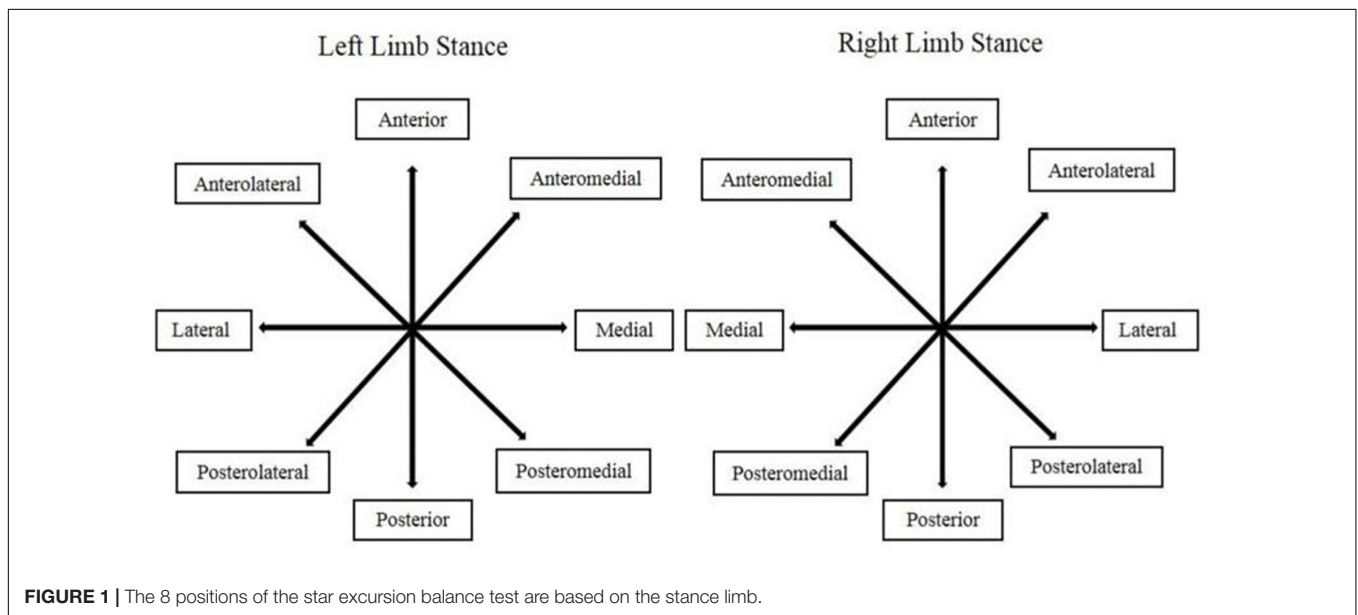
Group	Control			Paired t-test	Experimental			Paired t-test	ANOVA Group x Time	
	Pre	Post	% Δ	p value	Pre	Post	% Δ	p value	p value	Cohen's d
Anterior	83.1 ± 1.7	83.3 ± 1.9	0.26 ± 1.2	0.43	80.9 ± 3.6	83.9 ± 3.7	3.79 ± 2.4	0.00*	0.00*	1.94
Antero-lateral	89.9 ± 2.3	89.2 ± 3.0	-0.8 ± 0.7	0.02*	88.3 ± 4.4	88.8 ± 3.7	0.70 ± 2.3	0.32	0.50	0.19
Lateral	89.5 ± 2.6	88.7 ± 2.3	-0.92 ± 1.4	0.02*	88.8 ± 3.6	90.7 ± 2.7	2.18 ± 2.1	0.00*	0.00*	1.76
Postero-lateral	84.4 ± 8.8	83.9 ± 8.6	-0.59 ± 1.1	0.08	82.3 ± 7.1	86.2 ± 5.4	4.93 ± 3.8	0.00*	0.00*	2.13
Posterior	67.7 ± 7.9	67.6 ± 7.6	-0.11 ± 2.1	0.76	65.0 ± 5.9	71.4 ± 5.3	10.19 ± 6.2	0.00*	0.00*	2.43
Postero-medial	77.6 ± 4.1	77.7 ± 4.4	0.07 ± 1.8	0.85	74.9 ± 4.2	79.6 ± 3.9	6.39 ± 3.1	0.00*	0.00*	2.54
Medial	77.1 ± 3.8	76.6 ± 3.8	-0.51 ± 1.1	0.11	74.0 ± 5.0	78.4 ± 5.1	5.93 ± 2.8	0.00*	0.00*	3.21
Antero-medial	82.6 ± 4.5	81.8 ± 4.8	-0.97 ± 1.4	0.02*	79.2 ± 4.8	82.5 ± 5.0	4.08 ± 2.1	0.00*	0.00*	2.93

\* < 0.05.

**TABLE 6 |** Dynamic postural control (SEBT) of the non-dominant leg performance before and after intervention program.

Group	Control			Paired <i>t</i> -test	Experimental			Paired <i>t</i> -test	ANOVA Group × Time	
	Pre	Post	% Δ	<i>p</i> value	Pre	Post	% Δ	<i>p</i> value	<i>p</i> value	Cohen's <i>d</i>
Anterior	67.7 ± 4.1	67.0 ± 4.4	-0.96 ± 2.1	0.12	65.5 ± 4.2	70.2 ± 3.6	7.25 ± 4.2	0.00*	0.00*	2.70
Antero-lateral	87.3 ± 3.1	86.8 ± 2.8	-0.58 ± 1.1	0.07	86.2 ± 4.4	88.6 ± 4.3	2.80 ± 2.3	0.00*	0.00*	1.95
Lateral	89.5 ± 2.3	90.5 ± 2.7	0.76 ± 1.2	0.08	91.6 ± 3.1	92.6 ± 2.8	1.17 ± 1.8	0.04*	0.00*	0.10
Postero-lateral	91.0 ± 2.9	89.0 ± 4.9	-2.23 ± 4.3	0.07	89.5 ± 6.5	89.0 ± 7.6	-0.14 ± 9.4	0.84	0.54	0.23
Posterior	80.0 ± 4.0	80.1 ± 4.3	0.04 ± 1.2	0.85	77.0 ± 5.1	80.7 ± 4.1	4.96 ± 3.3	0.00*	0.00*	2.04
Postero-medial	82.5 ± 2.3	81.6 ± 2.6	-0.98 ± 1.6	0.04*	80.8 ± 3.1	84.0 ± 2.3	3.89 ± 2.0	0.00*	0.00*	2.86
Medial	74.0 ± 3.1	73.2 ± 3.3	-1.05 ± 0.9	0.00*	73.3 ± 4.0	77.6 ± 4.3	5.89 ± 2.5	0.00*	0.00*	3.76
Antero-medial	75.6 ± 4.2	74.6 ± 4.0	-1.21 ± 1.8	0.03*	74.2 ± 5.6	77.8 ± 5.6	4.86 ± 2.4	0.00*	0.00*	2.96

\* <0.05.



**FIGURE 1 |** The 8 positions of the star excursion balance test are based on the stance limb.

Other researchers (Ramirez-Campillo et al., 2014; Chaabene and Negra, 2017) interestingly proposed lower volume programs and still found significant gains in factors related to athletic performance. Moreover, Chaabene and Negra (2017) showed no significant difference of adaptations between high and low volume plyometrics programs in young soccer players, perhaps because the low volume training stimulus already elicits an optimal adaptive response in this population. In accordance with the findings of these studies, the present study was based on progressive and moderate intensity exercise in order to minimize the risk of overuse injuries in the young participants. Moreover, the singularity of the present study (compared to previous literature) is that it is unique by the combination of actions in the multidirectional planes to better meet the multi-directional needs of soccer activity.

Plyometric training (PT) improves exercise performance that involves SSC of muscle-tendon units (Markovic and Mikulic, 2010). Hirayama et al. (2017) demonstrated that PT improved the SSC exercise performance by the optimization of muscle-tendon behavior of the agonists, associated with an alteration

in the neuromuscular activity during SSC exercise and an increase in tendon stiffness. Furthermore, a decrease in the neuromuscular activity of the antagonists during the braking phase appears to play an important role in this improvement. These findings can explain why the present study showed a significant improvement ( $p < 0.05$ ) in both types of vertical jumps with two different regimes [concentric regime for the squat jump (SJ),  $\Delta = 11.14\%$ ] and plyometric regime for the counter movement jump (CMJ),  $\Delta = 9.91\%$ ]. But, it is important to point out that the characteristics of the muscle-tendon unit are different between youth and adults. Indeed, Mersmann et al. (2014) showed imbalanced development of muscle strength and tendon mechanical and morphological properties in adolescent athletes compared to middle-aged athletes. According to these specific characteristics in young people, the effect of a plyometric program on the SSC may be different compared to adults. But until now, to the best of the authors' knowledge, there has been no study on this topic in young athletes.

MPT induced significant improvements in vertical jump height, possibly reflecting increased strength, and/or power

of the leg extensor muscles (Michailidis et al., 2013), better coordination of agonists and antagonists, and/or a greater recruitment of motor units (Garcia-Pinillos et al., 2014). One weakness of the present study is that we did not measure strength itself, but have assessed proxies to strength (from vertical jumping height). Indeed, vertical jump height (during SJ and CMJ) is strongly correlated with the lower limbs' strength in young soccer players (Chamari et al., 2004). This suggests that the significant improvement in vertical jump height reflects a possible significant improvement in lower limb strength in the EG.

MPT also improved performance of the COD, corroborating the earlier findings of Miller et al. (2006) following 6 weeks of in-season PT. Such gains probably reflect increases in muscular power and movement efficiency (Miller et al., 2006), qualities that are important in team sports.

Finally, the present program enhanced postural control in multiple axes and inversely with the control group not performing MPT, the performance of DPC on several axes decreased. This suggests that when there is no specific preparation program to improve DPC in the sports season, this quality declines and consequently the risk of injury might potentially increase. This improvement in postural control for the experimental group could reflect either improvements in motor output of the lower extremity muscles (Mirwald et al., 2002), and/or changes in proprioception and neuromuscular control (Hewett et al., 2002). Dynamic balance was improved in both anterior-posterior and medial-lateral directions, in contrast with the study of Ramirez-Campillo et al. (2015), where vertical and horizontal exercises improved dynamic balance in the anterior-posterior but not in the medial-lateral direction. Taken all together these results corroborate the fact that an increase in the lower-extremity muscle power would be associated with enhancements of the postural performance (Paillard, 2017a). This underlines the importance of the present study with its' innovative multidirectional approach.

Although the present study points to the effectiveness of MPT, there remains a need to undertake a direct comparison between uni-, bi- and multi-directional plyometrics programs. Despite their importance in soccer, the present study did not include any goal keeper. Indeed, the latter players' training regimens and physical capacities are markedly different compared to outfield players. Therefore, (i) in order to avoid having eventual

outliers in our study population and (ii) for practical reasons of training organization on the field, we have chosen to exclude them from the study. Further studies on goal keepers are therefore warranted.

## PRACTICAL APPLICATIONS

The findings of the present study have important practical applications. It was shown for the first time that the MPT enhanced three important qualities that are relevant to the performance of young male soccer players (vertical jump height, CODP, and DPC). Such a program has the potential to be very much appealing to coaches as requiring little execution time while yielding valuable and relevant outcomes in the physical preparation of young soccer players.

## DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Manouba University Institutional Review Committee for the ethical use of human participants. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kind.

## AUTHOR CONTRIBUTIONS

MJ and GR performed the experiment and collected the data. All authors contributed to the study design, data analysis, and writing the manuscript.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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# Different Patterns of Cerebral and Muscular Tissue Oxygenation 10 Years After Coarctation Repair

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The purpose of this study was to assess whether the lower exercise tolerance in children after coarctation repair is associated with alterations in peripheral tissue oxygenation during exercise. A total of 16 children after coarctation repair and 20 healthy control subjects performed an incremental ramp exercise test to exhaustion. Cerebral and locomotor muscle oxygenation were measured by means of near infrared spectroscopy. The responses of cerebral and muscle tissue oxygenation index (cTOI, mTOI), oxygenated (O<sub>2</sub>Hb), and deoxygenated hemoglobin (HHb) as a function of work rate were compared. Correlations between residual continuous wave Doppler gradients at rest, arm-leg blood pressure difference and local oxygenation responses were evaluated. Age, length, and weight was similar in both groups. Patients with aortic coarctation had lower peak power output (P<sub>peak</sub>) (72.3 ± 20.2% vs. 106 ± 18.7%, *P* < 0.001), VO<sub>2peak</sub>/kg (37.3 ± 9.1 vs. 44.2 ± 7.6 ml/kg, *P* = 0.019) and %VO<sub>2peak</sub>/kg (85.7 ± 21.9% vs. 112.1 ± 15.5%, *P* < 0.001). Cerebral O<sub>2</sub>Hb and HHb had a lower increase in patients vs. controls during exercise, with significant differences from 60 to 90% P<sub>peak</sub> (O<sub>2</sub>Hb) and 70% to 100% P<sub>peak</sub> (HHb). Muscle TOI was significantly lower in patients from 10 to 70% P<sub>peak</sub> and muscle HHb was significantly higher in patients vs. controls from 20 to 80% P<sub>peak</sub>. Muscle O<sub>2</sub>Hb was not different between both groups. There was a significant correlation between residual resting blood pressure gradient and Δmuscle HHb/ΔP at 10–20W and 20–30W (*r* = 0.40, *P* = 0.039 and *r* = 0.43, *P* = 0.034). Children after coarctation repair have different oxygenation responses at muscular and cerebral level. This reflects a different balance between O<sub>2</sub> supply to O<sub>2</sub> demand which might contribute to the reduced exercise tolerance in this patient population.

**Keywords:** coarctation aortae, near infrared spectroscopy, exercise test, children, muscle oxygenation

## INTRODUCTION

Repair of coarctation of the aorta (CoA) has been performed for more than 50 years (Cohen et al., 1989; Corno et al., 2001). Although this surgical intervention aims to restore the functional capacity of these patients, exercise performance in this patient group remains impaired in adults (Trojnaraska et al., 2007) as well as in children (Rhodes et al., 2010). However, pulmonary gas exchange responses,

which should be considered as “whole-body” measurements, obtained during cardiopulmonary exercise tests cannot provide a sufficient insight into the origins of the lower exercise performance. In this context, residual coarctation, left ventricular dysfunction, and hypertension have all been proposed as possible underlying causes. In 1981, Eriksson et al. (Eriksson and Hanson, 1981) observed a disturbed blood flow regulation and impaired blood flow to the working leg muscles during exercise in adults after CoA repair. Johnson et al. (1995) studied blood flow measurements by duplex ultrasound and found an impaired lower limb blood flow in response to strenuous dynamic exercise, even without significant residual stenosis at rest. These studies indicate that also the relationship between O<sub>2</sub> delivery and O<sub>2</sub> utilization might be altered in CoA.

Near-infrared spectroscopy is a technique that measures the (changes in) concentration of oxygenated (O<sub>2</sub>Hb) and deoxygenated (HHb) hemoglobin (i.e., tissue oxygenation) during exercise in a non-invasive way. Together with the derived parameter TOI (i.e., tissue oxygenation index: O<sub>2</sub>Hb/(O<sub>2</sub>Hb + HHb) these parameters quantify the overall oxygenation at the level of the tissues. It has been suggested that HHb is a reflection of arterio-venous O<sub>2</sub> difference (DeLorey et al., 2003; Grassi et al., 2003). According to the principle of Fick, in this context HHb can provide information on the dynamic balance between O<sub>2</sub> delivery (QO<sub>2</sub>) and O<sub>2</sub> utilization (VO<sub>2</sub>) at the level of the tissues. In non-steady state conditions there are substantial changes in the ratios between QO<sub>2</sub> and VO<sub>2</sub> in different body regions (active/non-active muscles, brain, heart, and other organs, etc.). Therefore, studying the oxygenation patterns at different sites of the body during periods of changing metabolic demand is highly relevant to understanding key aspects of metabolic and vascular control.

At the level of the locomotor muscles HHb increases following a sigmoid pattern as exercise intensity increases during incremental ramp exercise (Boone et al., 2016; Vandekerckhove et al., 2016). The initial slow increase in HHb at the onset of the incremental exercise indicates faster QO<sub>2</sub> kinetics vs. VO<sub>2</sub> kinetics at the level of the muscle. In a second phase HHb increases more rapidly revealing an increased fractional O<sub>2</sub> extraction due to a relative slowing of QO<sub>2</sub> vs. VO<sub>2</sub>. Finally, a leveling-off in HHb occurs, at an intensity closely corresponding to the respiratory compensation point. Although it has been suggested that fractional O<sub>2</sub> extraction reaches its limits at this intensity, more recent studies (Inglis et al., 2017; Iannetta et al., 2018) suggest that a local redistribution of blood causes a matching between QO<sub>2</sub> and VO<sub>2</sub> with a leveling off in HHb as a consequence.

Also the oxygenation responses at the level of the brain might provide insights into the factors limiting exercise tolerance. It has been shown during incremental ramp exercise that cerebral O<sub>2</sub>Hb increases up to a point at high intensity exercise where a breakpoint occurs and a decline in O<sub>2</sub>Hb is initiated (Bhambhani et al., 2007; Rooks et al., 2010). Cerebral HHb remains stable during submaximal exercise but then shows a rapid increase from hard (>60%  $\dot{V}O_{2max}$ ) to maximal intensity (Rooks et al., 2010). These typical O<sub>2</sub>Hb and HHb response patterns indicate that cerebral blood flow increases in accordance with the increase

in work rate during incremental exercise (Panerai et al., 1999; Jorgensen et al., 2000; González-Alonso et al., 2004). In a recent study in Fontan patients it was shown that cerebral O<sub>2</sub>Hb did not increase during incremental exercise, which resulted in a progressive decrease in cerebral saturation (i.e., cerebral TOI), as HHb increased during the exercise (Vandekerckhove et al., 2019). These results confirm the potential role of brain oxygenation as a limiting factor to exercise tolerance, especially in patient populations (Brassard and Gustafsson, 2016).

Given the unknown etiology of the lower exercise tolerance in CoA patients after repair, assessing local oxygenation responses (O<sub>2</sub>Hb, HHb, and TOI) during incremental exercise could provide important insights into the underpinning mechanisms. In the past (Eriksson and Hanson, 1981; Johnson et al., 1995) it has been shown that tissue blood flow was affected in individuals following repair of aortic coarctation. Therefore, it can be expected that local oxygenation responses (brain, muscle) during incremental exercise differ from those of healthy controls. Thus, the purpose of the present study was to investigate oxygenation responses at the level of the brain and locomotor muscles during incremental exercise in children after CoA repair. We hypothesize first, that these children will have a lower exercise tolerance compared to healthy children. Second, we hypothesize altered oxygenation responses at the level of the brain and muscle that will be related to residual lesions in the patient group.

## MATERIALS AND METHODS

### Ethics Statement

This study was approved by the local ethical committee (Ghent University Hospital, Ghent, Belgium) and followed the ethical recommendations for the study of humans as suggested by the Declaration of Helsinki. All participants gave written informed consent prior to the start of the study.

### Participants

Sixteen children post aortic coarctation repair (13 boys, 3 girls) (CoA patients) and twenty healthy children (9 boys, 11 girls) volunteered to take part. The age and anthropometric characteristics of the two groups are presented in **Table 1**. The groups did not differ significantly for these characteristics. All patients were operated under the age of 4 years, with the majority operated under 1 year (12/16, median 6 weeks, 1 day – 4 years). Most patients (15/16) underwent resection of the coarctation site with end-to-end anastomosis, 1 patient underwent extended arch repair. CoA patients were in stable follow-up. They had normal blood pressures at rest, good left ventricular function (fractional shortening >28% in all patients), and no significant LV hypertrophy on echocardiography. All patients and controls were attending normal school and sports activities.

### Experimental Procedure

An incremental exercise test was performed on an electromagnetically braked cycle ergometer (Ergoline Ergoselect

**TABLE 1** | Age and anthropometric characteristics for coarctation aortae patients and healthy controls.

	Coarctation	Controls	P-value
Age (years)	13.0 ± 2.2	12.0 ± 1.8	<i>P</i> = 0.137
Body weight (kg)	47.5 ± 17.2	41.1 ± 11.0	<i>P</i> = 0.104
Body height (m)	1.57 ± 0.13	1.52 ± 0.11	<i>P</i> = 0.256
Type of surgery	End to end 15/16		
	Extended end to end 1/16		
Age at surgery (median, min-max)	6 weeks (1 day – 4 years)		
Residual gradient (mm Hg)	26.6 ± 7.3		
LV function (fractional shortening, %)	36.8 ± 5		
Septal thickness (diast) (Z-value)	8.25 ± 1.29 0.67 ± 0.70		
Posterior wall thickness (diast) (Z-value)	7.31 ± 1.49 0.55 ± 0.94		

Values are mean ± SD. LV, left ventricle; diast, diastole.

100K, Bitz, Germany). Following a 3 min warm-up at unloaded cycling, the work rate increased in a linear and continuous way (i.e., ramp exercise). The ramp slope (i.e., the increase in work rate per minute) was individualized and determined by dividing the individual body weight by 4 and rounding off to the closest natural number [0 Watt + (body weight/4) Watt.min<sup>-1</sup>]. This internally validated protocol leads to an optimal exercise duration of 8 – 12 min in healthy children, with reference values equal to the values of Wasserman et al. (Wasserman, 2012). Participants were asked to maintain a pedal rate of 60 revolutions per minute (rpm) and the test was terminated when they reached their self-determined point of full exhaustion or were unable to maintain the required pedal rate despite strong verbal encouragement. Echocardiographic measurements were reviewed and the residual doppler gradient (continuous wave) over the aortic coarctation zone at rest (mmHg) was defined.

## Experimental Measures

During the exercise tests, pulmonary gas exchange (VO<sub>2</sub>, oxygen uptake; VCO<sub>2</sub>, carbon dioxide production; VE, ventilation) was measured continuously on a breath-by-breath basis by means of a computerized O<sub>2</sub>-CO<sub>2</sub> analyzer-flowmeter combination (Jaeger Oxycon Pro, Germany). Respiratory exchange ratio (RER) was calculated by expressing VCO<sub>2</sub> relative to VO<sub>2</sub> (VCO<sub>2</sub>/VO<sub>2</sub>).

Blood pressure in the arm was measured every 3 min during the exercise phase and every 2 min during the recovery phase with an integrated blood pressure monitor (SunTech Tango) that uses 3D K-Sound Analysis. At the start and at maximal exercise, blood pressure was measured at the leg using the same technique. The difference between systolic pressure arm compared to leg was analyzed at rest and at maximal exercise. This is proven to be an important parameter for the degree of residual obstruction at the aortic arch (Dijkema et al., 2017).

Muscle and cerebral oxygenation (O<sub>2</sub>Hb and HHb) were measured by means of near infrared spectroscopy technology (NIRO-200NX, Hamamatsu Photonics K.K., Hamamatsu, Japan). This system consists of an emission probe emitting near-infrared light at three wavelengths (735, 810 and 850 nm) and a photon detector which measures the intensity of incident and transmitted light at a frequency of 2 Hz. For measurements of oxygenation, the probe was positioned longitudinally over the distal section of the left M. Vastus Lateralis and adhered to the skin. For measurements of cerebral oxygenation, the probe was placed over the left pre-frontal lobe, approximately 3 cm from the midline and just above the supra-orbital ridge (Kleinschmidt et al., 1996; Bhamhani et al., 2007). This device measures TOI as a reflection of mixed arterio-venous O<sub>2</sub> saturation (in%) at the location of the probe. Additionally, relative changes to baseline values in the concentration of O<sub>2</sub>Hb and HHb (in μmol) are recorded. Baseline cycling at 0 Watt was used as baseline values for O<sub>2</sub>Hb and HHb and were set to 0 μmol.

## Data Analysis

### Cardiopulmonary Exercise Test

The breath-by-breath data from the gas exchange responses were filtered upon exportation based on the following criteria: tidal volume < 0.2 and > 10 l.min<sup>-1</sup>; fraction of expired CO<sub>2</sub> < 1 and > 10% (Fontana et al., 2015). The VO<sub>2</sub>peak was calculated as the highest 30s average (i.e., moving average) VO<sub>2</sub> throughout the test. Since a leveling-off in VO<sub>2</sub> is often not reached in children (Armstrong and Welsman, 1994), the term VO<sub>2</sub>peak will be used throughout to avoid erroneous conclusions on maximal effort. The peak power output (Ppeak) was determined as the work rate attained at the termination of the exercise phase. The VO<sub>2</sub>peak and Ppeak were expressed relative to the norm values (predVO<sub>2</sub>peak, predPpeak), based on age and anthropometrics (Wasserman, 2012).

The gas exchange threshold (GET) was determined using the criteria of a disproportionate increase in carbon dioxide production (VCO<sub>2</sub>) to VO<sub>2</sub> (Beaver et al., 1986), a first departure from the linear increase in minute ventilation (VE) and an increase in VE/VO<sub>2</sub> with no increase in VE/VCO<sub>2</sub>. The disproportionate increase in VCO<sub>2</sub> is related to an increase in the buffering of H<sup>+</sup> due to an increased production of pyruvate from glycolytic processes in the cytosol of muscle fibers. The peak Respiratory Exchange Ratio (RERpeak) was determined as the highest 30s RER throughout the test, the peak heart rate (HRpeak) as the highest value obtained throughout the test.

### Cerebral and Muscle Oxygenation

The changes in TOI and in the concentration of cerebral and muscle O<sub>2</sub>Hb and HHb from baseline values (i.e., baseline cycling at 0 Watt) of each individual were expressed as a function of Ppeak by calculating the mean TOI, O<sub>2</sub>Hb and HHb response at 10%, 20%, 30%, . . . , 100% Ppeak. The values at these% Ppeak were calculated as the average of the O<sub>2</sub>Hb and HHb values 10s prior and 10s following the relative intensity.

Additionally, to quantify the relationship between muscle O<sub>2</sub> supply and O<sub>2</sub> demand, the change in muscle HHb

( $\Delta$ muscle HHb) as a function of the change in work rate ( $\Delta P$ ) ( $\Delta$ muscle HHb/ $\Delta P$ ) for each 10% Ppeak interval (i.e., from 0 to 10%, 10 to 20%, etc) was calculated.

To quantify the sudden changes in the pattern of the NIRS responses, breakpoints (BP) were determined. Therefore, the studies of Miura et al. (1998) and Spencer et al. (2012) were used as examples of typical responses in muscle O<sub>2</sub>Hb (BP at moderate and high intensity demarcating the point of an accelerated and attenuated decrease, respectively) and HHb (BP at high intensity at which muscle HHb levels off), respectively. The studies of Rooks (Rooks et al., 2010) and Bhambhani (Bhambhani et al., 2007) served as examples of the typical responses in cerebral O<sub>2</sub>Hb (BP at high intensity at which O<sub>2</sub>Hb starts to decrease) and HHb (BP demarcating the point of an accelerated increase). In case the determination of the breakpoints was not possible with the two-segment linear piecewise model of curve fitting in Sigmaplot (Systat Software Inc., San José, CA, United States), two experienced researchers analyzed the oxygenation responses visually to detect the BPs. When the analysis did not correspond between the two researchers, the data were re-evaluated together with a third researcher until a consensus was reached.

Finally, the amplitude of the NIRS responses was calculated as the difference between the NIRS value at baseline cycling and the highest (or lowest) obtained value throughout the test (i.e., either at the BP or Ppeak).

## Statistical Analysis

The statistical analysis was performed in SPSS 21.0 (IBM Corp., Armonk, NY, United States). The pulmonary gas exchange (VO<sub>2</sub>, VCO<sub>2</sub>) and NIRS (TOI, O<sub>2</sub>Hb, HHb) data were normally distributed, and therefore the data are presented as mean values  $\pm$  SD and parametric statistical analyses were performed. The parameters quantifying exercise tolerance (Ppeak, VO<sub>2</sub>peak, and GET) were compared between the CoA patients and healthy controls by means of Independent Samples *T*-tests. The predPpeak and predVO<sub>2</sub>peak in both patients and controls were compared to a reference value of 100% (Wasserman, 2012) by means of One Sample *T*-tests. The cerebral and muscle TOI, O<sub>2</sub>Hb, and HHb responses at the 10% Ppeak intervals (between 0% and 100% Ppeak) were compared between the CoA patients and healthy controls and between the intensities by means of Two-way Anova (Group x Intensity). Additionally, the  $\Delta$ muscle HHb/ $\Delta P$  values were compared at each relative intensity (% Ppeak) between patients and controls by means of Two-way Anova. In case of significant interaction or main effects *post hoc* Tukey tests were performed. Statistical significance was set at  $P < 0.05$ .

## RESULTS

### Exercise Tolerance

In Table 2 the parameters quantifying exercise tolerance, obtained from the incremental ramp exercise, are presented. When expressed relative to body weight, the CoA subjects had significantly lower Ppeak (Watt.kg<sup>-1</sup>) ( $P = 0.010$ ), VO<sub>2</sub>peak (ml.min<sup>-1</sup>.kg<sup>-1</sup>) ( $P = 0.019$ ), and GET (ml.min<sup>-1</sup>.kg<sup>-1</sup>)

**TABLE 2 |** Exercise tolerance (Ppeak, VO<sub>2</sub>peak, RERpeak, HRpeak, GET, and blood pressure) in coarctation aortae patients and healthy controls,

	Coarctation	Controls	P-value
Ppeak (Watt)	119 $\pm$ 49	125 $\pm$ 41	$P = 0.723$
Ppeak/kg (Watt.kg <sup>-1</sup> )	2.42 $\pm$ 0.65	3.04 $\pm$ 0.59	$P = 0.010^*$
% Predicted Ppeak (%)	72.3 $\pm$ 20.2	106 $\pm$ 18.7	$P < 0.001^*$
VO <sub>2</sub> peak (ml.min <sup>-1</sup> )	1792 $\pm$ 581	1790 $\pm$ 459	$P = 0.991$
VO <sub>2</sub> peak/kg (ml.min <sup>-1</sup> .kg <sup>-1</sup> )	37.3 $\pm$ 9.1	44.2 $\pm$ 7.6	$P = 0.019^*$
% Predicted VO <sub>2</sub> peak (%)	85.7 $\pm$ 21.9	112.1 $\pm$ 15.5	$P < 0.001^*$
RERpeak	1.14 $\pm$ 0.10	1.09 $\pm$ 0.06	$P = 0.382$
HRpeak (bts.min <sup>-1</sup> )	179 $\pm$ 19	193 $\pm$ 9	$P = 0.188$
GET (ml.min <sup>-1</sup> )	933 $\pm$ 371	964 $\pm$ 289	$P = 0.657$
GET/kg (ml.min <sup>-1</sup> .kg <sup>-1</sup> )	19.0 $\pm$ 4.7	23.6 $\pm$ 3.9	$P < 0.002^*$
Blood pressure rest (sys/dias) (mm hg)	121 $\pm$ 19/ 68 $\pm$ 12	110 $\pm$ 16/ 63 $\pm$ 16	$P = 0.561/P = 0.624$
Blood pressure max (sys/dias) (mm hg)	175 $\pm$ 22/ 69 $\pm$ 10		

Values are mean  $\pm$  SD. Ppeak, peak power output; VO<sub>2</sub>peak, peak oxygen uptake; RERpeak, peak respiratory exchange ratio; HRpeak, peak heart rate; GET, gas exchange threshold; BP, blood pressure. \*Indicates a significant ( $P < 0.05$ ) difference between coarctation and controls.

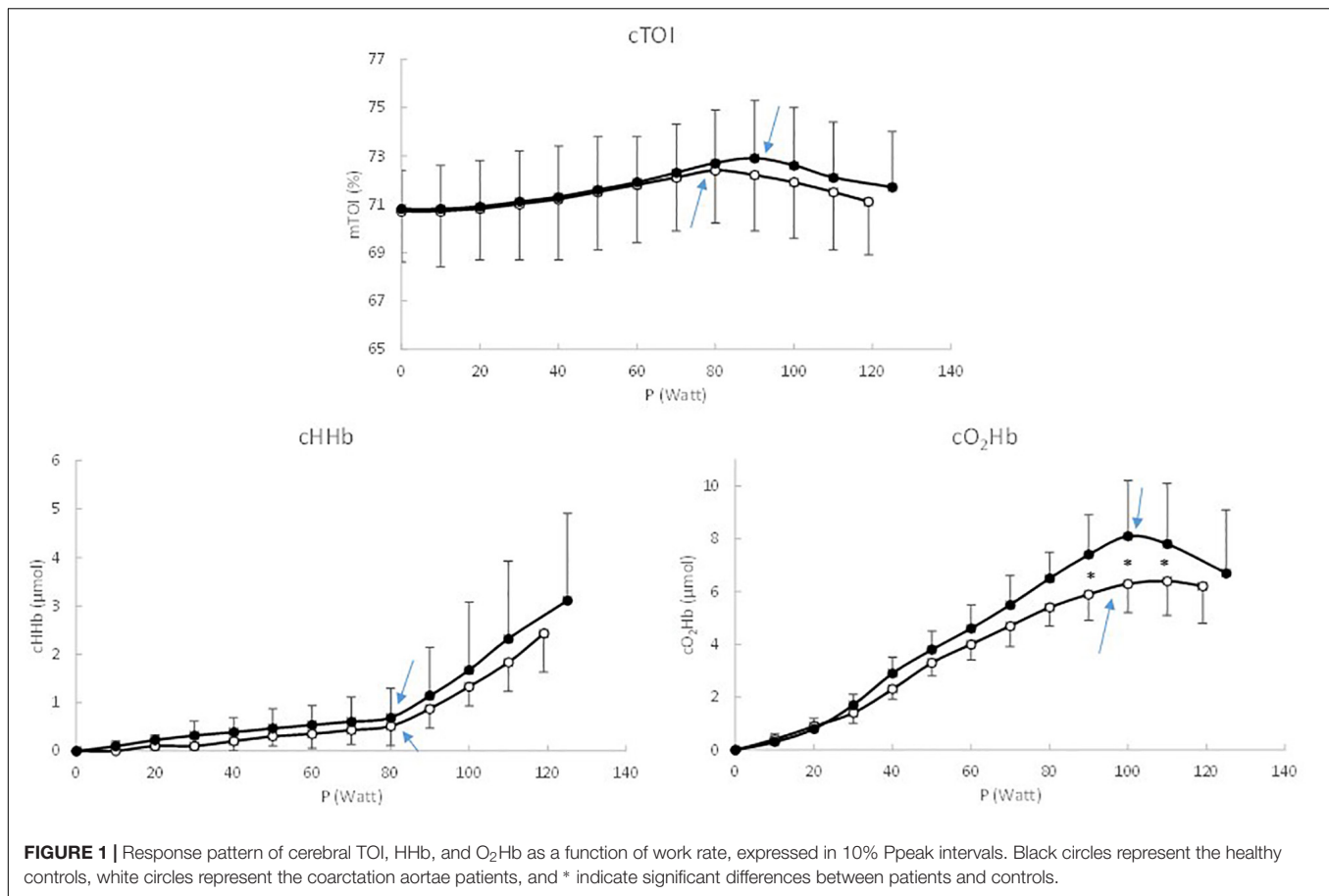
( $P = 0.002$ ). Also, the CoA patients had significantly lower Ppeak and VO<sub>2</sub>peak values compared the expected values for age, gender, stature and weight ( $P < 0.001$ ).

### Cerebral and Muscular Oxygenation

In Figures 1, 2, the cerebral and the muscular TOI, HHb and O<sub>2</sub>Hb patterns are presented as a function of intensity in 10% Ppeak intervals. For cerebral TOI there were no differences between patients and controls ( $P > 0.05$ ) at any intensity. However, the BP in cerebral TOI (at which TOI starts to decrease) occurred at a significantly lower absolute (78  $\pm$  33 Watt vs. 93  $\pm$  37 Watt, respectively,  $P = 0.027$ ) and relative intensity (65.5  $\pm$  9.7% Ppeak vs. 74.4  $\pm$  11.2% Ppeak, respectively,  $P = 0.014$ ) in patients compared to controls.

For the cerebral O<sub>2</sub>Hb ( $P = 0.038$ ) and HHb ( $P = 0.045$ ) a significant interaction effect (Intensity x Group) was demonstrated, indicating that the response pattern differed between CoA patients and healthy controls. For cerebral O<sub>2</sub>Hb, *post hoc* tests revealed that cerebral O<sub>2</sub>Hb was significantly lower ( $P < 0.05$ ) from 60 to 90% Ppeak in patients vs. controls. Also the BP occurred at a significantly lower absolute (89  $\pm$  36 Watt vs. 101  $\pm$  35 Watt, respectively,  $P = 0.027$ ) and but not relative intensity (74.8  $\pm$  12.1% Ppeak vs. 80.8  $\pm$  9.4% Ppeak, respectively,  $P = 0.102$ ) in patients compared to controls. For cerebral HHb, *post hoc* tests showed that the increase in cerebral HHb was more pronounced in healthy controls from 70 to 100% Ppeak. The BP did not occur at a different absolute (78  $\pm$  36 Watt vs. 78  $\pm$  32 Watt, respectively,  $P = 0.027$ ) and relative intensity (66.0  $\pm$  11.8% Ppeak vs. 66.8  $\pm$  10.4% Ppeak, respectively,  $P = 0.862$ ) in patients and controls.

For muscle TOI a significant main effect ( $P = 0.021$ ) of Group was found. *Post hoc* analysis showed that muscle TOI was significantly ( $P < 0.05$ ) lower in patients compared to controls



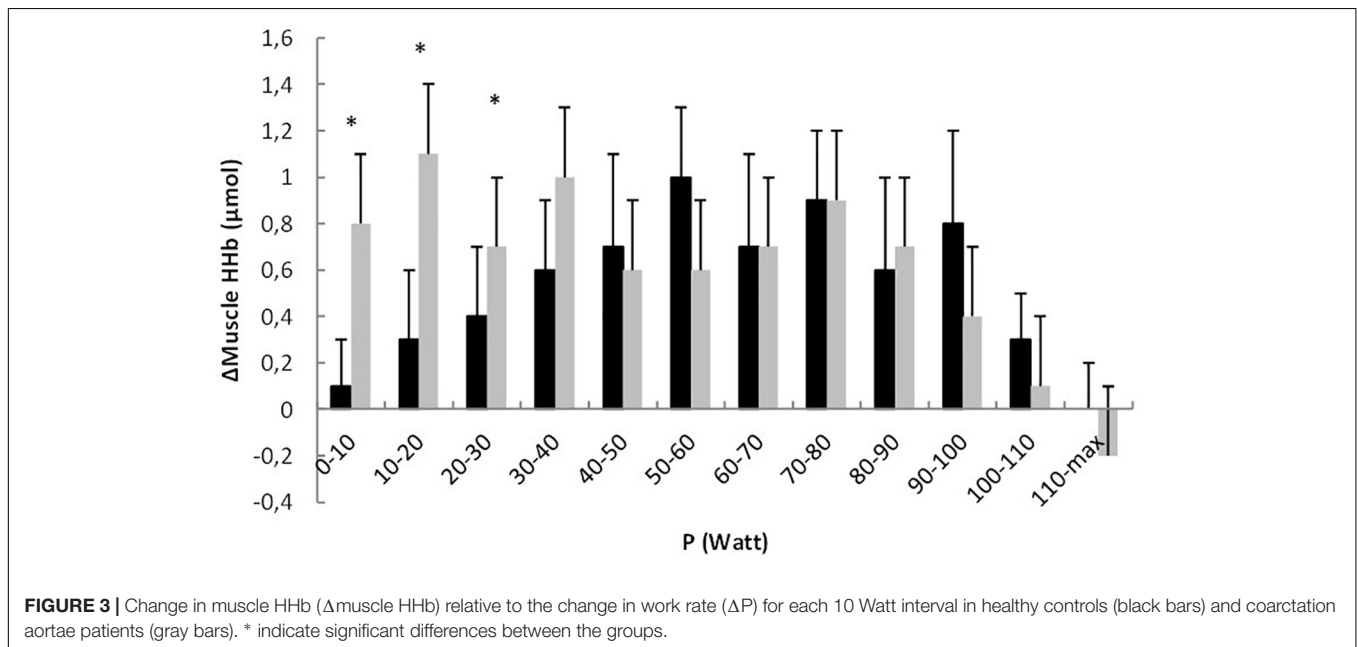
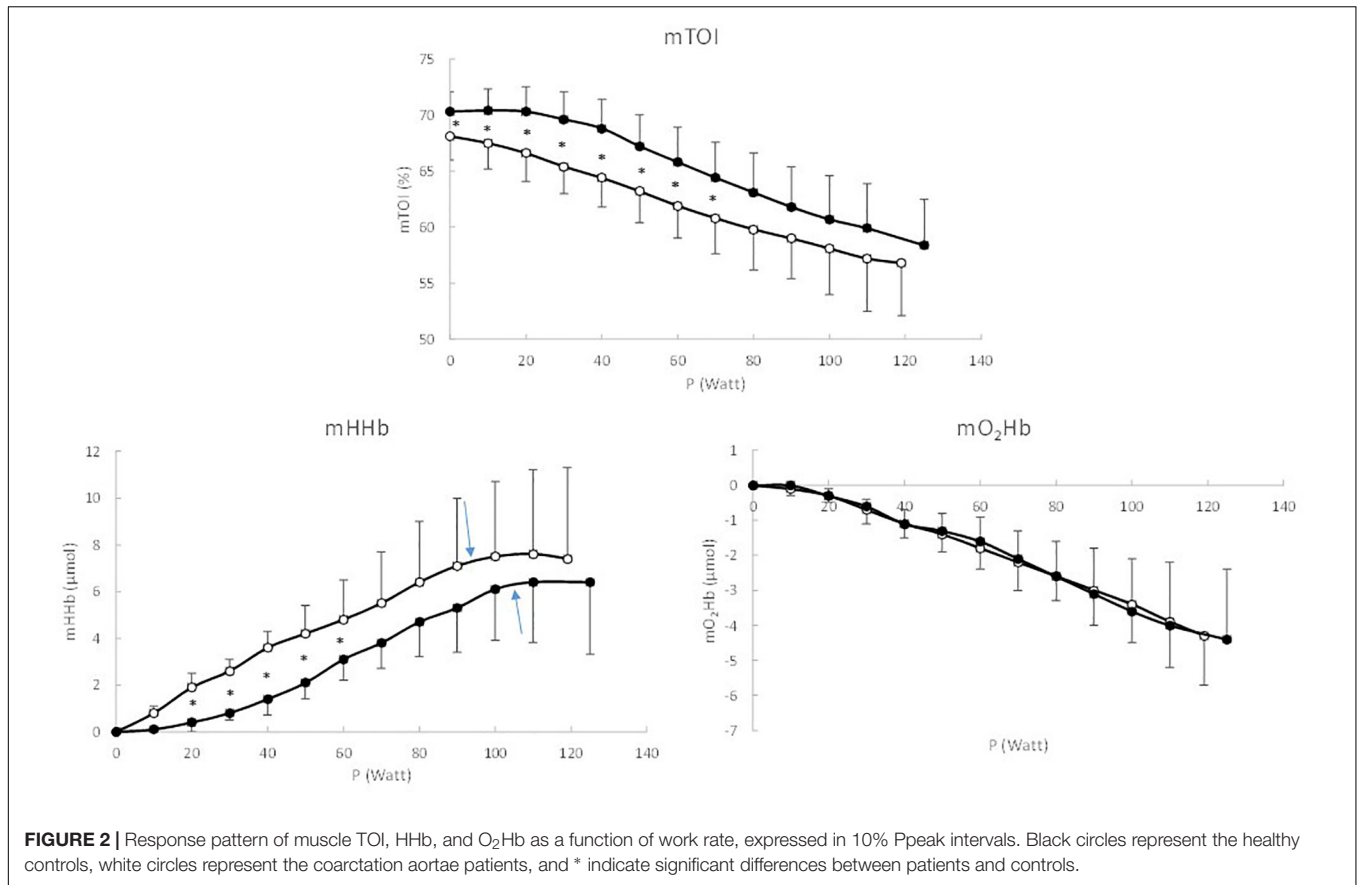
from 10 to 70% Ppeak. The total amplitude of the decrease in muscle TOI did not differ significantly ( $P = 0.739$ ) between both groups ( $-11.7 \pm 4.6\%$  vs.  $-11.6 \pm 5.5\%$  in patients and controls, respectively) and also the muscle TOI at Ppeak ( $56.8 \pm 4.0\%$  vs.  $58.4 \pm 5.1\%$  in patients and controls, respectively) did not differ significantly ( $P = 0.372$ ). Also for muscle Hb a significant ( $P = 0.010$ ) main effect of Group was found. Muscle Hb was significantly ( $P < 0.05$ ) higher in patients compared to controls from 20 to 70% Ppeak. The BP in muscle Hb occurred at a significantly lower absolute ( $92 \pm 33$  Watt vs.  $105 \pm 36$  Watt, respectively,  $P = 0.031$ ) and relative intensity ( $77.1 \pm 9.1\%$  Ppeak vs.  $84.4 \pm 9.7\%$  Ppeak, respectively,  $P = 0.039$ ) in patients compared to controls, whereas the maximal amplitude of the muscle Hb response ( $7.6 \pm 3.6$  μmol vs.  $6.8 \pm 3.6$  μmol, respectively,  $P = 0.466$ ) did not differ significantly between patients and controls.

In **Figure 3**  $\Delta$ muscle Hb is presented for each 10 Watt increase. It was observed that  $\Delta$ muscle Hb was significantly higher in CoA patients compared to healthy controls for the 0–10, 10–20, and 20–30, 30–40 Watt intervals ( $P < 0.05$ ). Muscle O<sub>2</sub>Hb did not differ significantly ( $P > 0.05$ ) between CoA patients and controls over the entire intensity range and at Ppeak ( $-4.3 \pm 1.4$  μmol vs.  $-4.4 \pm 2.0$  μmol, respectively,  $P = 0.792$ ). A clear BP could only be found in 4 of 16 CoA patients and 7 of 20 controls, therefore the BP in muscle O<sub>2</sub>Hb was not considered.

Finally, it was also found that some NIRS variables were correlated to clinical indices.  $\Delta$ Muscle Hb for the 10–20 and 20–30 Watt intervals (10–20 Watt:  $r = 0.40$ ;  $P = 0.039$  and 20–30 Watt:  $r = 0.43$ ;  $P = 0.034$ ) showed a weak but significant correlation with the residual gradient over the coarctation zone (**Figure 4**). The total amplitude of muscle Hb (i.e., the change between 0% Ppeak and 100% Ppeak) was significantly correlated ( $r = 0.61$ ,  $P = 0.017$ ) to the blood pressure difference between the arm and leg at maximal exercise.

## DISCUSSION

To the best of our knowledge, this is the first study to report the patterns of cerebral and muscular tissue oxygenation during incremental exercise in children with aortic coarctation. It was found that children with CoA had a lower exercise tolerance, as can be deduced from a lower VO<sub>2</sub>peak, Ppeak and GET, expressed relative to body weight. Additionally, there are different oxygenation patterns at the cerebral and muscle level compared to healthy children. More specifically, children with CoA had a less pronounced increase in cerebral O<sub>2</sub>Hb at high intensities, whereas muscle TOI and Hb showed a more rapid decrease and increase, respectively, especially at low to moderate exercise intensities. Also the breakpoints in cerebral TOI and

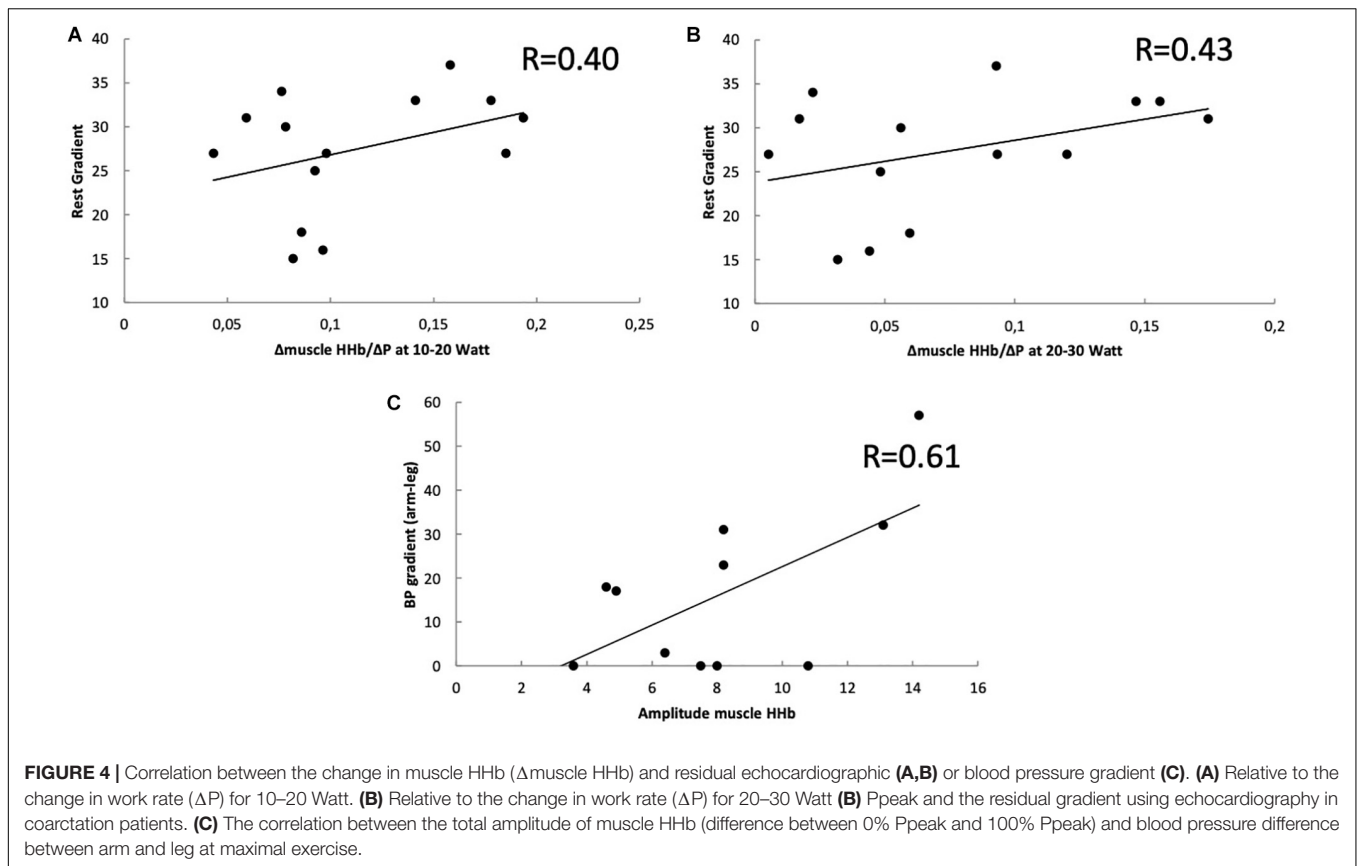


muscle HHb occurred at a lower absolute and relative intensity, indicating that peripheral oxygenation might contribute to the lower physical fitness levels observed in CoA patients compared to healthy controls.

### Exercise Performance

In accordance with previous studies (Trojnaraska et al., 2007; Hager et al., 2008), we found a lower exercise performance in patients after CoA repair. The CoA patients reached only





$72.3 \pm 20.2\%$  of the predicted Ppeak and  $85.7 \pm 21.9\%$  of the predicted  $\text{VO}_2$ peak, which was significantly lower compared to the healthy subjects. The GET, quantifying aerobic exercise tolerance, was also lower in CoA patients.

With regards to the potential underpinning mechanisms of lower exercise performance in CoA patients it has been suggested that there might be a reduced aortic compliance after CoA repair (Hager et al., 2008). Additionally, also a reduced cardiac output could potentially contribute to the reduced exercise performance. Left ventricular hypertrophy in patients with residual CoA can disturb diastolic function with a decreased cardiac output at high intensities. However, at the moment there is no scientific evidence of an affected cardiac output in CoA patients. The present study indicates that different flow distribution patterns and local changes in oxygenation could also contribute to diminished exercise performance (see below). The observation that children after CoA repair appear to have an earlier reliance on the anaerobic metabolism (Wong et al., 2017) supports the suggestion of a reduced functional capacity of the aerobic metabolism. However, at the moment the main origin of the limitation (i.e., convective  $\text{O}_2$  supply,  $\text{O}_2$  diffusive capacity) is unknown.

Additionally, it should be noted that next to underlying pathophysiological factors also deconditioning in relation to lower physical activity (due to an overprotective environment) might be a possible contributing factor to the lower exercise performance. However, a recent study of Stone et al. (2015)

showed that the physical activity levels of CoA children after repair were similar to those of healthy children.

## Cerebral Oxygenation

The lower exercise tolerance in CoA patients could at least in part be explained by a different oxygenation pattern at the peripheral level. Similar to healthy subjects (Rooks et al., 2010; Vandekerckhove et al., 2016) cerebral  $\text{O}_2\text{Hb}$  increased from low to high intensities, where a breakpoint occurred at which cerebral  $\text{O}_2\text{Hb}$  levels off or even decreased. Cerebral HHb showed a slow initial increase with a progressive speeding as work rate increased ( $>60\%$  Ppeak). In comparison to the healthy controls the amplitude of the responses was less pronounced for cerebral  $\text{O}_2\text{Hb}$ . The combination of both a lower cerebral  $\text{O}_2\text{Hb}$  and a similar HHb in CoA patients vs. controls at high intensity explains the lower cerebral TOI (i.e., mixed arterio-venous saturation) in CoA patients at high intensities. It is unclear what might be at the origin of this different oxygenation pattern at cerebral level. One study, although not in coarctation patients, reported that cerebral hemodynamics adapt very rapidly to changes in tension when hypertensive adult patients received antihypertensive medication (Zhang et al., 2007). A recent study described an increased intracranial arterial stiffness and decreased responsiveness to hypercapnic stimuli in adult CoA patients (Wong et al., 2017).

Patients after coarctation repair might suffer from residual narrowing and/or arterial stiffness distal from the origin of the

arteries supplying blood flow to the brains. This leads to a higher pressure and hypertension at the level of the brain. How the brain copes with higher pressures during exercise, and if an (over)protective mechanism is more active in children with CoA, is not known.

The results of the present study show a less pronounced increase in  $O_2$  supply (as reflected in the  $O_2Hb$  response), in combination with an earlier onset of the decrease (i.e., the breakpoint) in cerebral  $O_2Hb$  which resulted in an earlier decrease in cerebral TOI in CoA patients compared to controls. It can be speculated that this affected the “activity” of the motor cortex as such that the firing rate to the locomotor muscles was reduced which might have resulted in an earlier termination of the exercise test. In a recent study in our laboratory, it was observed that Fontan children had a fast decrease in cerebral TOI from the onset of the incremental ramp exercise (Vandekerckhove et al., 2019) and terminated the test with a reduced cerebral TOI compared to healthy controls. In this population it was speculated that the cerebral oxygenation might have been the main contributing factor to exercise termination. Whether this is also the case in the CoA children is questionable since cerebral TOI at Ppeak was similar to the rest values and did also not differ with the controls.

### Muscle Oxygenation

Also, the oxygenation at the locomotor muscles showed a different pattern in the two study groups. In the CoA patients, muscle HHb showed a more pronounced increase in the low to moderate intensity domain in combination with a lower TOI compared to healthy controls. As HHb is often considered as the most valuable NIRS parameter since it is a reflection of fractional  $O_2$  extraction (McNarry et al., 2015), this different pattern of HHb might reflect a disturbed relationship between  $O_2$  supply and  $O_2$  demand. In healthy subjects the HHb response to incremental ramp exercise shows a sigmoidal pattern (Carano et al., 1999; Boone et al., 2009; Vandekerckhove et al., 2016) with a rather slow increase in HHb at the onset of the incremental ramp exercise. This typical pattern in the HHb response at low to moderate intensities indicates that the blood flow to the locomotor muscles has increased to such an extent that the fractional  $O_2$  extraction does not need to increase (Ferreira et al., 2007; Boone et al., 2009). In the CoA patients however, this “sigmoidal” pattern is not present and the HHb response shows an immediate increase at the onset of exercise (Figure 2). This more pronounced increase in HHb in CoA patients is also expressed in Figure 3. This indicates that the balance between  $O_2$  supply and  $O_2$  demand might be altered at low to moderate intensities, highly likely related to a disturbed convective  $O_2$  delivery. This disturbed balance at low intensities is also reflected in the lower mTOI values during unloaded cycling.

Interestingly, we found a correlation between residual arch gradient and  $\Delta\text{muscleHHb}/\Delta P$  and between the total muscle HHb amplitude and blood pressure difference arm-leg at maximal exercise. The CoA children with higher residual obstruction at the descending aorta, have more pronounced increase in muscle HHb per increase in work rate and thus a more disturbed balance between  $O_2$  supply and  $O_2$  demand.

Exercise testing has been shown to be a useful tool for evaluation of residual coarctation after surgery (Carano et al., 1999; Das et al., 2009). Correia et al. (2013) showed that patients with higher residual gradient can develop hypertension during exercise, despite normal tension control at rest. A difference in exercise capacity between adults with higher residual gradient or normal gradient could not be shown (Trojnaraska et al., 2007), although exercise capacity is generally decreased in patients after CoA repair. The different mechanisms at the level of the muscles are unknown. Our findings demonstrate that there might also be metabolic differences at the muscular level in children after CoA repair, even more pronounced in children with higher residual stenosis. Surprisingly, this study also showed that CoA patients have a higher total amplitude of the muscle HHb response compared to healthy subjects, indicating a greater  $O_2$  extraction capacity in this patient population. It is highly likely that the greater reliance on  $O_2$  extraction, due to the disturbed balance between  $O_2$  supply and  $O_2$  demand even at low to moderate intensities, results in the greater capacity of the locomotor muscles to extract  $O_2$ .

Although the limited number of patients should be considered a limitation of the present study, the results shed a new light on the exercise tolerance of CoA patients. The evaluation of cerebral and peripheral oxygenation in this patient population provides useful information on the physical condition of the subjects and the efficacy of treatments and rehabilitation programs. Larger patient trials are needed to explore the influencing factors and causes which can possibly explain the different patterns in CoA patients. In this context, it would be useful to integrate NIRS measurements to assess whether the oxygenation patterns could provide a more comprehensive insight into the exercise performance of CoA patients.

### Conclusion

Children after coarctation repair have diminished exercise capacity in combination with different patterns of oxygenated and deoxygenated hemoglobin at the level of the brains and at the muscular level. This points toward diminished blood flow and oxygen transport at the level of the brains and increased oxygen extraction at the level of the muscles during exercise. The increased muscular deoxygenation is more pronounced in children with higher residual coarctation gradient and blood pressure gradient. The measurement of peripheral oxygenation during exercise might provide useful information with regards to the disease state of the individual patient.

### DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

### ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ghent University Hospital. Written informed

consent to participate in this study was provided by the participants' legal guardian/next of kin.

## AUTHOR CONTRIBUTIONS

KV, IC, DD, and JB contributed to the study design. KV, IC, JP, AM, KD, and TB contributed to the data collection. KV, IC, AM, TB, HD, KF, and JB contributed to the data analysis. KV, IC, TB, DD, AM, and JB contributed to the data interpretation. KV, IC, JP,

KD, and JB contributed to the writing of the manuscript. AM, KD, TB, JP, HD, and KF contributed to the revision of the manuscript.

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