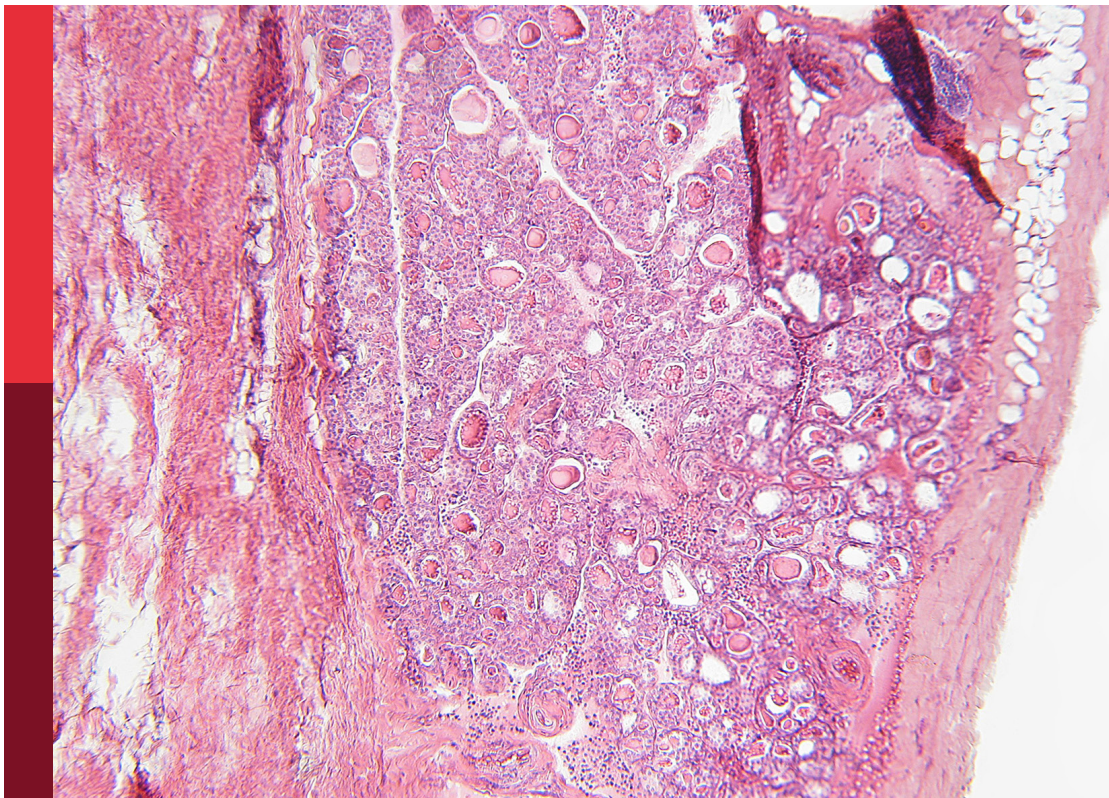
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Endocrine insights into heart disease

Edited by
Gabor Czibik, Li Chan, Eduardo Hertel Ribeiro and Daigo Sawaki

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Endocrine insights into heart disease

Topic editors

Gabor Czibik — Queen Mary University of London, United Kingdom
Li Chan — Queen Mary University of London, United Kingdom
Eduardo Hertel Ribeiro — Federal University of Minas Gerais, Brazil
Daigo Sawaki — Jichi Medical University, Japan

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EDITED AND REVIEWED BY
Gaetano Santulli,
Albert Einstein College of Medicine,
United States

*CORRESPONDENCE
Gabor Czibik
g.czibik@qmul.ac.uk

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Editorial: Endocrine insights into heart disease

Rogério Faustino Ribeiro Júnior[1], Daigo Sawaki[2], Li F. Chan[3] and Gabor Czibik[3]*

[1]Department of Physiological Sciences, Federal University of Espirito Santo, Vitoria, ES, Brazil, [2]Department of Clinical Pharmacology and Cardiology, Jichi Medical University, Shimotsuke, Japan, [3]Centre for Endocrinology, William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London, United Kingdom

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Editorial on the Research Topic:
Endocrine insights into heart disease

Apart from familial cardiomyopathies evolving based on disease-causing mutations of myocardial proteins, cardiovascular diseases do not develop in isolation. Common causes of and composite effects in cardiovascular disease tend to be more elusive. Some inducers include unhealthy diets promoting atherosclerosis or hypertension and age as well as sedentary lifestyle that are recognised determinants of cardiovascular disease. Increased incidence and prevalence of cardiovascular disease in patients with chronic liver or kidney disease underscore the importance of interdependent organ-organ interactions in health and disease. Sustained overactivation of the sympathico-adrenergic or renin-angiotensin-aldosterone systems (RAAS) straining the heart or vasculature illustrate classic endocrine influences over the cardiovascular system. These examples notwithstanding, relatively little is known about how dysfunction of one organ begets dysfunction in the cardiovascular system (1). Due to the reductionistic nature of scientific studies, there is a long way to the discovery of how seemingly disparate alterations impair cardiovascular health. This is particularly so when classic physiology does not come to the aid of our investigations.

There is much to be learned about an organism composed of symbiotic cells fated to live and die together. Questions include, but are not limited to how adverse influences work? Does a singular abnormality emerge as a loss-of-function of physiological activity (e.g. loss of kidney function altering extracellular volume or ion composition) or gain-of-function to induce detrimental changes (e.g., secretion of factors promoting organ damage)? How is the damage signal conveyed: via secretion, through bodily fluids, such as blood, the neural system or some other way? Which cells are responsible, and which ones are affected? How does the effect manifest? Are molecular effects mediated through receptors expressed by certain cell types or using other channels of cellular communication? Can we detect cardiovascular diseases from the blood? Are there easily measurable signs before a crippling disorder develops or is at a still reversible stage?

In line with these questions (to some of which we already have answers) there is a considerable clinical interest in discovering predictors (precede the condition, signifying its coming) or markers (simultaneous with the condition, and incrementally indicates degree

of severity) of cardiovascular disease. Criteria for good disease predictors and markers include that they draw attention to a specific condition to be prevented or stopped with great accuracy, i.e., with negligible chance for false negative and/or positive cases. By replacing or reducing the need for expensive equipment and/or specialised expertise, these diagnostic tools are meant to be simpler, and more cost-effective than traditional diagnosis. Functionally a good predictor/marker may be an epiphenomenon, a change that develops contemporaneously with the condition, but with no causal effect on the condition *per se*. It could be for example a product with a minor function co-secreted in stochiometric quantity with a more important factor (i.e., C-peptide co-secreted with insulin). The other kind of predictor/marker is intricately involved in the pathogenesis of the condition (such as insulin in glucose homeostasis and anabolism), without which the condition would develop very differently (type I and II diabetes mellitus). With regard to the diagnosis alone, it makes no difference whether the predictor/marker is pathogenically involved or not, as long as the condition is precisely reported. Research aiming to identify easily measurable, affordable disease predictors/markers could be especially useful for screening masses of people. This is a very important practical consideration, because in our overburdened healthcare systems, people, especially the underprivileged, are at a disadvantage to access diagnosis. A pathogenic involvement of predictors/markers is a bonus, as increased mechanistic understanding may be exploited therapeutically in the long run. For example, increased circulating levels of catecholamines and angiotensin II led to the development of new classes of medicines. Today, the countless human lives saved with beta blockers (2) and RAAS inhibitors (3) exemplify the potential of therapeutically targeting circulating messengers of harm.

Our goal with the call "*Endocrine insights into heart disease*" was to attract work to help connect the dots on a landscape spanning from primary abnormalities, through their responses down to cardiovascular disease. The exciting studies in this Research Topic cover an extended range of endocrine abnormalities, as briefly outlined below.

Classic hormones and cardiovascular health

Some of the papers offer novel insights into how imbalance of classic hormones may promote distinct cardiovascular conditions. Specifically, Liu et al. explored whether thyroid hormone replacement reverses diastolic dysfunction in adult patients with subclinical hypothyroidism. Whilst in this systematic review all 17 studies included reported normalised serum thyroid-stimulating hormone levels after levothyroxine treatment (n=568 patients), diastolic dysfunction was only partially rescued (e.g., E/A improved, but E/é did not). The authors admitted some study limitations (i.e., in sampling, inter-study variability in TSH values, limited observation times as well as unreported parameters with potential impact on diastolic function), which could be eliminated in a well-designed, large-scale randomised controlled study. do Val Lima et al. reported impaired myocardial contractility and oxidative phosphorylation in testosterone-deficient, orchidectomised rats. Intriguingly, mitochondrial dysfunction was restricted to interfibrillar mitochondria, i.e., those in charge of feeding sarcomeres with ATP, which could explain the link to contractile deficit. Myocardial effects of orchidectomy were not limited to mitochondrial bioenergetics, but also induced NADPH oxidase, a source of reactive oxygen species, whilst reduced expression of cellular antioxidant enzymes. Testosterone resupplementation rescued abnormalities. These observations support a unique sex-dependent role for testosterone in cardiac function, suggesting important clinical implications in patients undergoing castration due to disease or gender reassignment surgery. It will be interesting to see how and why testosterone controls interfibrillar but not subsarcolemmal mitochondria. Next, based upon the prothrombotic state observed in Cushing's syndrome, Brosolo et al. hypothesized that cortisol interferes with haemostasis in patients with essential hypertension. Consistent with the hypothesis, plasma samples showed higher D-dimer, prothrombin fragments 1 + 2 and von Willebrand factor levels across the upper tertile of cortisol and cortisol response to dexamethasone overnight suppression. Multivariate regression analysis established a positive relationship of the above coagulation factors to both cortisol and cortisol response to dexamethasone overnight suppression, independent of age, body mass index, blood pressure, and renal function. By identifying glucocorticoid-mediated haemostatic propensity as a surrogate mechanism to cause organ damage, essential hypertension seems to be more than its classic rheological consequences alone. Watanabe et al. studied fibroblast growth factor 23 (FGF23) and RAAS activity in C57BL6/j mice subjected to surgical pressure overload induced by transverse aortic constriction (TAC) vs. sham surgery. Sequential analysis revealed that cardiac FGF23 transcript but not circulating FGF23 levels increased in the early phase of pressure overload. By contrast, cardiac angiotensin-converting enzyme (ACE) activity and serum aldosterone levels increased in parallel with pressure overload. In addition to controlling left ventricular hypertrophy and RAAS activity, ACE inhibition with enalapril normalised cardiac FGF23 transcript levels. Liu et al. explored whether increased circulating Klotho levels increase or decrease cardiovascular risk. To explore this, they used patient data with cardiovascular disease from the National Health and Nutrition Examination database (NHANES; n=1905 patients). The authors found a U-shaped curve in terms of both all-cause and cardiovascular mortality, suggesting that an optimal Klotho dose may be required for survival. Finally, considering that α-Klotho (a member of the Klotho family) cleaves and activates FGF23, it is intriguing to speculate whether α-Klotho acts via FGF23 or any independent substrate (4).

Body composition and metabolism

Another important area revolves around body composition and metabolism. This is most timely due to the epidemic proportions obesity reaches, affecting our society in complex, elusive but

predominantly bad ways. The effect is not simply incremental, however, i.e., the more obese the worse. The obesity paradox indicates that obese patients show improved outcomes in myocardial infarction, stroke, or heart failure (5), despite obesity considered as a major risk factor for developing the same diseases. The subject remains highly contentious, hence more data is needed to shed light on this curious phenomenon. Wang et al. contributed to this debate with a systematic review investigating mortality after intracerebral haemorrhage and mortality. They found that in all 10 studies that met the inclusion criteria, obesity was protective, with 8 studies being statistically significant, whilst in 6 studies obesity associated with significantly lower short- and long-term mortality. Apart from the mass effect, subtleties of adipose tissue accumulation are illustrated by the subsequent two studies. First, Wu et al. studied the relationship between regional fat distribution and risk of developing atrial fibrillation. This large-scale, prospective study involved nearly half a million patients with evaluation of total and regional adipose tissue percentage along with incident atrial fibrillation. Over a follow-up of more than a decade almost 30000 patients developed atrial fibrillation. Curiously, fat mass, particularly that of the legs, associated with a reduced risk of developing atrial fibrillation. Second, Wang et al. explored the relationship between the volume of epicardial adipose tissue and premature ventricular complexes (PVCs). This retrospective study involving consecutive patients with PVC vs. matched control subjects found that larger epicardial adipose tissue mass was positively correlated with PVCs, and associated burden. Larger epicardial adipose tissue was observed in male patients with PVCs, greater body mass (BMI ≧24 kg/m (2)), diabetes mellitus, and impaired left ventricular relaxation. Therefore, epicardial adiposity, an adipose depot embracing the pumping heart, seems to extend myocardial predisposition to arrhythmias other than atrial fibrillation (6), probably through an arrhythmogenic secretome. Next, Cooper et al. was puzzled by the reasons for heterogeneous low-density lipoprotein (LDL)-cholesterol response to ketogenic diets. To study this, they observed lean, healthy premenopausal women with controlled periods of ketogenic and carbohydrate-containing regimes. Reintroduction of carbohydrates to ketogenic diet made notable hormonal (i.e., increased free T3 levels) and metabolic changes (i.e., increased fat mass, insulin levels). Amongst these, plasma LDL-cholesterol levels dropped when ketosis was interrupted by dietary carbohydrates. Intriguingly, LDL-cholesterol changes only correlated with body composition metrics, thyroid hormones, but not other metabolic parameters. The authors concluded that consistent with the increased lipid turnover to meet energy demands, lean individuals exposed to ketogenic diet were more susceptible to increases in circulating LDL-cholesterol levels. Beside LDL-cholesterol levels, insulin resistance and inflammation are other important risk factors for coronary heart disease. Dong et al. explored the utility of novel biomarkers of insulin resistance (triglyceride-glucose index: TyG), and inflammation (system immune-inflammation index: SII; & systemic inflammation response index: SIRI) in coronary heart disease among patients with non-alcoholic fatty liver disease (NAFLD). Besides established factors (i.e., triglyceride, LDL-cholesterol & neutrophiles promote, whilst HDL-cholesterol protect against coronary heart disease), logistic analysis suggested that TyG, SII and SIRI were all independent risk factors for coronary heart disease in NAFLD patients. Further analysis indicated that these innovative biomarkers, individually and especially combined, have high predictive value, therefore may aid diagnostic efforts. Our quest for the future will be to uncover the molecular underpinnings behind these exciting observations.

Effect of blood glucose +/- lipoprotein fractions on cardiovascular pathologies

Managing blood glucose is essential for reducing cardiovascular risks, but the intricate relationship between glycemic control and cardiovascular outcomes reveals deeper complexity, particularly involving different lipoprotein fractions. In particular, uncontrolled lipoproteins, through their varying biological effects, have been proposed to underlie divergent cardiovascular outcomes despite close glucose control. Studies highlight the distinct impacts of remnant- versus LDL-cholesterol in hypertension risk, demonstrate how apolipoprotein B mediates inflammation-related coronary heart disease, and emphasize the differential effectiveness of hypoglycemic agents in slowing carotid artery thickening, an early marker of cardiovascular disease. These observations underscore the necessity to adopt a more nuanced approach beyond standard blood glucose control and take specific lipid profiles and inflammatory markers into consideration in therapeutic decisions for cardiovascular disease.

Chen et al. took a deep dive into how SGLT2 inhibitors, originally designed to lower blood sugar in diabetes, protect patients with chronic kidney disease (CKD) from severe cardiovascular events. Remarkably, their analysis revealed that these medications significantly reduce hospital admissions for heart failure, the risks of heart-related and overall mortality. Even more reassuringly, these benefits did not come with increased rate of serious side-effects or urinary tract infections, though they did see an uptick in reproductive tract infections. This is invaluable knowledge for managing patients experiencing a combination of diabetes, kidney and heart disease. Shi et al. ventured beyond traditional markers like LDL-cholesterol, often tagged as "bad cholesterol," and highlighted the role of remnant cholesterol in hypertension. Their research underscored that higher levels of remnant cholesterol independently correlated with increased blood pressure—even in people whose LDL-cholesterol levels were within recommended limits. This finding challenges the traditional cholesterol paradigm, suggesting that clinicians should consider monitoring remnant cholesterol to more effectively identify and manage individuals at increased risk of hypertension and related cardiovascular diseases.

Taking a closer look at the inflammation-cholesterol nexus, Yang et al. investigated the roles of interleukin-1 receptor antagonist (IL-1Ra), a key player in inflammation, and

apolipoprotein B (apoB), a critical component of LDL particles. Their groundbreaking study revealed that genetically higher IL-1Ra levels increased the risk of coronary heart disease. However, this risk dramatically reversed once apoB was considered, indicating a role for apoB as a pivotal mediator in inflammation-driven cardiovascular disease. This discovery not only enhances our understanding of heart disease but also offers a promising target for potential treatments aiming to tackle both cholesterol and inflammation simultaneously. Finally, Lv et al. offered valuable, practical insights by comparing customary diabetes medications and their long-term effects on carotid intima-media thickness (cIMT), a critical marker of early artery stiffening and atherosclerosis. Their extensive network meta-analysis identified exenatide, alogliptin, and metformin as particularly effective choices to slow cIMT progression. This indicates that these drugs may carry unique cardiovascular protective properties beyond their blood sugar-lowering effects, providing clinicians with options for a more holistic approach to cardiovascular care.

Collectively, these studies reveal a new level of complexity: managing blood glucose is not equally effective across different aspects of cardiovascular health, largely because different lipoprotein fractions may undo any good achieved through prudent glucose control. Understanding these interactions can empower healthcare providers with more precise, individualized strategies to combat cardiovascular diseases linked with diabetes and metabolic conditions, ultimately improving patient outcomes and quality of life.

Miscellaneous clues

In this Editorial, we expose fascinating new insights revealed by recent research, exploring how hormones and other endocrine actors impact cardiovascular health. These studies help us better understand cardiovascular diseases, bringing us a step closer to easily accessible, early diagnosis and more effective treatments for improved patient care.

Tang et al. explored whether elevated serum levels of uric acid, the end-product of purine catabolism, challenge right ventricles of patients with connective tissue diseases. Using advanced cardiac imaging techniques, this study points out that higher serum uric acid levels are strongly linked to impaired right-sided heart function. This discovery underscores the importance of early diagnosis, and subsequent appropriate management of this condition, whilst providing mechanistic clues towards disturbed purine handling. Xu et al. shed light on an intriguing link between uterine fibroids and high blood pressure. Using a clever genetic analysis method, they established a direct connection between these benign tumors and elevated systolic blood pressure. This finding is particularly important for women's health, emphasizing the need for careful blood pressure monitoring in women with fibroids, especially during pregnancy. These findings also open the door to potential treatments that tackle uterine fibroids not just for their direct symptoms, but also as a means to control blood pressure.

Zou et al. used extensive hospital records to discover a link between abnormal serum osmolality (a measure of body fluid balance) and early mortality in heart failure patients. They found that, in a U-shape, both very low and very high levels increase the risk of death within the first month after hospitalization. This insight underscores how closely fluid and electrolyte management are tied to patient survival in acute heart conditions. Finally, Zhang et al. (2024) explored how the platelet-to-HDL cholesterol ratio may predict cardiovascular and mental health. Their study demonstrates a clear positive relationship between platelet-to-HDL cholesterol ratio levels, depression, and cardiovascular mortality. This finding suggests that platelet-to-HDL cholesterol ratio could serve as a simple and effective screening tool for depression-associated heart risks, ultimately improving early intervention efforts and patient outcomes.

Taken together, these studies offer crucial insights into the ways hormones and other endocrine signals affect our cardiovascular and overall health. By deepening our understanding of these mechanisms, we open new paths toward better diagnostics, targeted therapies, and ultimately, improved quality of life for patients battling cardiovascular diseases.

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EDITED BY
Rudolf Lucas,
Augusta University, United States

REVIEWED BY
Manuel Estrada,
University of Chile, Chile
Gabor Csanyi,
Augusta University, United States

*CORRESPONDENCE
Ivanita Stefanon
ivanita.stefanon@ufes.br

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Testosterone deficiency impairs cardiac interfibrillar mitochondrial function and myocardial contractility while inducing oxidative stress

Patrícia Ribeiro do Val Lima[1], Karoline Sousa Ronconi[1], Elis Aguiar Morra[1], Paula Lopes Rodrigues[1], Renata Andrade Ávila[1], Eduardo Merlo[2], Jones B. Graceli[2], Maylla Ronacher Simões[1], Ivanita Stefanon[1]* and Rogério Faustino Ribeiro Júnior[1]

[1]Department of Physiological Sciences, Federal University of Espirito Santo, Vitoria, ES, Brazil, [2]Department of Morphology, Federal University of Espírito Santo, Vitoria, ES, Brazil

Introduction: Clinical studies have shown that low levels of endogenous testosterone are associated with cardiovascular diseases. Considering the intimate connection between oxidative metabolism and myocardial contractility, we determined the effects of testosterone deficiency on the two spatially distinct subpopulations of cardiac mitochondria, subsarcolemmal (SSM) and interfibrillar (IFM).

Methods: We assessed cardiac function and cardiac mitochondria structure of SSM and IFM after 12 weeks of testosterone deficiency in male Wistar rats.

Results and Discussion: Results show that low testosterone reduced myocardial contractility. Orchidectomy increased total left ventricular mitochondrial protein in the SSM, but not in IFM. The membrane potential, size and internal complexity in the IFM after orchidectomy were higher compared to the SHAM group. However, the rate of oxidative phosphorylation with all substrates in the IFM after orchidectomy was lower compared to the SHAM group. Testosterone replacement restored these changes. In the testosterone-deficient SSM group, oxidative phosphorylation was decreased with palmitoyl-L-carnitine as substrate; however, the mitochondrial calcium retention capacity in IFM was increased. There was no difference in swelling of the mitochondria in either group. These changes in IFM were followed by a reduction in phosphorylated form of AMP-activated protein kinase (p-AMPK-α), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) translocation to mitochondria and decreased mitochondrial transcription factor A (TFAM). Testosterone deficiency increased NADPH oxidase (NOX), angiotensin converting enzyme (ACE) protein expression and reduced mitochondrial antioxidant proteins such as manganese superoxide dismutase (Mn-SOD) and catalase in the IFM. Treatment with apocynin (1.5 mM in drinking water) normalized myocardial contractility and interfibrillar mitochondrial function in

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the testosterone depleted animals. In conclusion, our findings demonstrate that testosterone deficiency leads to reduced myocardial contractility and impaired cardiac interfibrillar mitochondrial function. Our data suggest the involvement of reactive oxygen species, with a possibility of NOX as an enzymatic source.

KEYWORDS

testosterone, mitochondria, cardiac contractility, subsarcolemmal, interfibrillar, mitochondrial subpopulations, oxidative stress

Introduction

Androgen deficiency commonly occurs in middle-aged and older men (1, 2) and is sometimes observed in younger men with hypothalamic-pituitary or testicular disorders (3, 4). Since the discovery of the androgen receptor in the heart and cardiomyocytes (5), evidence suggests that testosterone regulates heart function by direct actions on the myocardium. Supraphysiological testosterone levels have been associated with sudden cardiac death (6), cardiac hypertrophy (7), thrombosis (8), and may increase the rate of atherosclerosis progression leading to coronary artery disease and myocardial ischemia (9, 8). However, the mechanisms mediating testosterone's myocardial actions remain unclear.

Clinical studies have shown that low levels of endogenous testosterone are also associated with cardiovascular diseases (10, 11) and a randomized trial showed that testosterone replacement improved functional exercise capacity, muscle strength, insulin and baroreflex sensitivity (2).

Testosterone plays an important role in the regulation of carbohydrate, fat and protein metabolism (12), which are strictly regulated by substrate availability and endocrine signaling (13). Therefore, changes in substrate availability, or testosterone levels could affect the supply of ATP from mitochondria. Since the heart has a limited storage capacity for ATP and very high-energy demand, the heart must continually generate ATP at a high rate to sustain contractile function, basal metabolic processes, and ionic homeostasis (14). Due to the intimate connection between oxidative metabolism and myocardial contractility, changes in mitochondrial function could result in reduced oxidative phosphorylation and contractility (15, 16).

In terms of energy production, cardiac cells contain two spatially distinct mitochondrial subpopulations: the subsarcolemmal subpopulation (SSM), located just below the sarcolemma and the interfibrillar subpopulation (IFM) which is located between the myofibrils (17–19). These two mitochondrial subpopulations not only differ in terms of their location, but their biochemistry (20). The literature suggests that SSM produces ATP to maintain the active transport of electrolytes and metabolites across the cell membrane, while IFM produce ATP to maintain the contractile function due to its location near the contractile machinery (17). Several studies demonstrated that the two subpopulations respond differently to physiological or pathological stimuli (17). Our hypothesis is that testosterone deficiency reduces cardiac and mitochondrial function in both subpopulations that are dependent on oxidative stress.

The present study aims to assess (1) cardiac contractility, (2) mitochondrial function, (3) oxidative phosphorylation, (4) morphology and mitochondrial membrane potential, and (5) permeability transition pore opening in the two subpopulations of cardiac mitochondria, thus providing new information on the effect of testosterone deficiency on cardiac contractility and mitochondrial function.

Materials and methods

Male Wistar rats (250 g,12 weeks old) were acquired from the Federal University of Espirito Santo (UFES), Brazil and randomly divided into 3 groups (n = 30) as follows: a) SHAM (control) - The animals of this group underwent the surgical procedure (anesthesia and surgical incision) without orchidectomy; b) OQT Group (orchidectomy) – This group underwent surgery with radical orchidectomy (bilateral) and c) OQT + T Group (orchidectomy + testosterone). These orchidectomized animals were treated with testosterone (2 mg/kg i.m. daily) or placebo as previously described (21). In order to understand the influence of oxidative stress, another group of OQT rats (n = 8) was treated with apocynin (1.5 mM in drinking water) for 12 weeks.

After 12 weeks of treatment, all rats were anesthetized with urethane (1.2 $g.kg^{-1}$). The thorax was opened, and blood was collected from the left ventricle (LV) and immediately placed on ice and centrifuged to obtain serum. The heart was removed, and the left and right ventricle removed and weighed. Sections of each ventricle were taken for biochemical analysis and stored at −80°C. The remaining cardiac tissue was used for mitochondrial isolation as described below. The liver, lungs, seminal vesicles, epididymal and retroperitoneal fat were also removed and weighed. The care and use of the laboratory animals was performed in accordance with *NIH* guidelines and the animal ethics committee of the Federal University of Espirito Santo (CEUA-UFES number: 072/2012) approved all procedures.

Preparation of left ventricle papillary muscles

The heart was quickly excised, and the posterior papillary muscle was secured utilizing a set of rings. One of these ends was

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affixed to maintain stability, while the other was linked to a force transducer (TSD125 – Byopac Systems, Inc; CA), which was further linked to an amplifier (DA100C Byopac Systems, Inc; CA). Both the transducer and the amplifier were housed within a glass container brimming with 20 mL of nutrition solution with specific proportions of electrolytes and glucose (NaCl 120 mM; KCl 5.4 mM; CaCl2 1.25 mM; MgCl2 1.2 mM; NaH2PO4 2 mM; Na2SO4 1.2 mM; NaHCO3 18 mM and glucose 11 mM). This solution was subjected to a 95% O2 and 5% CO2 mixture at 30°C, pH 7.4. Subsequently, the papillary muscles underwent electrical stimulation provided by a pair of silver electrodes, which delivered rectangular pulses of 15 ms duration at a voltage 1.5 times the threshold (10 – 20 mV, 0.5 Hz). The papillary muscles were stretched to the point of maximum active tension muscle length (Lmax). Following this, a stabilization period of 30 minutes was allowed before the beginning of the experimental protocols. Data was captured using an acquisition system (MP100 Byopac Systems, Inc; CA), registering at an impressive rate of 500 samples per second. At the end, the papillary muscles were weighed, and the developed isometric contraction force was adjusted according to the weight of the muscles. These included the isometric contraction, presented as force expressed in grams per milligram of muscle weight (F; g/mg), the activation and relaxation times (time from contraction initiation to maximum strength peak and maximum peak time to 50% of isometric relaxation respectively), as well as values obtained from positive and negative force time derivatives (+ and - dF/dt; g/mg/s). The assessment of inotropic response to calcium was analyzed using a calcium concentration-response curve, applying varied concentrations of extracellular calcium: 0.62, 1.25, 2.5, and 3.75 mM $CaCl_2$ in the perfusion solution. The resulting force variation was weight-corrected for the muscles and expressed in g/mg.

Mitochondrial isolation

SSM and IFM were isolated according to the method of Palmer et al. (20), with minor modification (19 – 22). Briefly, the LV was rinsed in ice-cold Chappel-Perry buffer (100 mM KCl, 50 mM MOPS, 5 mM $MgSO_4$, 1 mM ATP, 1 mM EGTA, 2 mg/ml BSA), blotted dry and then weighed. The ventricles were minced and homogenized in 1:10 (wt/vol) ice-cold Chappel-Perry buffer. The homogenates were centrifuged at 700 × g for 10 min. The supernatant containing SSM was extracted and centrifuged again at 10,000 x g to isolate SSM. The remaining pellet from the 700 x g spin was resuspended in KCl-MOPS-EGTA buffer containing 100 mM KCl, 50 mM MOPS, and 0.5 mM EGTA at pH 7.4, and treated with trypsin (5 mg/g) for 10 min at 4°C. The samples were incubated with trypsin inhibitor (albumin) and spun down at 700 x g for 10 min. The IFM-containing supernatant was then spun down at 10,000 x g for 10 min. The pellets were washed twice in ice-cold Chappel-Perry buffer and spun down at 10,000 x g for 10 min and then resuspended in ice KME buffer. The concentration of mitochondrial protein was measured by the Lowry method using bovine serum albumin as a standard.

Mitochondrial respiration

Mitochondrial respiration was assessed in both IFM and SSM as described previously (18 – 23). Isolated mitochondria (0.5 mg/mL) were respired in respiration buffer containing 100 mM KCl, 50 mM 3-(N-morpholino)propanesulfonic acid buffer (MOPS), 5 mM KH_2PO_4, 1 mM EGTA and 1mg/mL BSA/Fraction V, using a polarographic oxygen-sensing systems for measurement of oxygen consumption in liquid suspension (Qubit System, Kingston, ON, Canada). States 3 and 4 respiration were measured with glutamate + malate (10 and 5 mM, respectively), pyruvate + malate (10 and 5 mM, respectively), palmitoyl-L-carnitine (40 µM), succinate (20 mM) + rotenone (7.5 µM) or ascorbate (0.2 mM) + N, N,N',N'-tetramethyl-p-phenylenediamine (TMPD 0.5 mM) to assess respiration through the electron transport chain (ETC), exclusively. State 3 respiration (ADP-stimulated) was measured in the presence of 2 mM ADP. State 4 respiration (ADP-limited) was assessed after ADP consumption. Respiratory control ratio, the ratio of state 3 to state 4 was calculated to assess the control of oxygen consumption by phosphorylation. The ratio of ADP added in the chamber to the total amount of oxygen consumed in state 3 (ADP:O ratio) was calculated as an index of the efficiency of oxidative phosphorylation.

Mitochondrial size and membrane potential

Mitochondrial size and membrane potential were measured as previously described (24, 19). Briefly, isolated IFM and SSM were stained with MitoTracker Green FM (Molecular Probes, Carlsbad, CA) and assessed using a flow cytometer (FACSCanto II, BD Biosciences). To confirm differences in absolute mitochondria size, we included size calibration beads composed of microsphere suspensions ranging in size from 0.5 to 6 µm to serve as reliable size references. The arithmetic mean output from the forward scatter detector was used as an index of mitochondrial size. For membrane potential, mitochondria were incubated with 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazol carbocyanine iodide (JC-1) (Molecular Probes, Carlsbad, CA) at a final concentration of 0.3 µM. The shift to orange is due to dye aggregates forming upon polarization, causing a shift in emitted light from 530 nm (green) to 590 nm (orange).

Mitochondrial permeability transition pore opening

Ca^{2+}-induced MPTP was evaluated in SSM and IFM from LV myocardium using previously described method (18, 19). The capacity for Ca^{2+} uptake was evaluated in isolated mitochondria by following the extramitochondrial [Ca^{2+}] during a progressive, escalating exposure to Ca^{2+}. Briefly, using a 96-well fluorometric plate reader at 37°C, 250 µg/mL of mitochondrial protein was suspended in calcium free buffer (100 mM KCl, 50 mM MOPS, 5

mM KH_2PO_4, 1 mM $MgCl_2$, 5 mM EGTA) with glutamate + malate as substrate (5 and 2.5 mM). A bolus injection of 25 nmol free Ca^{2+} was injected every 7 min, and extramitochondrial [Ca^{2+}] was recorded every 40-s using 750 nM Calcium Green-5N.

Hormonal assay

Serum levels of testosterone and estrogen were measured according to the manufacturer. Testosterone levels were measured using the Testosterone ELISA kit (#29K116 42K034DRG Instruments GmbH, Germany). The assay detection limit for testosterone was 0.083 ng/mL. The intra-assay coefficient of variation for each assay was between 3.28% and 4.16%. The inter-assay coefficient of variation for each assay was between 4.73% and 9.94%. Estrogen levels were measured using the Estrogen ELISA kit (#42K026-3DRG Instruments GmbH,Germany) (25, 26). The assay detection limit for estradiol was 10.60 pg/mL. The intra-assay coefficient of variation for each assay was between 8.70% and 9.23%. The inter-assay coefficient of variation for each assay was between 6.87% and 14.91%.

Western blotting

Western blot was performed as previously described (27). Samples (25-80 μg for whole heart sample and 10-30 μg for isolated mitochondria) were separated using 7.5%, 10% or 12% SDS-PAGE. Proteins were transferred to nitrocellulose membranes and incubated with specific antibodies: goat polyclonal anti-Serca-2 (1:1000; Santa Cruz Biotechnology-USA), rabbit polyclonal anti-p-PLB^{Ser16} (1:2500; Bradrilla-UK), mouse monoclonal anti-PLB (1:3000; Santa Cruz Biotechnology-USA), rabbit polyclonal anti-PGC-1α (1:1000; Santa Cruz Biotechnology-USA), rabbit polyclonal anti-NRF1 (1:500; Santa Cruz Biotechnology-USA), mouse monoclonal anti-Mfn-1 (1:200; Santa Cruz Biotechnology-USA), mouse monoclonal anti-Mfn-2 (1:200; Santa Cruz Biotechnology-USA), rabbit polyclonal anti-p-AMPK-α (1:1000, Cell Signaling, USA), rabbit polyclonal anti-AMPK-α (1:1000, Cell Signaling, USA), mouse monoclonal anti-total OXPHOS (CI subunit NDUFB8, CII-30kDa, CIII-Core protein 2, CIV subunit I and CV alpha subunit, 1:2500, Abcam, USA), rabbit polyclonal anti-MCU (1:1000, from Sigma-Aldrich-USA), rabbit polyclonal anti-SOD-1 (1:6000; Sigma-Aldrich-USA), mouse monoclonal anti gp91phox (1:2500 from Transduction Laboratories-USA), rabbit polyclonal anti SOD-1 (1:2000, Sigma-Aldrich-USA), mouse monoclonal anti-Cyclophilin D (1:2000, Mitoscience, USA), mouse monoclonal anti-TFAM (1:1000, Santa Cruz Biotechnology-USA), mouse monoclonal anti-catalase (1:12000, Sigma-Aldrich-USA), rabbit polyclonal anti-SOD-Mn (1:5000, Millipore-USA), rabbit polyclonal anti-NOX-1 (1:1000, Sigma-Aldrich-USA), rabbit polyclonal anti-ACE-1 (1:200, Santa Cruz Biotechnology-USA).

After washing, the membranes were incubated with anti-mouse (1:5.000, Sigma-Aldrich-USA), anti-goat (1:5.000, Sigma-Aldrich-USA) or anti-rabbit (1:5000, Sigma-Aldrich-USA) immunoglobulin antibodies conjugated to horseradish peroxidase. The immunocomplexes were detected using ECL Prime (Amersham International-UK) and the bands were detected using a Chemidoc system (Bio-Rad, USA). The same membranes were then used to determine GAPDH expression using a mouse monoclonal antibody (1:5000; Santa Cruz Biotechnology-USA) or ponceau staining for isolated mitochondria.

Hydrogen peroxide production

Hydrogen peroxide production in isolated mitochondrial subpopulations IFM and SSM was determined using the oxidation of the fluorogenic indicator Amplex Red (Invitrogen) in the presence of horseradish peroxidase as previously described (28). The concentrations of horseradish peroxidase and Amplex Red in the incubation were 0.1 unit/mL and 50μM, respectively, and detection of fluorescence was assessed on a Molecular Devices Flex Station 3 fluorescence plate reader (Molecular Devices, Sunnyvale, CA) with 530-nm excitation and 590-nm emission wavelengths. Standard curves were obtained by adding known amounts of H_2O_2 to the assay medium in the presence of the substrates amplex red and horseradish peroxidase. H_2O_2 production was initiated in mitochondria using glutamate + malate (G + M) and succinate + rotenone (S + R) as substrates.

Protein oxidation detection

To identify carbonyl groups that are introduced into the amino acid side chains after oxidative modification of proteins, 2D-oxyblot analysis was performed (29). The derivative that was produced by reaction with 2,4-dinitrophenylhydrazine (DNPH) was immunodetected by an antibody specific to the attached DNP moiety of proteins using a commercial kit (Oxyblot, Millipore).

The DNPH derivatization was carried out on 20 μg of protein for 15 minutes according to the manufacturer's instructions. One-dimensional electrophoresis was carried out on a 12% SDS/polyacrylamide gel after DNPH derivatization. Proteins were transferred to nitrocellulose membranes which were then incubated in the primary antibody solution (anti-DNP 1: 150) for 2 h, followed by incubation in the secondary antibody solution (1:300) for 1 h at room temperature. The washing procedure was repeated eight times within 40 minutes. Immunoreactive bands were visualized by enhanced chemiluminescence (ECL; Amersham Biosciences) and immunodetection was carried out using a ChemiDoc system (Bio-Rad). To determine specificity, the oxidized proteins provided by the kit were included as a positive control. Treatment of samples with a control solution served as a negative control to the DNPH treatment. As an additional control, the anti-DNP antibody was omitted.

Statistical analysis

Values are shown as mean ± standard error of the mean (SEM). Differences were analyzed using one or two-way ANOVA followed by a Fisher test. $P < 0.05$ was considered significant.

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Results

Ponderal measurements

As shown in Table 1, castration of male animals for 12 weeks did not change body weight, left or right ventricle weight, demonstrating that endogenous testosterone does not affect the heart mass. As expected, castration decreased seminal vesicle weight after 12 weeks when compared to SHAM and testosterone replacement was able to restore this value.

Testosterone and estrogen serum levels

Testosterone levels decreased after 12 weeks in OQT animals and (SHAM: 2.09 ± 0,18 ng/mL; OQT: 0,53 ± 0.17 ng/mL* and OQT + T: 2.16 ± 0.32 ng/mL# n = 6 animals per group) and testosterone replacement restored to the SHAM level. Estradiol was also measured and was not different between groups (SHAM: 71.6 ± 5.2 pg/mL; OQT: 76 ± 5 pg/mL; OQT + T: 68 ± 7.71 pg/mL n = 6 animals per group).

Myocardial contractility

Myocardial contractility was analyzed using isolated papillary muscles at baseline and after exposure to different extracellular calcium concentrations. As expected, increasing extracellular calcium concentration resulted in a positive inotropic response in papillary muscles of all groups. However, the inotropic response to calcium at the concentrations of 0.62, 1.25, 2.5 and 3.75 mM were lower in the OQT group. (Figures 1A–C). Testosterone replacement prevented the reduction in contractile force because papillary muscles from OQT + T group presented similar inotropic responses as the SHAM group (Figures 1A–C). Figure 1C shows representative contractile responses recorded from papillary muscles paced at 0.5 Hz.

In myocardial cells, calcium handling depends upon Serca-2a and PLB function (30, 31). Serca2a and PLB protein expressions were measured in the hearts of our animals. As shown in Figure 1D, Serca2a protein expression was significantly decreased in the OQT group and this decrease was prevented by testosterone replacement. PLB protein expression was significantly increased in the OQT group and testosterone replacement prevented this increase in PLB protein expression (Figure 1E). We also measured phosphorylated phospholamban (P-PLB) at ser^{16}, the main target for PKA phosphorylation. P-PLB levels were significantly reduced in the OQT group, indicating that PLB activity was enhanced in the OQT group (Figure 1F).

Mitochondrial yield, size, and internal complexity

Yield refers to the amount of mitochondrial protein per gram of tissue. The yield for IFM was similar between the groups (Figure 2A); however, the yield for SSM was significantly increased in the OQT group when compared to SHAM (Figure 2D). Testosterone replacement prevented the increase in mitochondrial yield in the castrated animals. (Figure 2D).

To determine mitochondrial membrane potential and morphological differences between SHAM, OQT and OQT + T mitochondrial subpopulations, we used a gated flow cytometry method. Forward-scattered light (FSC) was used to estimate mitochondrial size, whereas assessment of side-scattered light (SSC) was used to estimate mitochondrial complexity, both of which were based on a logarithmic scale. Membrane potential of the mitochondrial subpopulations was analyzed by flow cytometry. Overall, baseline mitochondrial membrane potential (with endogenous substrate) was significantly increased in the IFM from OQT animals (Figure 2B), whereas the membrane potential was unchanged in the SSM (Figure 2E). We next incubated the samples with glutamate + malate (10 and 5 mM, respectively) to stimulate the ETC. While there was an initial increase in membrane

TABLE 1 Ponderal data from SHAM, OQT and OQT plus testosterone 12 weeks after castration and testosterone replacement therapy.

	groups		
	SHAM n=10	OQT n=10	OQT+T n=10
Body weight (g)	447 ± 48	429 ± 47	437 ± 31
Left ventricle (g)	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
Right ventricle (g)	0.2 ± 0.04	0.2 ± 0.05	0.2 ± 0.03
Liver (g)	15 ± 1.5	12.9 ± 1.6	12.9 ± 1.1
Lung (g)	2.2 ± 0.3	2.3 ± 0.4	2.3 ± 0.3
Epididymal fat (g)	7.3 ± 2.9	5.4 ± 1.7	5.5 ± 1
Retroperitoneal fat (g)	9.9 ± 3.9	12.7 ± 4.1	10.4 ± 3.4
Seminal vesicle (g)	1.1 ± 0.2	0.1 ± 0.02*	0.7 ± 0.2#
Tibia (mm)	40.3 ± 1.1	39.8 ± 1.3	39.9 ± 0.5

Data are expressed as mean ± S.E.M. *P<0.05 compared to SHAM. #P<0.05 compared to OQT group.

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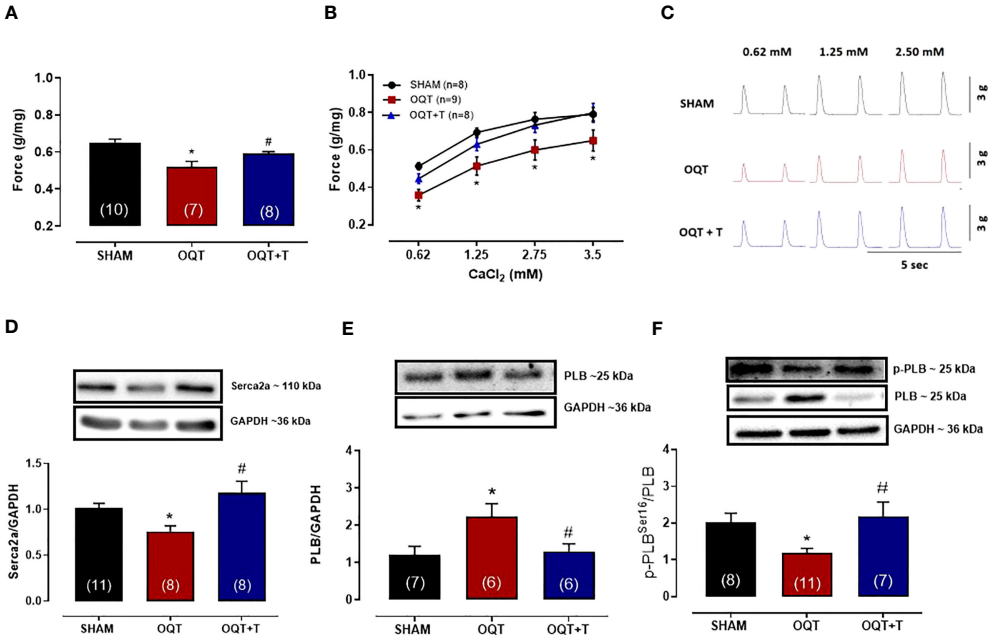


FIGURE 1
Testosterone deficiency decreases myocardial contractility after twelve weeks of hormone deprivation. The isometric force (g/g) in the left ventricular papillary muscles at baseline **(A)**, with different extracellular $CaCl_2$ (0.62,1.25, 2.5 and 3.75 mM) concentrations **(B)**. Representative myograph tracing recorded from papillary muscles with different extracellular $CaCl_2$ (0.62, 1.25 and 2.5 mM) concentrations from SHAM, OQT and OQT + T groups **(C)**. Western blot analyses of Serca2a protein expression **(D)**, total phospholamban **(E)** and phosphorylated phospholamban at Ser^{16} **(F)**. Results are presented as mean ± SEM values. Differences were analyzed using one-way ANOVA followed by Fischer *post-hoc* test. *P<0.05 compared to SHAM; #P<0.05 compared to OQT. Number of animals is indicated in parenthesis.

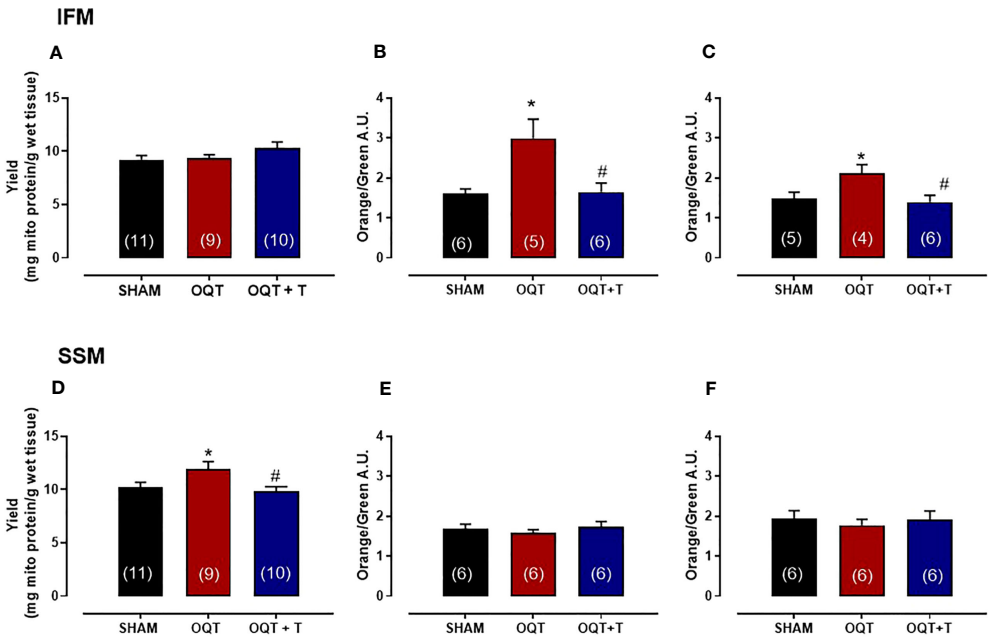


FIGURE 2
Testosterone deficiency increases mitochondrial membrane potential in interfibrillar mitochondria (IFM). Mitochondrial yield for isolated interfibrillar mitochondria (IFM) **(A)**, membrane potential with glutamate + malate (5 and 2.5 mM, respectively) **(B)** and membrane potential in the absence of substrate **(C)**. Mitochondrial yield for subsarcolemmal mitochondria (SSM) **(D)**, membrane potential with glutamate + malate (5 and 2.5 mM, respectively) **(E)** and membrane potential in the absence of substrate **(F)**. Isolated mitochondria stained with 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazol carbocyanine iodide (JC-1), which incorporates into intact mitochondria. Values for membrane potential are expressed as arbitrary units (AU). Serum testosterone levels (H) from SHAM, OQT and OQT + T groups. Results are presented as mean ± SEM values. Differences were analyzed using one-way ANOVA followed by Fischer *post-hoc* test. *P<0.05 compared to SHAM; #P<0.05 compared to OQT. Number of animals is indicated in parenthesis.

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potential in all groups when the substrates were added, membrane potential was significantly increased in the IFM, but not the SSM from the OQT groups (Figures 2C, F. For both substrates, the increase in IFM membrane potential was prevented by testosterone replacement (Figures 2B, C).

Mitochondrial morphology was assessed by flow cytometry to show relative morphological differences between the two subpopulations and confirm the success of the isolation. SSM were larger in size (FSC) and possessed greater internal complexity (SSC) compared with IFM, which were smaller and more compact (Figures 3A–D). Castration significantly increased the size and complexity of IFM compared to SHAM (Figures 3A, C). These increases were prevented by testosterone replacement. Conversely, the size and complexity of SSM were not affected by castration (Figures 3B, D).

To further investigate the mechanisms involved in mediating the changes in mitochondrial morphology, we developed western blots for mitofusins 1 and 2 which are involved in the regulation of mitochondrial fusion. Figures 3E, F show the expression of mitofusins 1 and 2, respectively. Mfn-1 protein expression was

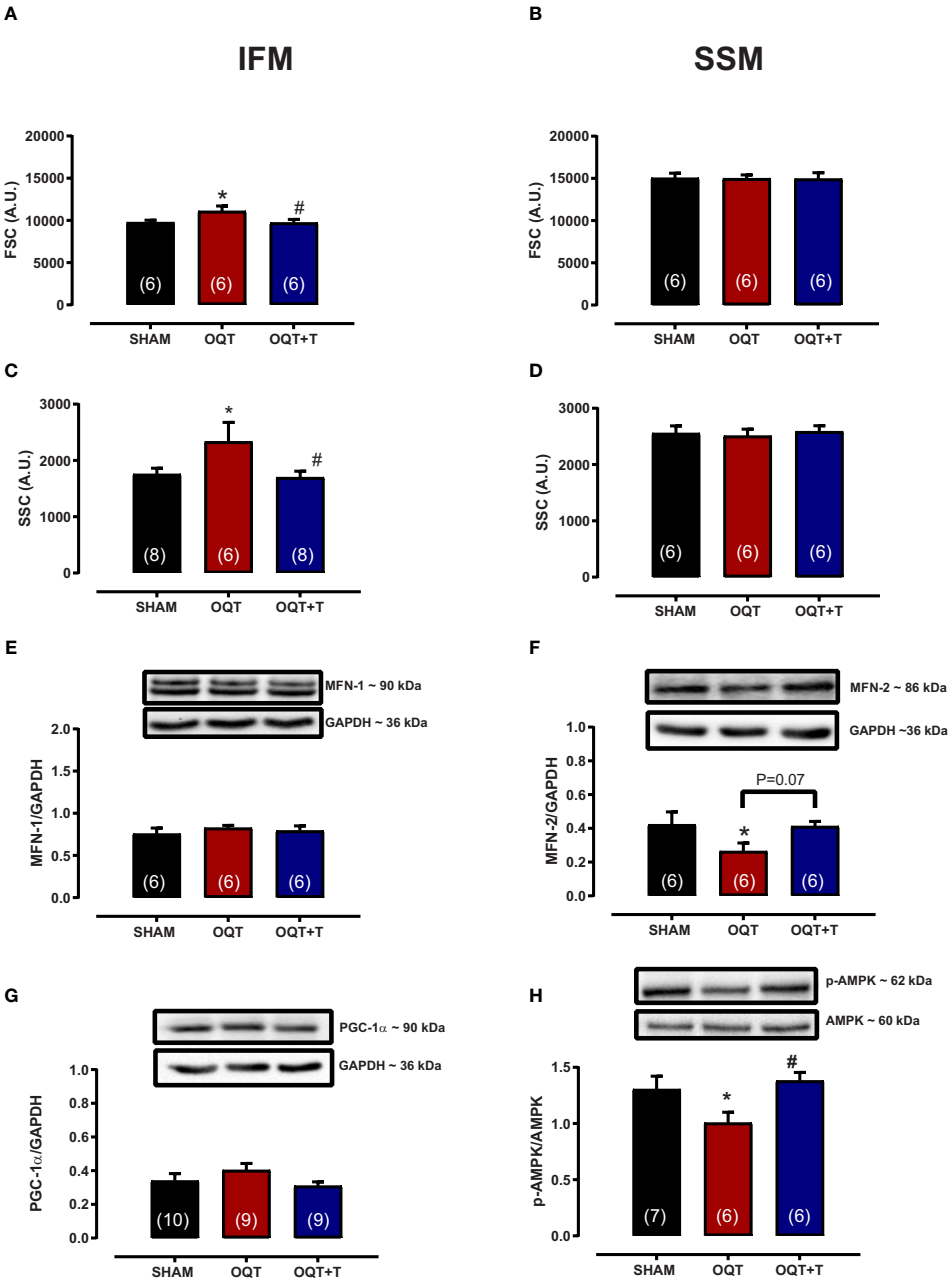


FIGURE 3
Testosterone deficiency increases interfibrillar mitochondrial internal complexity and size. Determination of the relative size **(A, B)** and internal complexity **(C, D)** of distinct mitochondrial subpopulations IFM and SSM using flow cytometric analyses. Western blot for mitofusion-1 **(E)**, mitofusion-2 **(F)**, PGC-1α **(G)**, and p-AMPK/AMPK **(H)** in whole heart tissue from SHAM, OQT and OQT+T groups. Results are presented as mean ± SEM values. Differences were analyzed using one-way ANOVA followed by Fischer *post-hoc* test. *P<0.05 compared to SHAM; #P<0.05 compared to OQT. Number of animals is indicated in parenthesis.

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not altered between the groups. Mfn-2 was decreased significantly in the OQT group (Figure 3F). As mitochondrial biogenesis is regulated by PGC-1α and PGC-1α translocation to mitochondria is regulated by AMPK-α, we also measured the expression and phosphorylation of these two proteins. PGC-1α protein expression was not altered after twelve weeks of hormone deprivation (Figure 3G), whereas AMPK-α protein phosphorylation was decreased in the OQT group and restored to control levels by testosterone replacement (Figure 3H).

Mitochondrial respiration

To assess the effects of testosterone deficiency on mitochondrial bioenergetics in the IFM and SSM, of the SHAM, OQT and OQT + T groups, we measured mitochondrial respiration with different substrates (glutamate + malate, pyruvate + malate, palmitoyl-L-carnitine, succinate + rotenone and ascorbate + TMPD) that use distinct oxidative pathways, mitochondrial transport and provide specific substrates to Complex I, Complex II and Complex IV.

The maximal rate of mitochondrial respiration (State 3) in IFM expressed as nanomoles of oxygen per mg of mitochondrial protein was lower in the OQT group when compared to SHAM and OQT +T with glutamate + malate, pyruvate + malate, rotenone + succinate, palmitoyl-L-carnitine and ascorbate + TMPD as substrates (Figures 4A–E). To further investigate if the reduction in state 3 in the OQT group was due to low expression of the ETC complexes, we developed western blots for complex I-V, which showed that testosterone deficiency increased complex I and II protein expression in IFM (Figure 4F). These increases were prevented by testosterone replacement.

We also measured state 3 with all substrates in SSM. State 3 was reduced with pyruvate + malate (Figure 5B) and rotenone + succinate (Figure 5C), but not with glutamate + malate (Figure 5A), palmitoyl-L-carnitine (Figure 5D) or ascorbate + TMPD (Figure 5E). Western blots for complex I-V did not reveal any differences in protein expression for the SSM (Figure 5F).

Mitochondrial permeability transition pore opening

Two standardized methods were used to assess Ca^{2+}-induced MPTP in LV mitochondria. MPTP was assessed from mitochondrial swelling induced by high [Ca^{2+}] as reflected by the decrease in absorbance at 540 nm following the addition of a bolus of Ca^{2+} to isolated mitochondria. Measurement of baseline absorbance before the addition of Ca^{2+} was performed to

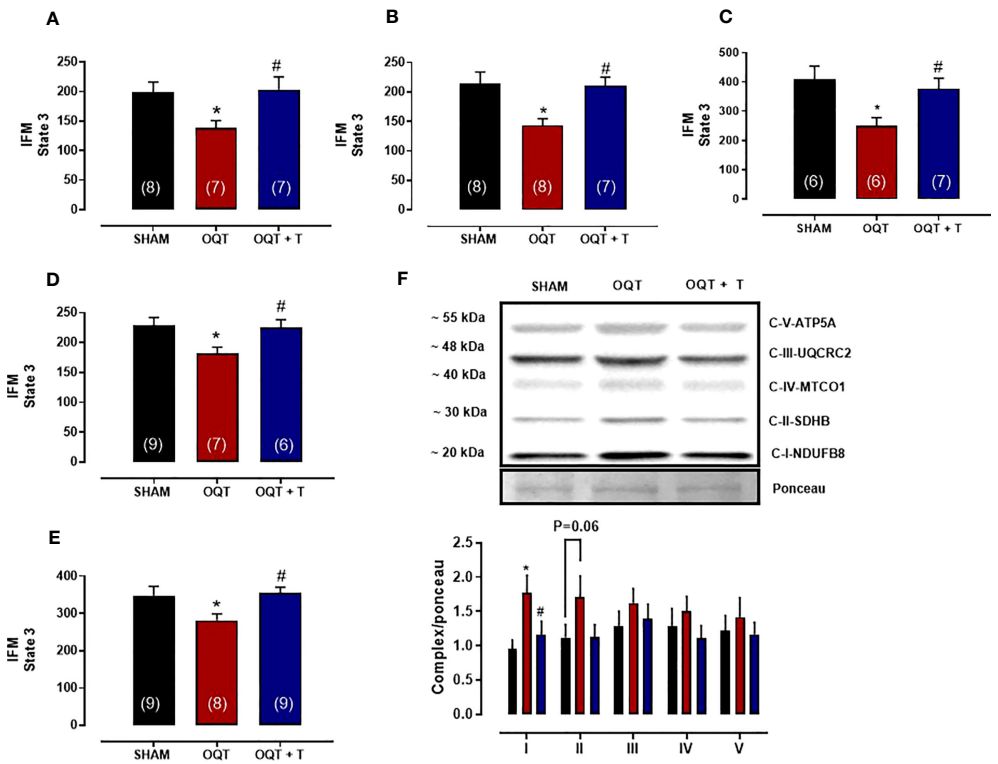


FIGURE 4
Testosterone deficiency decreases oxidative phosphorylation in interfibrillar mitochondria (IFM). Individual mitochondrial subpopulations were isolated from SHAM, OQT and OQT+T and polarographic measurements were performed to index oxygen consumption under state 3 and 4 respiration conditions. Respiration of individual electron transport chain (ETC) complexes was defined as the rate of oxygen consumed in the presence of specific substrates [glutamate and malate (complex I) **(A)**, β-oxidation **(B)**, ascorbate and N,N,N′,N′-tetramethyl-p-phenylenediamine (complex IV) **(C)**, pyruvate + malate **(D)** and succinate (complex II) **(E)**. All respiration rates are expressed in atoms O/mg mitochondrial protein^{-1}. min^{-1}. Western blot for total OXPHOS in IFM **(F)**. Results are presented as mean ± SEM values. Differences were analyzed using one-way ANOVA followed by Fischer *post-hoc* test. *P<0.05 compared to SHAM; #P<0.05 compared to OQT. Number of animals is indicated in parenthesis.

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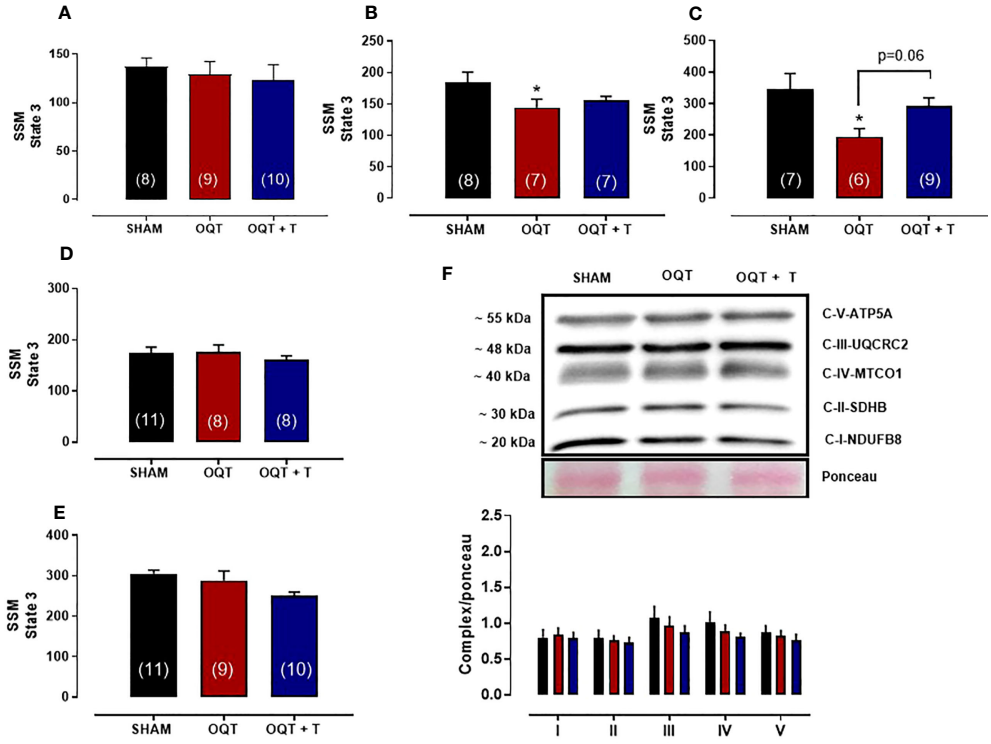


FIGURE 5
Testosterone deficiency decreases oxidative phosphorylation in subsarcolemmal mitochondria (SSM). Individual mitochondrial subpopulations were isolated from SHAM, OQT and OQT+T and polarographic measurements were performed to index oxygen consumption under state 3 and 4 respiration conditions. Respiration of individual electron transport chain (ETC) complexes was defined as the rate of oxygen consumed in the presence of specific substrates [glutamate and malate (complex I)] **(A)**, β-oxidation **(B)**, ascorbate and N,N,N′,N′-tetramethyl-p-phenylenediamine (complex IV) **(C)**, pyruvate + malate **(D)** and succinate (complex II) **(E)**. All respiration rates are expressed in atoms O/mg mitochondrial protein^{-1}. min^{-1}. Western blot for total OXPHOS in subsarcolemmal mitochondria **(F)**. Results are presented as mean ± SEM values. Differences were analyzed using one-way ANOVA followed by Fischer *post-hoc* test. *P<0.05 compared to SHAM. Number of animals is indicated in parenthesis.

determine basal values. There was a decrease in absorbance with addition of either 100 or 500 nmol Ca^{2+}/mg protein in all groups, but no difference was found between the groups (data not shown).

Ca^{2+}-induced MPTP was also assessed by measuring the ability of isolated mitochondria to take up added Ca^{2+}. As shown in Figure 6 (top panel), IFM from the OQT group had significantly enhanced Ca^{2+} retention capacity compared to the SHAM and OQT + T groups. The Ca^{2+} uptake in SSM was the same in all groups (Figure 6, bottom panel).

To further elucidate whether these alterations in mitochondrial Ca^{2+} retention capacity were due to changes in cyclophilin D or MCU, we developed western blots for these two proteins. As shown in Figure 6, there were no differences in protein expression in either IFM (top panel) or SSM (bottom panel).

Mitochondrial antioxidant enzymes, TFAM and PGC-1α translocation to mitochondria

PGC-1α trans-activates nuclear respiratory factor 1, which in turn, activates mtDNA transcription factor A (TFAM) that regulates mtDNA transcription and replication. Subsequently, NRF-1 promotes transcription of mitochondrial transcription factor TFAM, which targets mtDNA-encoded genes. TFAM protein expression was significantly reduced after castration in IFM (Figure 7A), but not in SSM (Figure 7B). Again, the reduction in TFAM could be prevented by testosterone replacement (Figure 7A).

In the IFM, the expression of mitochondrial Mn-SOD (Figure 7C) and catalase (Figure 7E) were significantly decreased after castration and this reduction was prevented by testosterone replacement. In SSM, mitochondrial SOD-Mn expression (Figure 7D) was unchanged by castration, while catalase levels (Figure 7F) were significantly increased. The PGC-1a protein expression decreased in both IFM (Figure 7G) and SSM (Figure 7H) OQT group.

Orchidectomy increases NADPH oxidase protein expression and mitochondrial protein oxidation

It is well known that NADPH oxidase transfers an electron to molecular oxygen to form superoxide anion. We examined the role of NADPH oxidase isoforms and mitochondrial protein oxidation in the hearts from our groups. Western blots showed significant increases in NOX-1 and $gp91^{phox}$ in the hearts of the OQT group

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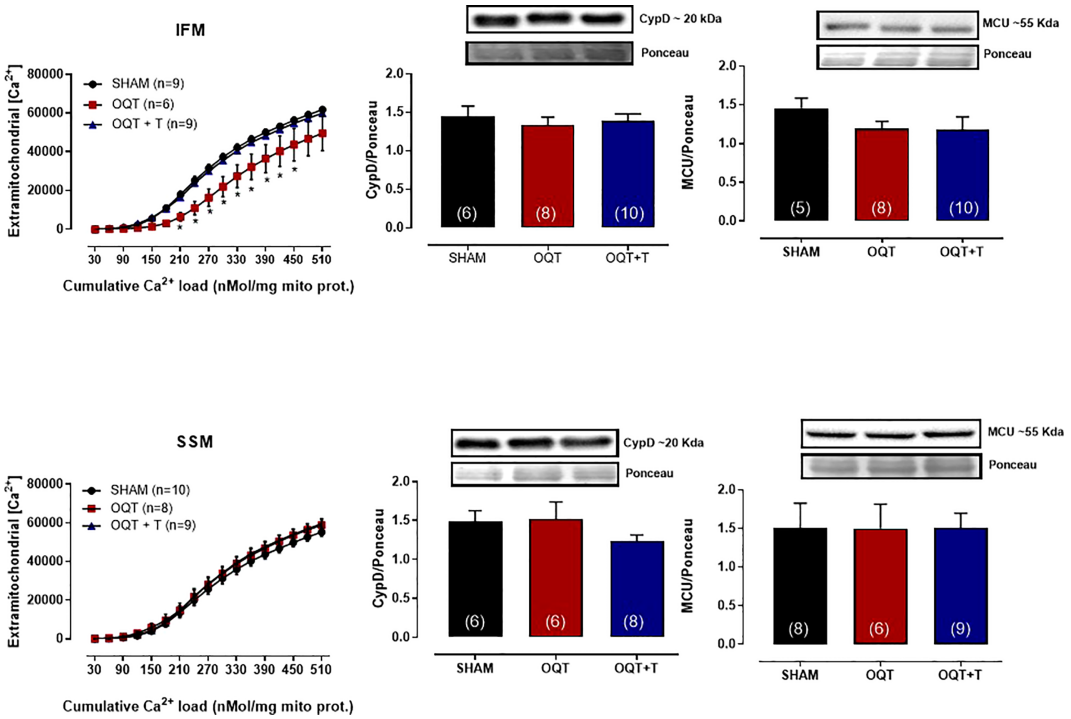


FIGURE 6
Testosterone deficiency increases mitochondrial calcium retention capacity in interfibrillar mitochondria. Calcium-retention capacity of the two distinct mitochondrial subpopulations isolated from SHAM, OQT and OQT + T. Mitochondria were primed for mitochondrial permeability transition pore (MPTP) opening with a progressive exposure to (Ca^{2+}). 25 nmol free Ca^{2+} was injected every 7 min, and extramitochondrial [Ca^{2+}] was recorded every 2 s using 750 nM Calcium Green-5N. Western blot analyses for cyclophilin D and mitochondrial uniporter (MCU) in interfibrillar mitochondria (top graphs) and subsarcolemmal mitochondria (bottom graphs). Results are presented as mean ± SEM values. Differences were analyzed using one and two-way ANOVA followed by Fischer *post-hoc* test. *P<0.05 compared to SHAM. Number of animals is indicated in parenthesis.

and testosterone replacement restored this increase to SHAM levels (Figures 8A, B). We next analyzed protein expression for SOD-Cu-Zn (Figure 8C) and angiotensin converting enzyme (ACE-1) (Figure 8D). SOD-Cu-Zn in the heart was not changed after castration; however, ACE-1 protein expression was significantly increased in the OQT group. This increase was blocked by testosterone replacement. As reactive oxygen species can promote protein carbonylation, we developed western blots to detect DNPH-derivatized proteins in both mitochondrial subpopulations. As shown in Figures 9A, B, orchidectomy significantly increased mitochondrial protein carbonylation in IFM, but not in SSM. The increased protein carbonylation in IFM was prevented by testosterone replacement (Figure 9A).

Apocynin treatment prevents OQT induced myocardial contractility dysfunction

In order to understand the influence of oxidative stress, a separate group of OQT rats (n=8) was treated with apocynin (1.5 mM in drinking water) for 12 weeks after castration. Myocardial contractility was analyzed using isolated papillary muscles at baseline (Figure 10A) and with different extracellular calcium concentrations (Figure 10B). Increasing extracellular calcium concentration elicited a positive inotropic response in the papillary muscles of all groups; however, the inotropic response was significantly smaller in the OQT group. Treatment of the castrated rats with apocynin prevented the reductions in the inotropic responses to calcium (0.62, 1.25, 2.5 and 3.75 mM) (Figures 10A, B). Treatment with apocynin also prevented the castration-related decreases in state 3 oxidative phosphorylation, independent of substrate (Figures 10C–F).

Apocynin treatment prevents OQT induced mitochondrial H_2O_2 production

The ability of mitochondria to generate H_2O_2 was evaluated in isolated interfibrillar mitochondria (IFM) subpopulations (Figures 10G, H) and subsarcolemmal mitochondria (SSM) subpopulations (Figures 10I, J) from the left ventricular (LV) myocardium using the amplex red assay. Testosterone deficiency resulted in an increase in hydrogen peroxide production in IFM (Figure 10G) when using the glutamate + malate substrate. Treatment with apocynin prevented oxidative stress-induced mitochondrial H_2O_2 production in the IFM subpopulation when using the glutamate + malate substrate.

Discussion

This investigation assessed the effects of testosterone deficiency on myocardial contractility and the function of the spatially distinct

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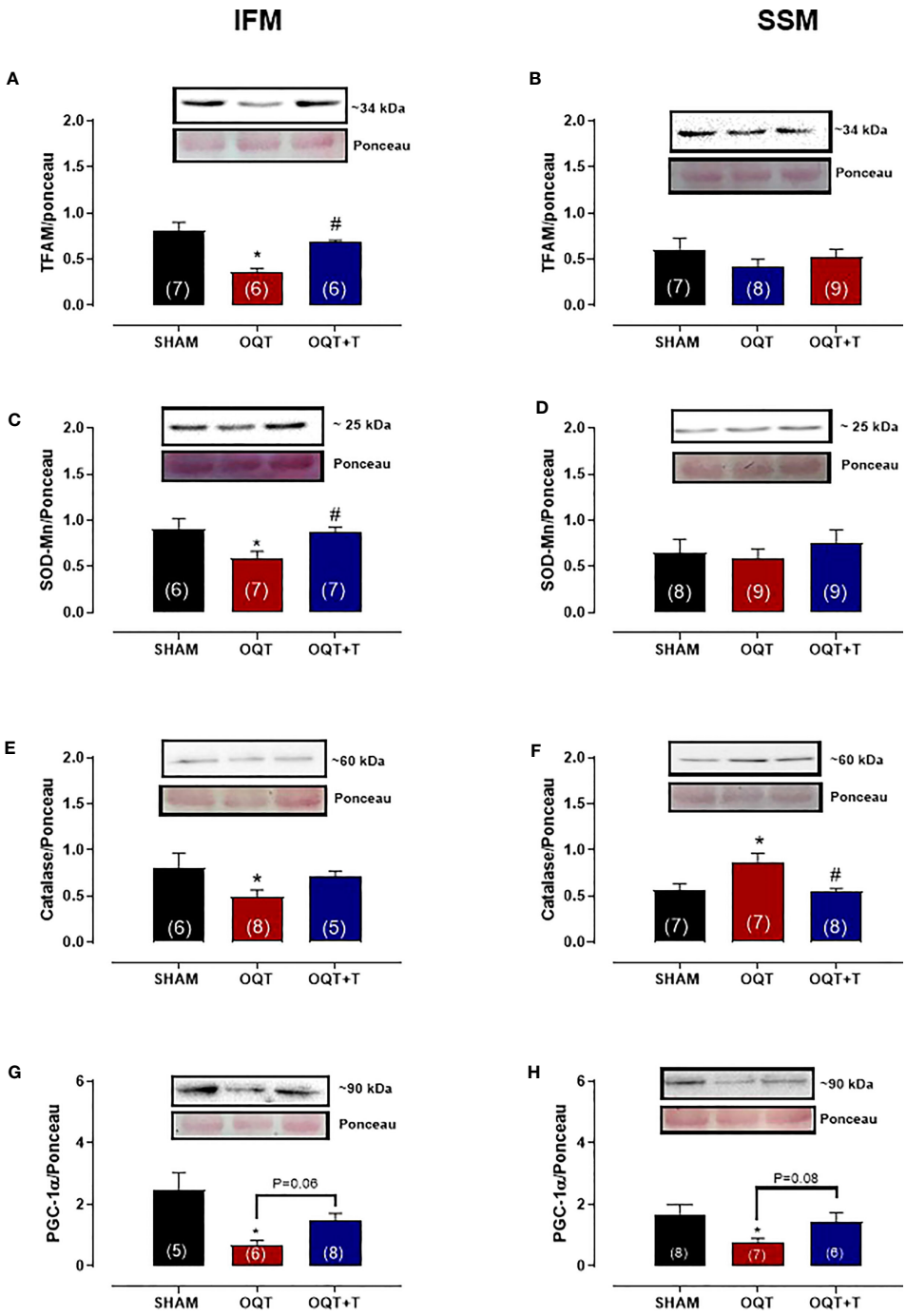


FIGURE 7
Testosterone deficiency decreases interfibrillar mitochondrial antioxidant enzymes and transcription factors. Western blot analyses for mitochondrial transcription factor A (TFAM) **(A, B)**, mitochondrial superoxide dismutase (SOD-Mn) **(C, D)**, mitochondrial catalase **(E, F)** and mitochondrial peroxisome proliferator-activated receptor γ coactivator 1α **(G, H)**. Results are presented as mean ± SEM values. Differences were analyzed using one-way ANOVA followed by Fischer *post-hoc* test. *P<0.05 compared to SHAM; #P<0.05 compared OQT. Number of animals is indicated in parenthesis.

subpopulations of cardiac mitochondria. Our data showed that orchidectomy significantly decreased myocardial contractility after twelve weeks of hormone deprivation. In addition, while the yield of SSM in the heart was increased after testosterone deprivation, the structure and function of these mitochondria was not substantially altered. In contrast, the reduction in testosterone elicited significant changes in the IFM, including increased membrane potential, mitochondrial size and internal complexity. Moreover, the rate of oxidative phosphorylation in the IFM was significantly reduced in the OQT animals independent of the substrate analyzed, for complex I, II and IV. IFM from OQT animals also had a higher Ca^{2+} retention capacity, implying that IFM from orchidectomized animals are less susceptible to permeability transition pore opening. Finally, we provided data using apocynin suggesting that oxidative

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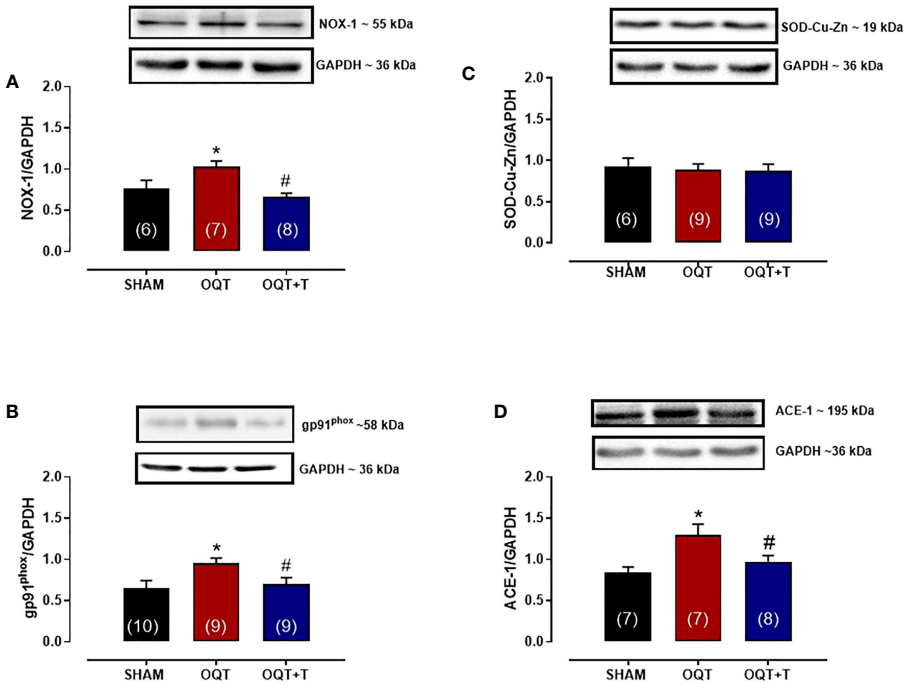


FIGURE 8
Testosterone deficiency increases NADPH oxidase (NOX-1), gp91phox and ACE-1 protein expressions. Western blot analyses for NOX-1 **(A)**, gp91^{phox} **(B)**, superoxide dismutase Cu -Zn **(C)**, angiotensin converting enzyme 1 **(D)** in cardiac tissue from SHAM, OQT and OQT + T groups. Results are presented as mean ± SEM values. Differences were analyzed using one-way ANOVA followed by Fischer *post-hoc* test. *P<0.05 compared to SHAM; #P<0.05 compared OQT. Number of animals is indicated in parenthesis.

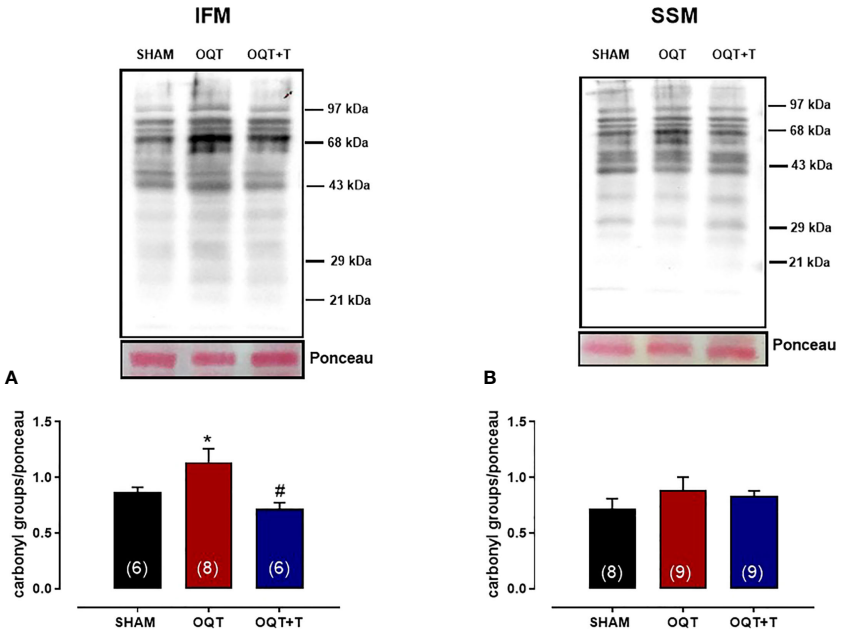


FIGURE 9
Testosterone deficiency increases interfibrillar mitochondrial protein oxidation. Immunodetection of carbonyl groups that are introduced into the amino acid side chain after oxidative modification of proteins in the interfibrillar mitochondria **(A)** and subsarcolemmal mitochondria **(B)**. Results are presented as mean ± SEM values. Differences were analyzed using one-way ANOVA followed by Fischer *post-hoc* test. *P<0.05 compared to SHAM; #P<0.05 compared OQT. Number of animals is indicated in parenthesis.

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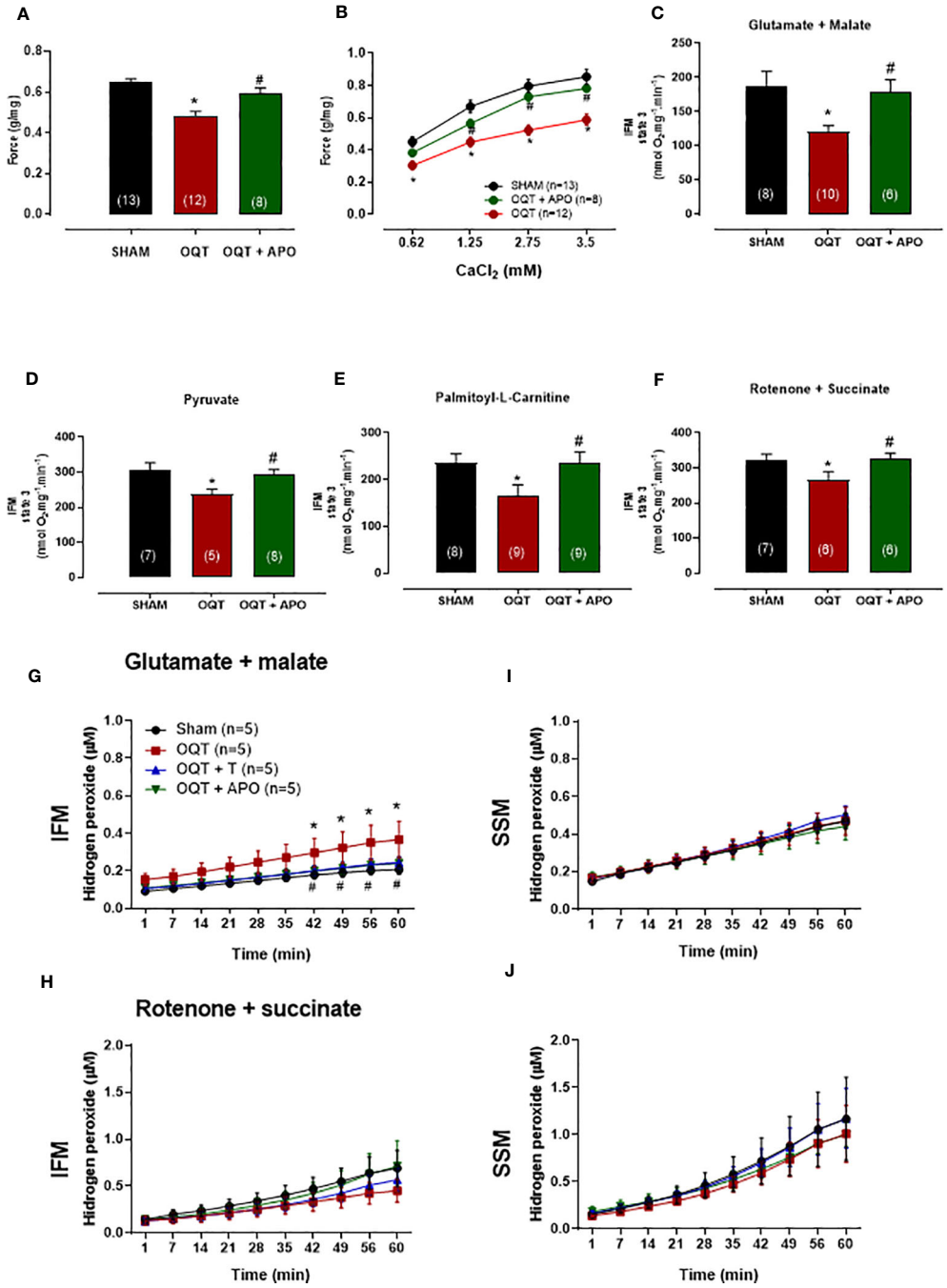


FIGURE 10
Oxidative stress on myocardial contractility was analyzed using apocynin in drinking water. Apocynin prevented the reduction of myocardial contractility **(A, B)** and mitochondrial function **(C–F)**. The isometric force (g/g) in the left ventricular papillary muscles at baseline **(A)**, and at different extracellular $CaCl_2$ (0.62, 1.25, 2.5 and 3.75 mM) concentrations **(B)**. Individual mitochondrial subpopulations were isolated from SHAM, OQT and OQT+T and polarographic measurements were performed to index oxygen consumption under state 3 respiration conditions. Respiration of individual electron transport chain (ETC) complexes was defined as the rate of oxygen consumed in the presence of specific substrates [glutamate and malate (complex I) **(C)**, pyruvate **(D)**, β-oxidation in the presence of specific substrate palmitoyl-L-carnitine **(E)**, and rotenone + succinate (complex II) **(F)**. All respiration rates are expressed in Atoms O.mg mitochondrial protein^{-1}. min^{-1}. Testosterone deficiency increases hydrogen peroxide production in intermyofibrillar mitochondria. **(G, H)** heart mitochondria for isolated interfibrillar mitochondria (IFM) and **(I, J)** heart mitochondria for isolated subsarcolemmal mitochondria (SSM) subpopulations were added to the mixture of Amplex Red and basal H_2O_2 formation was measured (µM). Differences were analyzed using one-way ANOVA followed by Fischer *post-hoc* test. *P<0.05 compared to SHAM; #P<0.05 compared to OQT. Number of animals is indicated in parenthesis (n).

stress is involved in the cardiac dysfunction and increased IFM protein oxidation produced by testosterone deficiency.

It is well known that testosterone plays a significant role in cardiac contraction coupling and that the lack of this hormone decreases myocardial contractility due to a decrease in Serca2a activity (32–35). Our data shows that orchidectomy reduces myocardial contractility in a time dependent manner. We previously showed that, myocardial contractility is preserved 8 weeks after orchidectomy in rats (36). However, in the present study, 12 weeks after orchidectomy, myocardial contractility was significantly reduced, corroborating previous findings in the literature (32). Furthermore, our data also show that phospholamban protein expression is increased and its phosphorylation at Ser^{16} (37) is reduced, indicating that Serca2a activity is reduced after 12 weeks of deficiency.

Myocardial force generation relies on the energy supplied by ATP, which is mainly produced by mitochondria. Approximately 95% of this ATP comes from oxidative phosphorylation processes and is delivered to consuming sites such as myosin-ATPase (60-70%), Serca2a (30-40%) and Na^+/K^+-ATPase (8, 27) [51). Efficient matching of energy production and consumption is crucial for beat-to-beat adaptation to changes in cardiac workload and involves important molecules and ions such as ADP and Ca^{2+} (38, 39). Furthermore, the fine-tuning of energy production and expenditure is also regulated by gene transcription (39). It has been hypothesized that IFM supply ATP for contraction, whereas SSM are involved primarily in providing ATP for active transport of electrolytes and metabolites across the sarcolemma (17) (20, 40, 41). Here we showed that testosterone deficiency induced IFM dysfunction after 12 weeks as evidenced by increased size, reduced bioenergetics and increased protein oxidation. The increase in mitochondrial size could be due to reduced Mfn-2 protein expression. Wang et al. also demonstrated that testosterone deficiency decreased Mfn-2 protein expression and corroborated our findings (21) showing that IFM have increase size and greater calcium retaining capacity. The literature demonstrates that knockout mice for Mfn-2 have larger mitochondria and greater calcium retention capacity (42).

Mitochondrial calcium signaling plays a key role in the regulation of cellular energy metabolism, cardiac excitation–contraction coupling and the generation of ATP for contraction. In the mitochondrial matrix, Ca^{2+} plays an important role in energetics by activating the F1/FO ATPase (43) and several Ca^{2+} sensitive dehydrogenases in the tricarboxylic acid cycle, including pyruvate dehydrogenase, 2-oxoglutarate (α-ketoglutarate) dehydrogenase, and the NAD^+-linked isocitrate dehydrogenase (44, 45). Activation of these enzymes results in increased NADH production, which is critical for matching energy supply with demand during increased workload (46). The mechanism that triggers calcium influx across the inner mitochondrial membrane is the large negative membrane potential (-180 mV) that drives cytosolic calcium entry into the matrix (47). The higher membrane potential in the OQT group could be responsible for the increased calcium uptake we observed in the IFM. Furthermore, the higher calcium concentration in the matrix most likely does not increase NADH production due to the decreased oxidative phosphorylation and cytochrome c oxidase activity that has been demonstrated by us and others (48). It has been proposed that IFM generate ATP for the contractile machinery. Thus, it is possible that the reduced force generation we observed in the OQT animals is the result of the reduced state 3 activity found in this group. The lower activity of the electron transport chain could be due to posttranslational modification, such as oxidation which most likely, underlies the increase in carbonylation we observed in IFM.

PGC-1α is implicated in the regulation of the mitochondrial genome. Recently, it has been demonstrated that PGC-1α is located within mitochondria, where it co-localizes within the D-loop of mtDNA with mitochondrial transcription factor A (TFAM) (49). Furthermore, AMPKα phosphorylates PGC-1α, which in turn translocates to mitochondria and forms a complex with TFAM. TFAM also exists in subcellular compartments from which it can translocate to mitochondria. TFAM regulates nuclear DNA such as Serca2a expression in cardiomyocytes. The transcription of genes for mitochondrial ATP production and the genes for proteins that consume large amounts of ATP (Serca2a) are regulated by the same transcription factor. Oxidative stress decreases TFAM levels in both the nucleus and the mitochondria (39). Correspondingly, our results demonstrated that phosphorylated AMPK-α is reduced, while PGC-1α protein expression did not change in whole heart tissue after castration. Protein content was reduced in both mitochondrial subpopulations, indicating that PGC-1α translocation is reduced in our model. We also detected decreased TFAM protein expression in both IFM and SSM. Oxidative stress was also increased as indicated by NOX-1 and $gp91^{phox}$ overexpression. It has been proposed that NADPH oxidase causes mitochondrial damage in aging and hypertrophic cardiomyocytes (50). NOX-2 also stimulates mitochondrial ROS by activating reverse electron transfer in mitochondria (51). Conversely, inhibition of mitochondrial ROS production improves cardiac and mitochondrial dysfunction in heart failure (28). Similarly, our data show that inhibition of NOX normalizes cardiac and mitochondrial function in the face of low testosterone.

The effects of testosterone deficiency on SSM function have been demonstrated previously (9); however, these effects are controversial (52), possibly due to the fact that previous studies only analyzed one mitochondrial subpopulation. The literature describes that cardiac cells contain discrete pools of mitochondria that are characterized spatially by their subcellular location (17). The effects of testosterone deficiency on IFM function have not been previously reported. One interesting aspect of our data is that catalase expression was increased in SSM but reduced in IFM. We also found that protein oxidation was increased in IFM but did not change in SSM. These findings could explain the slight dysfunction found in SSM. As previously reported, overexpression of mitochondrial catalase attenuates pressure overload-induced heart failure (53). One question that remains to be answered is why the two distinct subpopulations of mitochondria respond differently to the same stimulus. Further investigation is needed to answer this question.

The present research offers valuable insights but has some limitations. One such limitation is that using apocynin to analyze NOX lacks specificity for NOX enzymes. Apocynin has been widely

recognized as a NOX inhibitor, but it can also interact with other enzymes or cellular components, potentially leading to off-target effects. Therefore, while apocynin can provide valuable insights into the overall involvement of NOX in a biological process, it cannot definitively establish the specific isoform or enzymatic source of ROS. The second important limitation is predominantly due to the inherent differences between human and rat physiology, rendering the translatability of the findings to humans uncertain. Differences span from metabolism, implying potential divergence in dose-response relationships, to ethical and logistical constraints limiting the scope of measures such as organ weight alterations. Confounding factors may also impact study outcomes. The age of animal subjects and strain-specific attributes may affect results, reflecting the dynamic nature of testosterone levels, as well as cardiac and mitochondrial functions. External factors such as housing conditions, diet, stress levels, and comorbid conditions, which are commonly present in humans suffering from low testosterone, like obesity and diabetes, might influence the results. These confounders may not be adequately accounted for in an animal study. While providing insightful preliminary data, these limitations necessitate cautious interpretation of the findings. For comprehensive understanding and clinical relevance, validation through carefully designed human clinical trials is indispensable.

In conclusion, our data show that low testosterone reduces cardiac contractile function. Moreover, our data suggests that the contractile deficit may reflect an oxidative stress-mediated decrease the ability of the IFM to supply ATP to the contractile filaments.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by the animal ethics committee of the Federal University of Espirito Santo (CEUA-UFES number: 072/2012).

Author contributions

PL, KR, EAM, PR, RÁ, EM, JG, MS, and RR performed the experiments, analyzed the data, and discussed the results. IS and RR were involved in the study conception, data analyses and interpretation, discussing the results, and writing the paper. IS is the corresponding author. All authors contributed to the article and approved the submitted version.

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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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EDITED BY
Daigo Sawaki,
Jichi Medical University, Japan

REVIEWED BY
Anthony Martin Gerdes,
New York Institute of Technology, United States
Shuhang Xu,
Nanjing University of Chinese Medicine, China

*CORRESPONDENCE
Gejing Liu
546503789@qq.com

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Effect of thyroid hormone replacement treatment on cardiac diastolic function in adult patients with subclinical hypothyroidism: a meta-analysis

Gejing Liu[1]*, Man Ren[1], Yingshi Du[1], Ruoyu Zhao[2], Yu Wu[3], Yongming Liu[1] and Liang Qi[4]

[1]Department of Geriatrics, First Hospital of Lanzhou University, Lanzhou, China, [2]Department of Gastroenterology and Oncology, Gansu Provincial Cancer Hospital, Lanzhou, China, [3]Institute of Evidence-Based Medicine, School of Basic Medical Sciences, Lanzhou University, Lanzhou, China, [4]Department of Cardiovascular Surgery, First Hospital of Lanzhou University, Lanzhou, China

Background: Although subclinical hypothyroidism (SCH) is related to abnormalities in left ventricular diastolic function, the use of levothyroxine as a regular treatment remains debatable. This meta-analysis aimed to determine whether thyroid hormone replacement therapy affects cardiac diastolic function in patients with SCH as measured by echocardiography.

Methods: This meta-analysis included a search of the EMBASE, PubMed, Web of Science, and Cochrane Library databases from their inception to May 18, 2023, for studies analyzing cardiac morphology and functional changes in patients with SCH before and after thyroid hormone replacement. The outcome measures were cardiac morphology and diastolic and overall cardiac function, as assessed using ultrasound parameters (including ventricular wall thickness, chamber size, mitral wave flow, tissue Doppler, and speckle tracking). The quality of the studies was assessed using the Newcastle–Ottawa Scale. The standard mean differences (MDs) and 95% confidence intervals (CI) were calculated using fixed- or random-effects models.

Results: Seventeen studies met the inclusion criteria. A total of 568 patients participated and completed the follow-up. All studies specifically stated that serum thyrotropin levels returned to normal by the end of the study period. Compared with baseline levels, no significant morphological changes were observed in the heart. In terms of diastolic function, we discovered that the ratios of E-velocity to A-velocity (E/A) had greatly improved after thyroid hormone replacement therapy, whereas the ratios of the mitral inflow E wave to the tissue Doppler e' wave (E/e') had not. Global longitudinal strain (GLS) increased significantly after treatment with levothyroxine.

Conclusion: In adult patients with SCH, thyroid hormone supplementation can partially but not completely improve parameters of diastolic function during the observation period. This meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2020

statement, an updated guideline for reporting systematic reviews (11) and was registered with INPLASY (INPLASY202320083).

Systematic review registration: https://inplasy.com/inplasy-2023-2-0083.

KEYWORDS

diastolic function, subclinical hypothyroidism, meta-analysis, echocardiography, levothyroxine

Introduction

Subclinical hypothyroidism (SCH) exists when free thyroxine (FT4) levels are within the defined reference range but thyroid stimulating hormone (TSH) levels are elevated outside the reference range (1). It is a common disorder which affects approximately 10% of the adult population, and around 1 in 3 patients with SCH are asymptomatic (2). Inadequate serum thyroid hormone levels impair cardiac function and may result in multiple cardiovascular risk factors, such as endothelial dysfunction, increased intima-media thickness, increased vascular resistance, and pericardial effusion (3). Additionally, the risk of heart failure increases with higher serum thyrotropin levels, with the incidence increasing as TSH levels increase, particularly above TSH ≥10 mIU/L, even after adjusting for cardiovascular risk factors (4).

Previous studies have demonstrated that patients with SCH had a higher prevalence of left ventricular diastolic dysfunction (LVDD) than controls (5, 6). SCH diastolic dysfunction mainly manifests as an impaired left ventricular (LV) relaxation pattern, such as longer deceleration time, isovolumic relaxation time, and higher LV filling, compared to euthyroidism (7). The potential mechanisms responsible for diastolic dysfunction of the left ventricle in SCH are related to endothelial dysfunction, arterial stiffness, and the inflammatory state and are driven by TSH apoptosis-derived microparticles (8). Although the association between SCH and LV diastolic function is relatively well established, the benefits of thyroid hormone replacement therapy for SCH in improving cardiac diastolic function remain unclear. One study revealed that treatment with levothyroxine reversed diastolic abnormalities in SCH to comparable levels to those in controls (9). However, a randomized controlled trial (RCT) in older adults with mild SCH showed that diastolic heart function did not differ after treatment with levothyroxine compared to a placebo (10). Considering the potential benefits of thyroid hormone supplementation, the risk of overtreatment should also be considered. More substantive evidence of sufficient cardiovascular benefits from thyroid hormone supplementation for patients with SCH is required. This meta-analysis aimed to explore the value of levothyroxine application in terms of cardiac diastolic function in adult patients with SCH to provide a basis for the clinical use of hormone replacement therapy in the SCH population.

Methods

Data sources and searches

This meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2020 statement, an updated guideline for reporting systematic reviews (11) and was registered with INPLASY (INPLASY202320083). Systematic literature searches were conducted in the PubMed, EMBASE, Cochrane Library, and Web of Science databases from their inception to May 18, 2023. Variations of the terms 'Heart' or 'Cardiac' were combined with variants of the terms 'Hypothyroidism' and 'Levothyroxine' (the search strategy is shown in Table 1). All the references included in the study were traced back.

Inclusion and exclusion criteria

All potential studies were identified by reading the titles and abstracts. Studies that were deemed suitable for inclusion were selected. The full text was then read further to determine the final inclusion criteria and exclude irrelevant literature.

The inclusion criteria for each study were as follows. First, the clinical outcomes of each study must have been continuous variables that describe the cardiac structure or function which were obtained using echocardiography. Second, the mean and SD of each outcome must have been available as values from baseline and after treatment or as the amount of change after treatment. Third, all studies must have been conducted in adults with SCH rather than in patients with specific diseases or special populations (e.g., children or pregnant women). Fourth, the authors explicitly mentioned that previously elevated serum thyrotropin levels returned to normal after treatment.

Studies were excluded if they met any of the following criteria: first, participants taking medications related to thyroid dysfunction (amiodarone, thyroid hormone replacement, and/or antithyroid drugs); second, subjects with comorbidities that could affect cardiac metrics, such as serious cardiovascular disease, hypertension, pulmonary heart disease, or diabetes mellitus; third, studies which focused on other unfavorable outcomes, such as right ventricular function and left atrial strain; fourth, studies which used methods other than echocardiography to measure cardiac function. The language used was restricted to English. Reviews, letters to the

TABLE 1 Search strategy for PubMed and Embase.

Database	
PubMed	"hypothyroidism"[MeSH] OR "hypothyr*"[Title/Abstract] OR "thyroid deficien*"[Title/Abstract] OR "thyroid insufficien*"[Title/Abstract] AND "thyroid hormones"[MeSH] OR "thyroid hormon*"[Title/Abstract] OR "Levothyro*" [Title/Abstract] OR "Levothroid" [Title/Abstract] OR "Levoxine" [Title/Abstract] OR "Eltroxine" [Title/Abstract] OR "Euthyrox" [Title/Abstract] OR "Eutirox" [Title/Abstract] OR "thyronin*"[Title/Abstract] OR "thyroxin*"[Title/Abstract] OR "tyroxin*"[Title/Abstract] OR "L-T3"[Title/Abstract] OR "L-T4"[Title/Abstract] OR "FT3"[Title/Abstract] OR "FT4"[Title/Abstract] OR "t3 hormon*"[Title/Abstract] OR "t4 hormon*"[Title/Abstract] OR "T3"[Title/Abstract] OR "T4"[Title/Abstract] OR "substitution therap*"[Title/Abstract] AND "Echocardiography"[MeSH Terms] OR "stroke volume"[MeSH] OR "ventricular function "[MeSH] OR "diastole"[MeSH] OR "atrial remodeling"[MeSH] OR "Echocardiography"[Title/Abstract] OR "ventricular remodeling"[Title/Abstract] OR "cardiac remodeling"[Title/Abstract] OR "cardiac adaptation"[Title/Abstract] OR "lv geometry"[Title/Abstract] OR "left ventricular geometry"[Title/Abstract] OR "cardiac geometry"[Title/Abstract] OR "cardiac dimension"[Title/Abstract] OR "left ventricular function"[Title/Abstract] OR "systolic function"[Title/Abstract] OR "ejection fraction"[Title/Abstract] OR "diastolic function"[Title/Abstract] OR "atrial remodeling"[Title/Abstract] OR " strain "[Title/Abstract] 548 hits (18-05-2023)
Embase	'thyroid hormone'/exp OR 'hypothyr*':ab,ti OR 'thyroid deficien*':ab,ti OR 'thyroid insufficien*':ab,ti AND 'hypothyroidism'/exp OR 'thyroid hormon*':ab,ti OR 'levothyro*':ab,ti OR 'levothroid':ab,ti OR 'levoxine':ab,ti OR 'eltroxine':ab,ti OR 'euthyrox':ab,ti OR 'eutirox':ab,ti OR 'thyronin*':ab,ti OR 'thyroxin*':ab,ti OR 'tyroxin*':ab,ti OR 'l-t3':ab,ti OR 'l-t4':ab,ti OR 'ft3':ab,ti OR 'ft4':ab,ti OR 't3 hormon*':ab,ti OR 't4 hormon*':ab,ti OR 't3':ab,ti OR 't4':ab,ti OR 'substitution therap*':ab,ti AND 'echocardiography'/exp OR 'heart ventricle remodeling'/exp OR 'heart stroke volume'/exp OR 'heart function'/exp OR 'heart atrium remodeling'/exp OR 'Echocardiography':ab,ti OR 'ventricular remodeling':ab,ti OR 'cardiac remodeling':ab,ti OR 'cardiac adaptation':ab,ti OR 'lv geometry':ab,ti OR 'left ventricular geometry':ab,ti OR 'cardiac geometry':ab,ti OR 'cardiac dimension':ab,ti OR 'left ventricular function':ab,ti OR 'systolic function':ab,ti OR 'ejection fraction':ab,ti OR 'diastolic function':ab,ti OR 'atrial remodeling':ab,ti OR ' strain ':ab,ti 5067 hits (18-05-2023)

editor, case reports, and case series without follow-ups were excluded. The screening criteria are shown in Figure 1.

Ultrasound measurements

Cardiac function was classified into three categories: cardiac morphology, diastolic function, and ventricular strain.

Quality assessment

The Newcastle–Ottawa Scale (NOS), with a total of eight items, was chosen for the quality assessment of non-randomized research. The NOS includes study-specific criteria. Four of these items were used to score the selection of patients with SCH (four points). To assess the comparability of patients with SCH before and after therapy, one question was used (two points). The availability of cardiac outcomes was evaluated using three elements (three points). Finally, only studies with at least five points were included in the quantitative analysis. Following the screening process, all 17 candidate papers met the requirements and were used for further analysis, as indicated in Table 2. Two reviewers rated the included studies separately, and differences were resolved by consensus. No study was omitted based on the risk of bias assessment. Publication bias was visually examined using funnel plots.

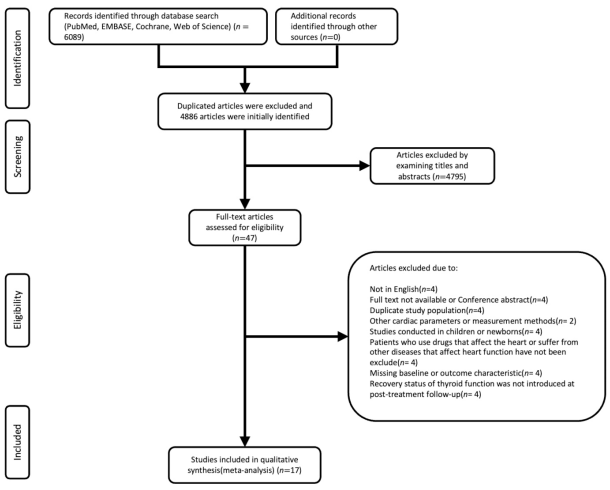


FIGURE 1
The general process of literature searching.

Data reporting and statistical analysis

The means, SDs, and number of subjects for each echocardiographic index at baseline and after thyroid hormone supplementation were extracted from each study. For the meta-analysis, we calculated mean differences before and after treatment for ventricular wall thickness, left atrial diameter (LAD), left ventricular mass index (LVMi), the ratio of E-velocity to A-velocity (E/A), the ratio of the mitral inflow E wave to the tissue Doppler e' wave (E/e'), and LV strain, using Review Manager version 5.4 (The Nordic Cochrane Centre, Cochrane Collaboration, Copenhagen, Denmark) with inverse variance weights. The heterogeneity among the studies was estimated with χ^2 heterogeneity and the I^2 test. The I^2 statistic was calculated to assess the proportion of the total variation in the study estimates due to heterogeneity. If I^2 was greater than 25%, a random-effects model was used, and a fixed-effects model was used if I^2 was < 25%. Sensitivity analysis was performed by removing each study individually. A P-value of p <0.05 was considered statistically significant.

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TABLE 2 The Newcastle–Ottawa Scale for the assessment of the quality of studies.

Number	Study	Selection			Comparability		Exposure			Scores
		Adequate definition of cases	Representativeness of the cases	Selection of controls	Definition of controls		Ascertainment of exposure	Same method	No-respons rate	
1	R.Arem 1996	1		1	1	1	1	1	1	8
2	Bernadette 1999	1		1	1	1	1	1	1	7
3	Chen 2016	1	1	1	1	1	1	1	1	8
4	Fabio 2001	1		1	1	1	1	1	1	7
5	Franzonia 2006	1		1	1	1	1	1	1	7
6	Huseyin 2006	1		1	1	1	1	1	1	7
7	Gulbanu 2011	1	1	1	1	1	1	1	1	8
8	Marijana 2014	1	1	1	1	1	1	1	1	8
9	Mehmet 2004	1		1	1	1	1	1	1	7
10	Milena 2021	1		1	1	1	1	1	1	7
11	Sahar 2021	1	1	1	1	1	1	1	1	8
12	Shatynska 2016	1		1	1	1	1	1	1	7
13	Tanase 2014	1		1	1	1	1	1	1	7
14	Valentina 2018	1	1	1	1	1	1	1	1	8
15	Fatma 2011	1		1	1	1	1	1	1	7
16	Owen 2006	1		1	1	1	1	1	1	7
17	CAMCI 2022	1		1	1	1	1	1	1	7

Results

Characteristics of studies

The search yielded 6089 records. After removing duplicates, 4886 records were screened for eligibility. The screening of titles and abstracts resulted in 47 potentially eligible studies, in which cardiac structure and function were measured before and after levothyroxine treatment. Two studies (12, 13) reported overall and cardiac function in the same patient population. As both outcome measures were relevant for this review, we included both studies, but handled them as a single study. There were also two articles (14, 15) that included individuals from the same sample with overlap; we chose the one (15) with the highest number of participants. In total, 17 studies (9, 13, 15–29) and 568 patients with SCH met the inclusion criteria and were included in this review (Figure 1).

Most patients with SCH included in the study were female and were all over 18 years old. The duration of follow-up varied across studies, ranging from 1 to 12 months. The characteristics of the studies are listed in Table 3.

Effects of levothyroxine treatment on Cardiac Morphology in SCH Patients

As shown in Figure 2, five metrics for assessing the cardiac morphology were included in the quantitative analysis. These indicators included interventricular septal thickness at diastole (IVSd), left ventricular posterior wall thickness at diastole (LVPWd), left ventricular end-diastolic diameter (LVEDd), LVMi, and LAD. According to the results, there were no statistically significant differences between the five indicators before and after treatment (MD for IVSd 0.12 [95% CI, -0.05–0.29], p =0.17; MD for LVPWd 0.14 [95% CI, -0.03–0.31], p =0.12; MD for LVEDd 0.20 [95% CI, -0.48–0.87], p =0.57; MD for LVMi

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TABLE 3 characteristics of included studies.

Number	Study	Country	No. patient	Final participants	Mean age	Gender (M/F)	Thyrotropin threshold	TSH (before therepy)	TSH(atfer therepy)	Treatment duration
1	R.Arem 1996	American	8	8	36.4±6.02	6/2	>5.5mIU/L	14.8±9.5	3.0±1.6	3 months
2	Bernadette 1999	Italy	26	10	36 ± 12	2/24	>3mU/L	8.6±4.8	——	6 months
3	Chen 2016	China	32	32	40.7±9.7	12/20	>4.2uIU/ml	5.62±1.34	3.86±1.07	12months
4	Fabio 2001	Italy	20	10	32.6±12.1	2/18	>3.6mIU/l	5.44±2.41	1.32±0.47	6 months
5	Franzonia 2006	Italy	42	16	52.2±15.1	13/29	>3.6mIU/l	8.8±1.7	——	6 months
6	Huseyin 2006	Turkey	22	22	48±13	7/15	4.0ng/ml	13.3±9.1	——	105±60days
7	Gulbanu 2011	Turkey	22	22	18-60	22F	4.2uIU/ml-9.9uIU/ml	7.17±1.74	2.28±0.63	3 months
8	Marijana 2014	Serbia	54	54	41±6	54F	>4mIU/L	8.8±2.7	2.15±0.7	1years
9	Mehmet 2004	Turkey	45	23	40.2±9.3	4/19	>4.0mU/L	8.47±1.9	3.34±1.7	6 months
10	Milena 2021	Serbia	35	35	51.6±15.4	6/29	4mIU/L-10mIU/L	6.9±2.1	——	3 months
11	Sahar 2021	Egypt	36	36	40.2±8.6	4/32	>4.5IU/L	11(10-7)	2.2(1.9-2.4)	6 months
12	Shatynska 2016	Ukraine	33	33	51.21±4.32	9/24	>4.0mU/L	11.82±0.56	——	6 months
13	Tanase 2014	Romania	75	75	60.86±12.56	8/67	>4.2 mUI/L	5.80±1.50	3.11 ± 0.56	6 months
14	Valentina 2018	Macedonia	54	54	43.1±12.4	2/52	>4.2mU/L	8.1±1.3	2.8±2.6	5 months
15	Fatma 2011	Turkey	27	27	35.4±11.4	3/24	>4.2mIU/mL	7.01±2.36	3.16±0.33	1~1.5 months
16	Owen 2006	England	19	19	49.2±3.8	19F	>5.5mU/L	8.8(5.7-21.6)	1.3(0.4-2.8)	6 months
17	CAMCI 2022	Turkey	70	70	44.1 ± 9.4	34/33	>4.0 mIU/L	7.95 ± 1.44	2.28 ± 0.7	6 months

0.47 [95%CI, -0.78, 1.71], p=0.46; MD for LAD -0.05 [95% CI, -0.87–0.77], p =0.91).

Effects of levothyroxine treatment on diastolic function in SCH Patients

Figure 3 includes two indicators of LV diastolic function. After treatment with levothyroxine, E/A significantly improved (MD for E/A -0.09 [95% CI, -0.12– -0.05], p <0.001), while E/e' did not significantly improve (MD for E/e' -0.09 [95% CI, -0.27–0.44], p =0.64).

Effects of levothyroxine treatment on myocardial strain in SCH Patients

GLS showed significant improvement after levothyroxine treatment (MD for GLS -1.11 [95% CI, -1.63– -0.59], p <0.001) (Figure 4).

Discussion

This meta-analysis provided a comprehensive overview of the effects of levothyroxine on cardiac diastolic function in patients with SCH, assessed by echocardiography, based on 17 studies. Compared with before therapy, structural cardiac indices showed no significant changes after levothyroxine treatment in patients with SCH. In terms of diastolic function, although E/A improved, E/e' did not improve after treatment. In our study, GLS also improved after levothyroxine treatment.

Our study showed that the IVSd, LVPWd, LVEDd, and LVMi in patients with SCH did not change significantly after treatment with levothyroxine. These LV structural indicators also represent LV remodeling. This result is consistent with that of another systematic evaluation based on echocardiography, cardiac magnetic resonance imaging, and myocardial radionuclide

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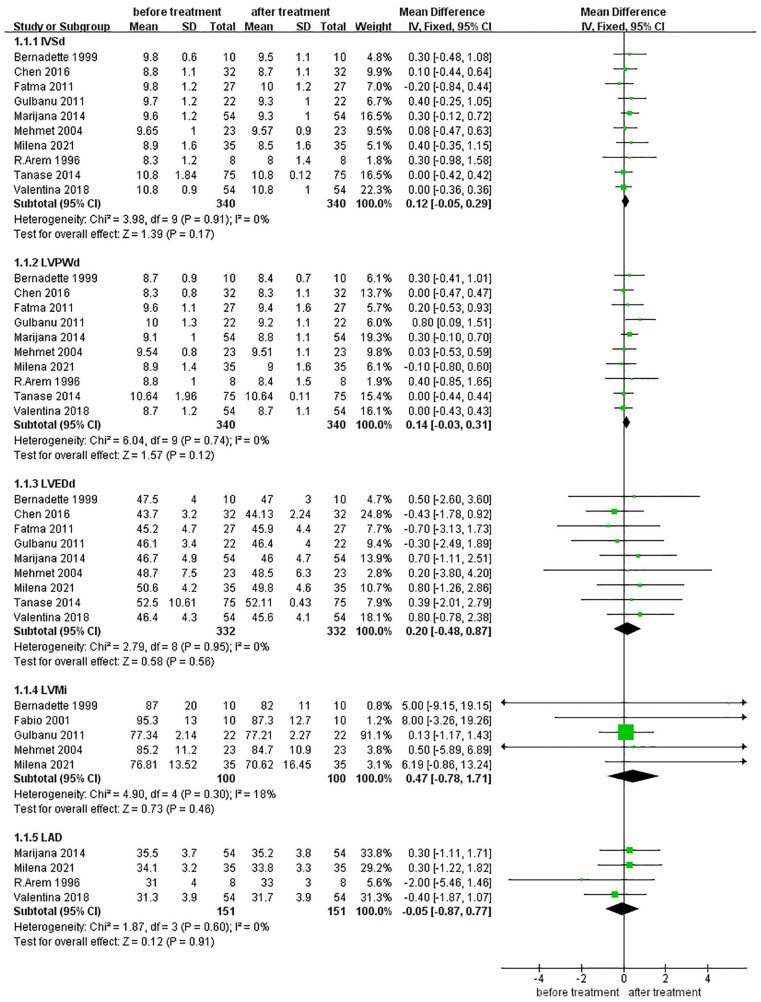


FIGURE 2
Comparison of cardiac morphology before and after levothyroxine treatment for SCH. IVSd, Interventricular Septal Thickness at Diastole: LVPWd, left ventricular posterior wall Thickness at Diastole; LVEDd, left ventricular end diastolic dimensions; LVMi, Left Ventricular mass index: LAD, left atrial diameter. LV mass index is the result of dividing the heart mass by the body surface area (g/m2).

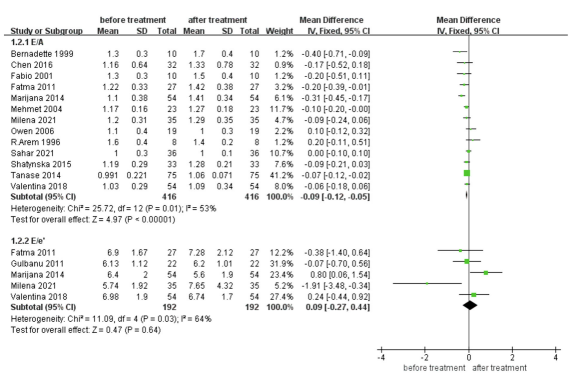


FIGURE 3
Comparison of Left ventricular diastolic function before and after levothyroxine treatment for SCH. E/A, the ratio of Left ventricular early diastolic mitral valve blood flow spectrum to the peak velocity of mitral valve blood flow formed by LA contraction in late diastole of LV. E/e' the ratio of the mitral inflow E wave to the tissue Doppler e' wave.

imaging, which found no significant morphological changes in the heart after treatment with levothyroxine in patients with SCH (30). Some studies have suggested that the LV mass of both adult and pediatric patients with SCH is significantly increased compared to that in the normal population (14, 31) and that this increases with the duration of the disease among those with TSH≥10.0 (32). However, other studies have reported no significant association between SCH and LV mass (33). Therefore, we consider that there may be two possible reasons: first, the TSH level of the patients in our study was mildly increased, so the LVMi at baseline may not have changed significantly compared with the normal population; second, the treatment time may have been insufficient to allow for significant changes in LV morphology.

In addition to LV thickness and mass, another vital structural index used to evaluate LV diastolic function was the LAD. However, this meta-analysis found no significant changes in LAD before and after levothyroxine treatment. The anteroposterior diameter of the left atrium is a commonly used clinical index to measure its size; however, a two-dimensional index cannot fully reflect the true size

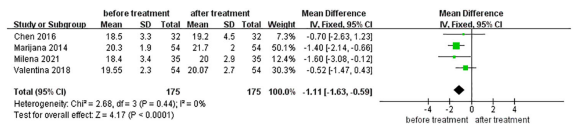


FIGURE 4
Comparison of Left ventricular Global longitudinal strain (GLS) before and after levothyroxine treatment for SCH.

of the left atrium. At present, the left atrial volume index (LAVi) is a more accurate measurement index. Malhotra et al. (6) found that increased LAVi could reverse levothyroxine therapy in a population with LVDD. This study included 67 patients with SCH without underlying heart disease. After echocardiographic analysis, eight patients with LVDD were selected and treated with levothyroxine for 6 months. Their LAVi decreased from 31.17 ± 3.257 to 26.98 ± 2.668 (p =0.013). However, Nakova et al. (14) found that treatment with levothyroxine in average patients with SCH did not improve LAVi. We speculate that levothyroxine does not change the size of the left atrium in patients with SCH without LVDD.

For diastolic function, we found that E/A ratio increased after treatment with levothyroxine, but E/e' did not change. E/A is a traditional index that has been widely used to evaluate LV relaxation, LV diastolic stiffness, and LV filling pressures (34). The E/A ratio was significantly lower in patients with SCH than in controls (6). Hormone replacement therapy in patients with SCH can increase the early diastolic left atrioventricular pressure gradient and improve the LV diastolic pattern. E/e' is a commonly used index to evaluate LV diastolic function, LV filling pressure, and LA pressure (35, 36). Compared with blood flow Doppler, tissue Doppler is less load-dependent and has a minimal effect on LV filling pressure (37). However, there are few large evidence-based analyses using E/e' to evaluate the effect of hormone replacement therapy in patients with SCH. According to a nested TRUST trial, diastolic heart function did not differ after treatment with levothyroxine compared to a placebo (10). However, in contrast to our study, participants in the TRUST trial were all aged ≥65 years, and functional indicators representing LV diastolic function other than E/e' (such as E/A, E deceleration time) did not significantly improve. This may be due to the normal range of TSH increasing to higher values with aging (38). Some studies focusing on cardiovascular outcomes and cognitive function suggest that levothyroxine treatment is not beneficial in SCH patients aged 65 years or older (39, 40).

Our meta-analysis included a study by Tadic et al. (15) which differed from other included studies with their finding that E/e' was significantly improved from baseline (6.4 ± 2 *VS.* 5.6 ± 1.9). In contrast to other studies, their observation duration was one year, rather than within six months. Therefore, further age-stratified randomized controlled studies and long-term observations are required to determine whether levothyroxine can improve E/e'.

Speckle tracking–based echocardiography (STE) has emerged as an interesting and promising tool for evaluating myocardial function. As LV diastolic and longitudinal systolic disturbances may stem from a common pathological background, they may develop in parallel. The presence of impaired GLS reinforces this diastolic dysfunction (41). GLS increased in patients with SCH after levothyroxine treatment. Although a decrease in GLS indicates impaired LV systolic function, an increasing number of studies have shown that it is related to diastolic function. Tschöpe and Senni found that GLS is a clinically and prognostically relevant parameter in patients with HFpEF (42). The 2019 Heart Failure Association (HFA) of the European Society of Cardiology (ESC) guidelines recommend including a cut-off point of 16% in absolute values as a secondary criterion for diagnosing HFpEF (43). Unlike tissue Doppler spot tracking, there are fewer sampling sites and fewer angle dependences (44). The improvement in LV long-axis strain recorded by spot tracking can better explain the improvement in LV function in patients with SCH after treatment with levothyroxine.

This study had some limitations. First, most included patients were middle-aged women, and we could not perform a subgroup analysis based on age and sex. Therefore, we could not draw detailed conclusions from specific populations. Future studies should clarify specific conclusions for patients with SCH of different ages, sexes, and SCH severity. Second, the differential diagnosis of elevated serum thyrotropin levels has been reported in various studies. Therefore, the heterogeneity caused by these factors may have affected our conclusions. Third, there are currently limited research data or observation times for some key indicators that evaluate diastolic function, such as LAVi and E/e'. Therefore, further studies with prolonged follow-ups are required to confirm whether SCH-induced myocardial morphological changes can be reversed by levothyroxine supplementation. Fourth, our study only included cardiac diastolic functions measured by echocardiography in patients with SCH. We did not further explore the lipid levels, BNP levels, carotid intima-media thickness, and cardiovascular outcomes of SCH patients after treatment with levothyroxine. Large RCTs should be conducted to confirm whether levothyroxine supplementation improves cardiovascular outcomes in all adult patients.

Conclusion

In this study, we investigated the changes in cardiac diastolic function after thyroid hormone replacement therapy in adults with SCH. We found that the LV structure did not change significantly after hormone replacement therapy. Some parameters representing LV diastolic function improved after treatment; however, other parameters did not show significant improvement. The results of this study suggest that whether the use of levothyroxine replacement therapy is beneficial for the improvement of cardiac diastolic function in adults with SCH, is still unknown. However, as only some parameters showed improvement, there is a need for future studies to confirm that the cardiovascular benefits of levothyroxine replacement therapy apply to different age groups of SCH patients.

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Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Author contributions

GL: Conceptualization, Funding acquisition, Writing – original draft, Writing – review & editing. MR: Data curation, Investigation, Software, Writing – original draft. YD: Formal Analysis, Investigation, Supervision, Writing – review & editing. RZ: Investigation, Project administration, Writing – review & editing. YW: Methodology, Supervision, Writing – review & editing. YL: Investigation, Resources, Writing – review & editing. LQ: Writing – review & editing.

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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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EDITED BY
Daigo Sawaki,
Jichi Medical University, Japan

REVIEWED BY
Alessandro Pingitore,
National Research Council, Italy
Oscar Lorenzo,
Health Research Institute Foundation Jimenez Diaz (IIS-FJD), Spain

*CORRESPONDENCE
Yingwei Chen
zzyingweichen@126.com
Yihong Sun
yihongsun72@126.com

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The relationship between frequent premature ventricular complexes and epicardial adipose tissue volume

Zhe Wang[1], Siqi Jiao[2], Jiawei Chen[1], Hehe Guo[3], Lichen Ren[3], Liping Sun[1], Yihong Sun[2]* and Yingwei Chen[1]*

[1]Department of Cardiology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China, [2]Department of Cardiology, Peking University China-Japan Friendship School of Clinical Medicine, Beijing, China, [3]Department of Radiology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China

Background: Epicardial adipose tissue (EAT) is related to atrial fibrillation. The association between EAT volume and premature ventricular complexes (PVCs) remains unclear. Our study aimed to investigate the effect of EAT volume on the risk of frequent PVCs and burden levels of PVCs.

Methods: This observational study retrospectively recruited consecutive patients who had consultation between 2019 and 2021 at the First Affiliated Hospital of Zhengzhou University. Frequent PVC patients (n = 402) and control patients (n = 402) undergoing non-contrast computed tomography (CT) were enrolled. We selected evaluation criteria for the conduct of a 1:1 propensity score matching (PSM) analysis. Multivariable logistic analysis was used to investigate factors related to frequent PVCs. Furthermore, the determinants of EAT volume and the burden levels of PVCs were evaluated.

Results: Patients with PVCs had a significantly larger EAT volume than control patients. EAT volume was significantly larger in male PVC patients with BMI ≥24 kg/m^2, diabetes mellitus, and E/A ratio <1. EAT volume was independently associated with PVCs. Moreover, the larger EAT volume was an independent predictor for the high burden level of PVCs. We revealed that the risk of high PVC burden level was increased with the rising of EAT volume by restricted cubic splines.

Conclusions: EAT volume was larger in frequent PVC patients than in control patients, regardless of other confounding factors. A large EAT volume was independently associated with high burden levels of PVCs. EAT volume may be a new mechanism to explain the pathogenesis of PVCs.

KEYWORDS

premature ventricular complexes, epicardial adipose tissue, computed tomography, burden levels, propensity score matching

Introduction

Premature ventricular complexes (PVCs) are common diseases in clinical practice. The burden levels of PVCs are one of the most important indicators of PVC risk (1). Frequent PVCs are usually accompanied by symptoms and are thought to cause arrhythmia-induced cardiomyopathy (2). The risk factors associated with frequent PVC have not been completely revealed (3).

Epicardial adipose tissue (EAT) is an adipose tissue that accumulates between the epicardium and myocardium. EAT is 20% of the heart mass under physiologic conditions (4). EAT can be considered an endocrine organ with local effects on the heart through vasocrine or paracrine secretion of pro-inflammatory and profibrotic cytokines, which promote the development of arrhythmias (5). One study found that large pericardial fat is strongly associated with ventricular arrhythmias in 50 patients with heart failure (6). Another study revealed that EAT thickness is increased, by transthoracic echocardiography measurement, in 50 patients with frequent PVCs, compared with control subjects (7). However, the role of EAT in PVCs remains unclear. The purpose of this study was to investigate the relationship between EAT volume and frequent PVCs.

Methods

Study population

This observational study retrospectively recruited consecutive patients who had consultation between January 2019 and December 2021 at the Department of Cardiology, First Affiliated Hospital of Zhengzhou University. Data were extracted from the hospital's files and were anonymized. The study population consisted of patients diagnosed with PVCs and presented to our hospital for routine follow-up. The inclusion criteria were as follows: 1) patients who underwent non-contrast CT, 2) patients symptomatic with frequent PVCs (>10,000/24 h), 3) patients >18 years of age, and 4) absence of severe valvular heart disease and hypertrophic heart disease. The exclusion criteria were follows: 1) patients with a history of prior radiofrequency ablation, 2) patients with poor/insufficient CT images, 3) patients with atrial fibrillation (AF), and 4) patients with coronary heart disease, including angina pectoris, myocardial infarction, percutaneous coronary intervention, and coronary artery bypass surgery. The control group (n = 402) matched with the PVC patients during the same time period, which had no detected PVCs using the 24-h Holter monitoring. Patients with a diagnosis of PVCs were identified using the International Classification of Disease (ICD) code in our hospital's electronic health record systems. Frequent PVC was defined as a total PVC count of >10,000 beats during a 24-h Holter monitoring. Non-contrast CT was performed for patients who wanted examination for various reasons including smoking histories, shortness of breath, or screening for lung disease in our institution. The study complied with the Declaration of Helsinki. The study protocol was authorized by the local institution's ethics committee and waived the need for written informed consent (2022-KY-0288).

Clinical and laboratory data

The following data were collected from all patients: demographic parameters, comorbidities, echocardiography parameters [left ventricular ejection fraction (LVEF), left atrial diameter (LAD), E/A ratios], CT parameter (EAT volume), and burden levels of PVCs on admission. PVC burden = total ventricular premature beats/total beats × 100%. Body mass index (BMI) ≥24 kg/m^2 was defined as overweight or obese. Smokers and drinkers were either former or active smokers and drinkers.

CT acquisition

All individual examinations use a dual-source CT system (Somatom Force, Siemens Healthineers, Forchheim, Germany). Non-contrast CT was performed with 120 kV, and the tube current depended on body habitus and heart rate. The images were reconstructed with a slice thickness of 0.5 mm, a reconstruction increment of 0.5 mm with a medium soft-tissue convolution kernel (B26F), and a reconstructed matrix size of 512 × 512.

EAT volume quantification

EAT volume was quantified using a dedicated semiautomatic software (syngo.via Frontier Cardiac Risk Assessment, version 1.2.3, Siemens Healthineers, Germany), as shown in Figure 1. Previous studies used the sophisticated software to quantify EAT volume (8–10). EAT is defined as non-contrast CT density ranging from −195 Hounsfield units (HU) to −45 HU (8, 10, 11). The software automatically delineates and identifies EAT and manually adjusts the contour of EAT volume if necessary. EAT volume measurement was performed by two independent radiologists who were unaware of the patient's clinical data.

Statistical analysis

The values of the continuous variables were described as the mean ± standard deviation or median (Q1, Q3 quartiles) and compared among the groups using Student's t-test or Mann–Whitney U test, depending on whether the data were normally distributed. Categorical variables are presented as numbers (percentage) and compared between two groups by the Pearson chi-squared test or Fisher exact test. The propensity score matching (PSM) 1:1 was applied in the observational case–control study to reduce bias in selecting the case controls. Matching of the two groups was performed for basic characteristics (age, sex), and variables subjected to univariate analysis showed p <0.10 (BMI ≥ 24 kg/m^2, hypertension, diabetes mellitus, fasting blood glucose, total cholesterol, E/A ratio <1, LVEF, and LAD). We calculated the standardized mean difference to assess the balance of baseline characteristics after PSM. Receiver operating characteristic (ROC) curve analysis was performed to analyze discriminative power,

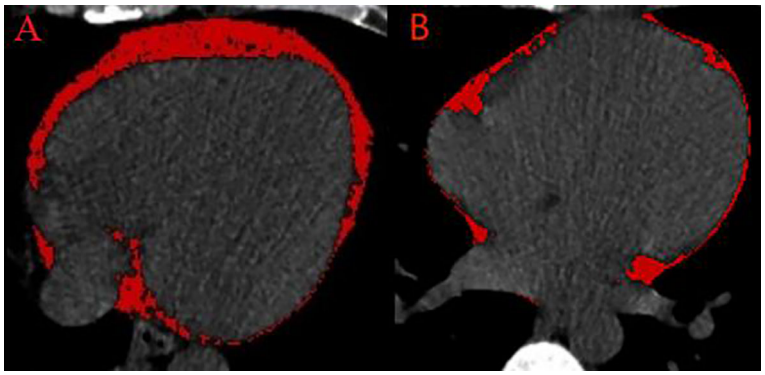


FIGURE 1
Semiautomated EAT volume analysis on non-contrast CT. **(A)** A patient with frequent PVCs. **(B)** A control patient. CT, computed tomography; EAT, epicardial adipose tissue; PVCs, premature ventricular complexes.

specificity, and sensitivity to predict the frequent PVC risk based on the EAT volume.

The associations between EAT volume and continuous variables were determined using Pearson's correlation analysis. The cutoff values of the PVC burden levels were determined by the median index. Variables subjected to univariable analysis showed *p*-value <0.10, and important clinical risk factors were included in the multivariable analysis. Multivariable logistic analysis was performed to investigate factors associated with PVC risk and burden levels after adjusting other confounders. We also used restricted cubic splines (RCS) with four knots at the 5th, 25th, 75th, and 95th centiles to flexibly model the relationship between EAT volume and PVC burden levels. Furthermore, we compared EAT volume by sextile of the PVC burden levels, with the first sextile serving as the reference. Statistical analysis was performed using R language version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria). A two-sided *p*-value of <0.05 was considered statistically significant.

Results

Baseline clinical features

Four hundred ninety-nine frequent PVC patients were screened for eligibility. Four hundred and two PVC patients were included in the final analysis, as shown in Supplementary Figure 1. The gap time between the non-contrast CT and the 24-h Holter monitoring scan performed was 2 (1–3) days in frequent PVC patients. The patients with frequent PVCs had significantly higher proportions of hypertension, diabetes mellitus, E/A ratio <1, and BMI ≥24 kg/m^2, compared with control patients. In the unadjusted cohort, PVC patients also had significantly larger age, BMI, fasting blood glucose, total cholesterol, LVEF, and LAD, compared with the control patients.

PSM was used to reduce bias in the selection of potential clinical confounders. Following the 1:1 matching protocol, differences in clinical characteristics between the two groups were eliminated. Compared with the control patients, patients' age, hypertension, diabetes mellitus, E/A ratio <1, BMI ≥24 kg/m^2, BMI, fasting blood glucose, total cholesterol, LVEF, and LAD showed no significant differences between the two groups in the PSM cohort, as shown in Table 1. Based on the propensity score, PSM achieved an optimal balance between the two groups. The standardized mean difference values were all under 0.1, which indicated that confounding factor bias was attenuated (Supplementary Figure 2).

EAT volume characteristics among PVC patients

The distribution of EAT volume is shown in Figure 2. PVC patients had significantly larger EAT volume compared with control patients in the unadjusted cohort and PSM cohort. The cutoff points of EAT volume for the prediction of frequent PVCs were calculated as 102.81 ml, and the AUC was 0.660 (95% CI: 0.622–0.697). The specificity and sensitivity were 60.2% and 66.9%, respectively, as depicted in Supplementary Figure 3. EAT volume was significantly correlated with age ($r = 0.396$, $p = 0.001$), BMI ($r = 0.388$, $p < 0.001$), and LAD ($r = 0.322$, $p < 0.001$) in the 402 PVC patients (Supplementary Table 1). EAT volume was significantly larger in male PVC patients with BMI ≥24 kg/m^2, diabetes mellitus, and E/A ratio <1, as described in Figure 3. Notably, the multivariable logistic analysis found that EAT volume (per 10 ml increase) was an independently associated factor for PVCs after adjusting different confounders, as indicated in Table 2.

EAT volume and burden levels of PVCs

The 402 PVC patients were divided into two groups—those with high burden levels of PVCs and those with low burden levels of PVCs—according to the median burden level of 19.32%. Compared with patients with low burden levels of PVCs, patients with high burden levels had significantly larger age, LAD, and EAT volume

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TABLE 1 Baseline demographics and clinical characteristics of the study patients.

Variables	Unadjusted cohort			PSM cohort		
	PVCs (*n* = 402)	Control (*n* = 402)	*p*-value	PVCs (*n* = 294)	Control (*n* = 294)	*p*-value
Age, years	47.9 ± 12.2	45.7 ± 12.6	0.012	46.4 ± 12.4	46.8 ± 12.4	0.687
Female	209 (51.7)	194 (48.0)	0.290	155 (52.7)	149 (50.7)	0.620
BMI ≥ 24 kg/m^2	242 (60.2)	207 (51.5)	0.013	161 (54.8)	165 (56.1)	0.740
BMI, kg/m^2	24.9 ± 3.4	24.3 ± 4.0	0.049	24.4 ± 3.2	24.8 ± 4.0	0.260
Smoker	67 (16.7)	57 (14.3)	0.344	49 (16.7)	41 (14.1)	0.388
Drinker	59 (14.7)	60 (14.9)	0.921	45 (15.3)	43 (14.6)	0.817
Hypertension	66 (16.4)	37 (9.2)	0.002	32 (10.9)	35 (11.9)	0.697
DM	33 (8.2)	19 (4.7)	0.045	16 (5.4)	15 (5.1)	0.854
WBC, 10^9/L	6.3 ± 1.7	6.2 ± 1.9	0.375	6.3 ± 1.7	6.2 ± 1.8	0.390
FPG, mmol/L	5.0 ± 1.3	4.8 ± 1.0	0.023	4.8 ± 1.0	4.9 ± 1.1	0.564
TC, mmol/L	4.3 ± 0.7	4.0 ± 0.8	<0.001	4.2 ± 0.7	4.2 ± 0.8	0.366
TG, mmol/L	1.2 (0.9–18)	1.2 (0.8–1.7)	0.222	1.2 (0.8–1.7)	1.3 (0.8–1.7)	0.443
UA, μmol/L	297.4 ± 88.0	289.8 ± 94.7	0.241	289.8 ± 80.2	293.9 ± 97.0	0.577
HS-CRP (>2 mg/L)	48 (11.9)	40 (10.0)	0.187	35 (13.8)	30 (11.6)	0.465
LVEF	62.4 ± 3.5	63.0 ± 1.5	0.001	62.9 ± 3.2	63.0 ± 1.5	0.634
LAD, mm	33.6 ± 5.9	30.7 ± 2.6	<0.001	31.4 ± 3.7	31.2 ± 2.6	0.415
E/A ratio <1	199 (49.5)	162 (40.3)	0.009	131 (44.6)	134 (45.6)	0.804
EAT volume, ml	135.6 ± 64.3	103.4 ± 56.0	<0.001	126.6 ± 60.5	109.7 ± 58.2	0.001

BMI, body mass index; DM, diabetes mellitus; EAT, epicardial adipose tissue; FPG, fasting blood glucose; HS-CRP, high-sensitivity C-reactive protein; LAD, left atrial diameter; LVEF, left ventricular ejection fraction; TC, total cholesterol; TG, triglycerides; PSM, propensity score matching; PVCs, premature ventricular complexes; UA, uric acid; WBC, white blood cell.

and higher proportions of being a smoker, having diabetes mellitus, and having an E/A ratio <1 (Supplementary Table 2). Variables included in the univariable analysis showed *p*-value <0.10, and clinical risk factors (fasting blood glucose, total cholesterol) were included in the multivariable analysis, which included age, female gender, smoker, fasting blood glucose, total cholesterol, diabetes mellitus, LAD, E/A ratio <1, and EAT volume (per 10 ml increase). EAT volume (per 10 ml increase; HR: 1.06; 95% CI: 1.01–1.10; p = 0.008) remained independently associated with high burden levels of PVCs, as described in Table 3. The risk of high PVC burden levels increased with the rising of EAT volume (p for non-linearity = 0.084) by RCS, using the median of EAT volume as the reference (123.38 ml), as illustrated in Figure 4. Moreover, EAT volume with high PVC burden levels significantly increased in PVC patients with burden levels of sextiles 3–6, with sextile 1 serving as the reference (Figure 5).

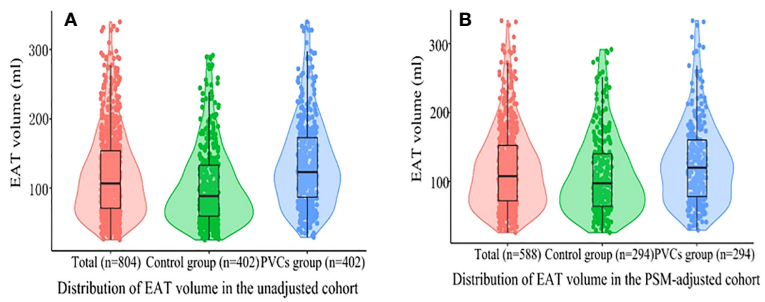


FIGURE 2
Violin plots of the distribution of EAT volume. **(A)** All patients in the unadjusted cohort; **(B)** all patients in the PSM cohort. Different color dots represent measured EAT volume; the top of the box, the 75th percentile; the horizontal line, the 50th percentile (median); the bottom of the box, the 25th percentile; the line represents the distribution area of the 95% confidence interval of the data. EAT, epicardial adipose tissue; PSM, propensity score matching; PVCs, premature ventricular complexes.

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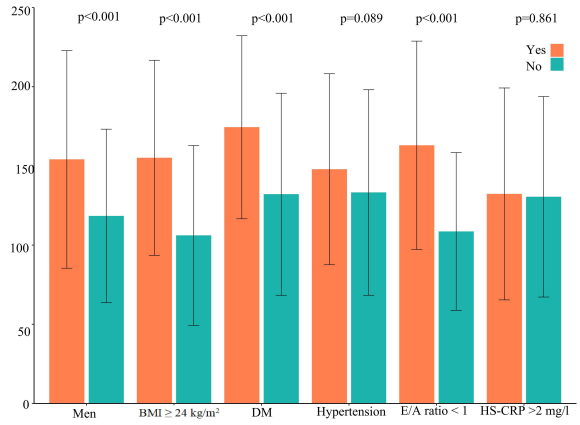


FIGURE 3
EAT volume in frequent PVC patients according to cardiovascular risk factors. DM, diabetes mellitus; HS-CRP, high-sensitivity C-reactive protein; EAT, epicardial adipose tissue; PVCs, premature ventricular complexes.

Discussion

There are several major findings in our study. First, EAT volume was increased in frequent PVC patients compared with control patients. The association was independent of other risk factors. Second, the larger EAT volume was independently associated with high PVC burden levels. There was a linear relationship between increased EAT volume and the risk of high PVC burden levels. These findings indicated that EAT volume might be a useful marker to assess the risk of PVCs and high PVC burden levels.

There is no border zone between the EAT and the myocardium, which indicated that EAT plays a vital role in arrhythmias. In the myocardium, a combined effect of structural, electrical, and autonomic remodeling may contribute to arrhythmias (12). The myocardium is affected by some metabolites, which are released from the EAT (13). Non-contrast CT can measure EAT volume to stratify cardiovascular risk with accuracy and reproducibility similar to CCTA (8). EAT volume by non-contrast CT can reduce extra costs or radiation exposure. There are no puncture

TABLE 2 Association between EAT volume and frequent PVCs.

Variables	Unadjusted cohort		PSM cohort	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Crude model (no adjustment)	1.10 (1.07–1.12)	<0.001	1.05 (1.02–1.08)	0.001
Adjusting for age and sex	1.12 (1.08–1.15)	<0.001	1.07 (1.04–1.11)	<0.001
Adjusting for age, sex, and BMI	1.13 (1.09–1.17)	<0.001	1.10 (1.06–1.14)	<0.001
Adjusting for variables[a]	1.13 (1.09–1.16)	<0.001	1.09 (1.05–1.14)	<0.001
Adjusting for variables[b]	1.08 (1.04–1.11)	<0.001	1.09 (1.05–1.13)	<0.001
Adjusting for variables[c]	1.10 (1.06–1.14)	<0.001	1.10 (1.06–1.14)	<0.001

Variables[a] included age, sex, BMI, smoker, and drinker; variables[b] subjected to univariate analysis of the unadjusted cohort showed p-value <0.10, which included age, hypertension, DM, BMI, TC, FPG, LAD, LVEF, and E/A ratio <1; variables[c] that included variables[a] and variables[b], which included age, sex, BMI, smoker, drinker, hypertension, DM, TC, FPG, LAD, LVEF, and E/A ratio <1.
BMI, body mass index; DM, diabetes mellitus; EAT, epicardial adipose tissue; FPG, fasting blood glucose; LAD, left atrial diameter; LVEF, left ventricular ejection fraction; TC, total cholesterol; PSM, propensity score matching; PVCs, premature ventricular complexes.

TABLE 3 Multivariable logistic analysis of risk factors for the burden levels of PVCs.

Variables	OR (95% CI)	*p*-value
Age	1.00 (0.98~1.02)	0.936
Female	1.07 (0.66–1.73)	0.781
Smoker	1.69 (0.90–3.16)	0.101
FPG	0.98 (0.82–1.17)	0.825
TC	0.87 (0.65–1.16)	0.336
DM	1.74 (0.75–4.01)	0.197
LAD	1.03 (0.99–1.07)	0.189
E/A ratio <1	1.08 (0.65–1.80)	0.762
EAT volume (per 10 ml increase)	1.06 (1.01–1.10)	0.008

Variables subjected to univariable analysis showed p-value <0.10, and clinical risk factors (FPG, TC) were included in the multivariable analysis, including age, female gender, smoker, FPG, TC, DM, LAD, E/A ratio <1, and EAT volume (per 10 ml increase).
DM, diabetes mellitus; EAT, epicardial adipose tissue; FPG, fasting blood glucose; LAD, left atrial diameter; PVCs, premature ventricular complexes; TC, total cholesterol; WBC, white blood cell.

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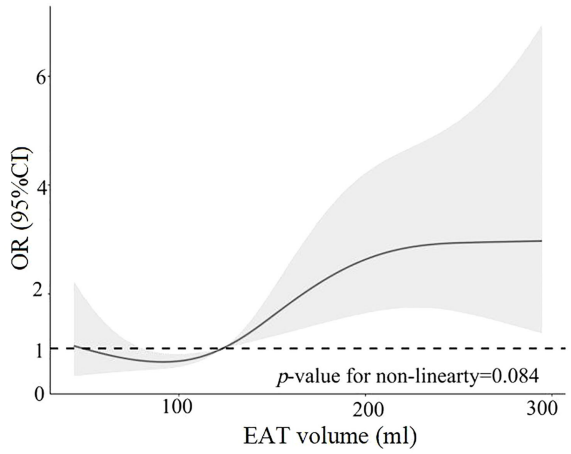


FIGURE 4
Restricted cubic spline analysis of high PVC burden risk as a function of EAT volume. EAT, epicardial adipose tissue; PVCs, premature ventricular complexes.

injuries and contrast agent injection-related complications (14). EAT volume quantification in non-contrast CT is feasible and reliable (15). Our study used sophisticated software to quantify the EAT volume.

An increasing number of studies tried to investigate the relationship between EAT and arrhythmias (13). A meta-analysis study described that large EAT volume increases the prevalence and progression of AF (16). The accumulation of EAT could promote the progression from paroxysmal to persistent AF by prolonging action potential durations (12). Sepehri et al. revealed that a large anterior interventricular groove of EAT thickness is an independent risk factor for the recurrence of ventricular tachycardia in patients following ablation (17). Another study revealed that EAT thickness is larger in patients with frequent

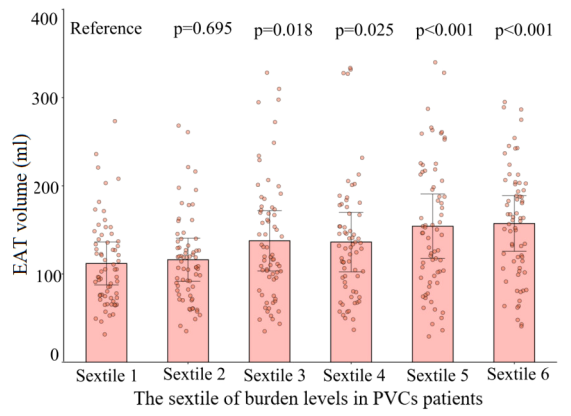


FIGURE 5
Comparison of the volume of EAT with different burden levels of PVCs. The dots represent measured EAT volume. EAT volumes in sextiles 2 to 6 of PVC burden were compared with those in the sextile 1 group. Sextile 1: burden levels < 12.9%; sextile 2: 12.9% ≤ burden levels < 16.1%; sextile 3: 16.1% ≤ burden levels < 19.3%; sextile 4: 19.3% ≤ burden levels < 24.8%; sextile 5: 24.8% ≤ burden levels < 32.0%; sextile 6: burden levels ≥ 32.0%. EAT, epicardial adipose tissue; PVCs, premature ventricular complexes.

PVC ablation failure (18). QRS widening indicates slow ventricular conduction or ventricular hypertrophy, and increased EAT is associated with widened and fragmented QRS waves. The QTc interval represents the time between ventricular depolarization and repolarization. EAT accumulation was found to be associated with prolonged QTc interval. Further studies need to investigate the relationship between EAT and ventricular repolarization.

We considered that there is insufficient direct evidence to assess the relationship between EAT volume and PVCs. Decisions based on baseline PVC burden, rather than on the so-called etiology, seem to be more appropriate for determining treatment modalities (19). Our study first revealed that patients with frequent PVCs had a large EAT volume by non-contrast CT measurement, compared with control patients. Considering that the thickness of EAT cannot accurately reflect the overall level of EAT, CT was used in our study to measure the volume of EAT. Our study suggested that EAT volume, as a non-invasive imaging biomarker, may potentially assess this risk of PVCs.

In our study, male patients with diabetes mellitus, BMI ≥24 kg/m^2, and E/A ratio <1 had a larger EAT volume in the frequent PVC cohort. Recent studies demonstrated these results (20, 21). Both obesity and type 2 diabetes are important risk factors for AF, possibly because they both cause an expansion of EAT, which is the source of pro-inflammatory adipocytokines that can lead to microvascular dysfunction and fibrosis of the myocardium. In obese and T2D patients, the EAT is thickened and dysfunctional, promoting cardiovascular damage (22). Importantly, the accumulation of cardiac adipose tissue in patients with obesity and type 2 diabetes affects the atria and the ventricles (23). A large EAT volume may be associated with impaired left cardiac diastolic function in PVC patients (24). Accumulation of EAT can lead to adjacent myocardial microcirculation dysfunction and impaired ventricular dilation (23). It indicated that a larger EAT volume might represent higher levels of cardiometabolic risk factors. This also suggested that these cardiovascular risk factors may be risk components for a larger EAT volume (20). Multiple risk factors for PVCs have been shown to act synergistically to promote the development of arrhythmias (25). The mechanisms underlying the relationship between EAT volume and cardiometabolic risk still require more in-depth study. Furthermore, our study revealed the independent association between the burden levels of PVCs and EAT volume. EAT volume may potentially quantify the burden levels of PVCs. EAT may alter the local electrophysiological properties and provide a means of risk assessment.

Several potential mechanisms can be proposed to explain the relationship between EAT volume and PVCs. Firstly, EAT can release inflammatory cytokines. EAT is associated with a high-level inflammation state (26). Second, the accumulation of EAT may cause abnormalities in Ca^{2+} handling and in excitation–contraction coupling, which lead to the disruption of intercellular electrical conduction in myocytes and slow cardiac electrical conduction (12, 27). Another hypothesis linking EAT to PVCs is related to adipose cells. EAT infiltration in the myocardium formats an anatomical obstacle for cardiac excitation, which can promote the degeneration of adjacent cardiomyocytes (28). EAT accumulation could release excessive free fatty acids and promote myocardial lipotoxicity, which leads to mitochondrial dysfunction, and increase reactive oxygen species production, which increases susceptibility to

arrhythmias (29). To elucidate these mechanisms, it is necessary to investigate the relationship between the various bioactive substances expressed in EAT and pathological conditions.

Given the important role of EAT in the development of arrhythmias, it is considered a promising therapeutic target. The systematic review and meta-analysis provide evidence that exercise, diet, and bariatric surgery can reduce cardiac adipose tissue (30). In an echocardiographic study of subjects before and after bariatric surgery, weight loss was associated with a 30% reduction in visceral fat area and a 14% reduction in EAT thickness (31). Cardiometabolic drugs have significant benefits in reducing EAT, such as glucagon-like peptide-1 receptor agonists, sodium-glucose cotransporter 2 inhibitors, metformin, and statins, especially for young patients with high BMI (32). Furthermore, we should note that whether the reduction of EAT can confer benefits for patients' clinical outcomes needs further investigation.

Limitations

Our study presented some limitations. First, this single-center study was a cross-sectional, observational study, which may lead to selection bias. It is difficult to determine any causal relationship between PVCs and EAT volume. Second, patients with coronary artery disease and AF were excluded from the study, and we considered the close association of these diseases with EAT, which leads to the inaccuracy of the research results. Third, we selected patients with high burden levels of PVCs with more symptoms and impaired cardiac function, limiting the generalizability of the results. However, it is more meaningful to study patients with frequent PVCs, which need more treatment and follow-up. Moreover, our study did not include any reference standard, such as histopathology, to confirm absolute EAT volume. However, we applied a sophisticated CT software for automatic quantification of EAT volume. Finally, we were not able to assess the potential association between PVCs and the ventricular region of EAT. To address this issue, further research may be needed using CMR to quantify the EAT volume in particular areas and investigate the impacts of different EAT areas on PVC patients. Our study could not include all confounding factors, which may alter the power of the statistical analysis and the study's conclusion. Further prospective studies involving matched groups are needed to confirm the findings.

Conclusions

EAT volume was larger in frequent PVC patients compared with control patients. A large EAT volume was independently associated with high burden levels of PVCs. EAT volume may be a new mechanism to explain the pathogenesis of PVCs.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the First Affiliated Hospital of Zhengzhou University. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because this is an observational study that does not involve drug and device interventions.

Author contributions

ZW, YS, and YC conceived and designed the study. ZW, SJ, JC, HG, and LR participated in the acquisition of data. ZW, SJ, and JC analyzed the data. ZW drafted the manuscript. LS, YS, and YC revised the manuscript. All authors contributed to the article and approved the submitted version.

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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1219890/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Frequent PVCs patient selection and study design. CT, computed tomography; PSM: propensity score matching; PVCs, premature ventricular complexes.

SUPPLEMENTARY FIGURE 2
Standardized mean differences of different cohorts in this graph. BMI, body mass index; DM. diabetes mellitus; EAT, epicardial adipose tissue; FPG, fasting blood glucose; LAD, left atrial diameter; LVEF, left ventricular ejection fraction; TC, total cholesterol.

SUPPLEMENTARY FIGURE 3
Receiver operating characteristic curve illustrating the accuracy of EAT volume for frequent PVCs risk. EAT, epicardial adipose tissue; PVCs, premature ventricular complexes.

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EDITED BY
Rogerio Faustino Ribeiro Junior,
Escola Superior de Ciências da Santa Casa de Misericórdia de Vitória (EMESCAM), Brazil

REVIEWED BY
Blake Cochran,
University of New South Wales, Australia
Xiangyuan Pu,
Zhejiang University, China

*CORRESPONDENCE
Zhuye Gao
zhuyegao@126.com
Hao Xu
xuhaotcm@hotmail.com

†These authors have contributed equally to this work

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Remnant cholesterol associates with hypertension beyond low-density lipoprotein cholesterol among the general US adult population

Liu Shi[1,2], Dongmei Zhang[3], Jianqing Ju[2], Anlu Wang[2], Tianyi Du[1,2], Xuanye Chen[1,2], Yewen Song[2], Zhuye Gao[2*†] and Hao Xu[2*†]

[1]Graduate School, Beijing University of Chinese Medicine, Beijing, China, [2]National Clinical Research Center for Chinese Medicine Cardiology, Xiyuan Hospital, China Academy of Chinese Medical Sciences, Beijing, China, [3]Dongzhimen Hospital, Beijing University of Chinese Medicine, Beijing, China

Background: Previous findings have indicated that elevated low-density lipoprotein cholesterol (LDL-C) and remnant cholesterol (RC) are associated with hypertension. We aim to explore whether higher RC levels may be associated with hypertension beyond LDL-C in the general US adult population.

Methods: This study included 10,842 adults from the National Health and Nutrition Examination Survey (NHANES) 1999–2018. Weighted multivariable logistic regression models were used to estimate the odds ratios (ORs) of hypertension for LDL-C and RC. We also performed analyses examining the association between hypertension and LDL-C vs. RC concordant/discordant groups.

Results: A total of 4,963 (41.54%, weighted) individuals had hypertension. The weighted median levels were LDL-C: 118mg/dL, RC: 20mg/dL. At lower LDL-C clinical cut-point, the proportion of discordantly high RC dramatically increased. After multivariable adjustment, log RC was associated with higher prevalence of hypertension [OR 2.54, 95% confidence interval (CI) 2.17–2.99]. Participants with the highest tertile of RC were more likely to have hypertension (OR 2.18; 95% CI 1.89-2.52) compared with those with the lowest tertile of RC. This association remained marked after including body mass index (BMI), LDL-C, high-density lipoprotein cholesterol (HDL-C) or triglycerides. The association between LDL-C and hypertension was absent after adjusting for BMI, RC or triglycerides. Compared with low LDL-C/low RC group, the discordant low LDL-C/high RC group was associated with hypertension (OR 2.04; 95% CI 1.72-2.42), whereas the high LDL-C/low RC group was not, regardless of BMI, HDL-C or triglycerides. Similar results were observed when examining discordance among different clinical cut-points, except for the cut-point of LDL-C 70 mg/dL and RC 13 mg/dL. To better understand the association, we performed an additional analysis, which showed that among participants with apolipoprotein B < median (92mg/

dL), those with discordant RC ≥ median (20mg/dL) had significantly higher odds of having hypertension (OR 1.73; 95% CI 1.38-2.17).

Conclusion: RC was associated with hypertension beyond LDL-C in the general US adult population. This association went beyond increased triglycerides levels, and lipoproteins other than apoB may be involved.

KEYWORDS

remnant cholesterol, hypertension, low-density lipoprotein cholesterol, apolipoprotein B, dyslipidemia

Introduction

Hypertension is one of the most critical risk factors for heart disease and stroke, two of the leading causes of premature death in the United States (1). Nearly 47.3% of US adults have hypertension (2). The total costs of hypertension as a risk factor could increase to 30% of the total expenditure for cardiovascular disease, by 2035 (3).

Hypertension and hypercholesterolemia are important and prevalent disorders in atherosclerotic cardiovascular disease (ASCVD). More than half of hypertensive patients have hypercholesterolemia (4). Abnormal lipid metabolism induces insulin resistance, chronic inflammatory response, and impaired endothelial function, all of which may affect blood pressure (5). Triglyceride-rich lipoproteins (TRLs) and their cholesterol-rich remnants are now recognized as powerful causative factors of atherosclerosis and metabolic disorders, with predictive ability exceeding that of low-density lipoprotein cholesterol (LDL-C) (6–8). Remnant cholesterol (RC) is the cholesterol content of TRLs and its metabolic remnants. Previous studies have shown that both LDL-C and RC were associated with the onset of hypertension (9–11). A Chinese cohort-based study revealed that RC was associated with the risk of atherosclerosis and atherosclerotic advancement, even in individuals with favorable LDL-C levels (12). Another community-based cohort indicated that there was a threshold effect of LDL-C on the new-onset hypertension, and the risk was increased only at normal LDL-C concentrations (13).

These unanticipated observations have spurred calls to validate these findings further in other populations settings and to re-assess the independent pathogenic role of RC. In the present study, we intended to examine the association of RC and LDL-C with hypertension in the general US adult population with not receiving lipid-lowering treatment, and to further identify the concordant/discordant LDL-C and RC associations with hypertension.

Methods

Study setting and population

We used data collected in the National Health and Nutrition Examination Survey (NHANES), which is conducted by the National Center for Health Statistics (NCHS) of the Center for Disease Control and Prevention. NHANES is a nationally representative survey of the civilian, noninstitutionalized US population, to evaluate the health and nutritional status. All participants provided written informed consent. The NHANES study protocols were approved by the NCHS ethics review board. Data were collected consecutively but released in 2-year cycles (14). Specific information is available at the NHANES website (https://www.cdc.gov/nchs/nhanes/index.htm). We examined data from 10 sequential cycles covering the periods 1999-2018. There were 17,398 participants aged 18 years or older with plasma lipid data available as well as complete questionnaire information and physical examination data. We excluded participants who were pregnant (N=567). Considering that some medications for cancer may affect blood pressure, we also excluded participants who had cancer (N=2,071). Next, we excluded those did not self-report medication use (N=19) or currently on lipid-lowering treatment (N=3,429) that may affect plasma lipid levels. Participants with missing key laboratory data (N=36) were also excluded. Considering the complex, multi-stage, probability sampling design of NHANES, we further excluded individuals with missing weights (N=434). The final analytical cohort included 10,842 participants.

Hypertension diagnosis

Blood pressure measurements were taken by the mobile examination center examiners using a standardized protocol. Hypertension was diagnosed based on the questionnaires and physical examination results. Three or 4 sometimes consecutive blood pressure determinations were obtained. Traditional averages did not represent the final blood pressure results. If only one blood pressure measurement was available, that measurement is the average. If there were multiple blood pressure readings, the first reading was always not included in the average. Participants were regarded as having hypertension if they met one of the following 3 criteria were considered to have hypertension: (1) the average systolic blood pressure ≥130 mmHg or the average diastolic blood pressure ≥80 mmHg; (2) the answer to the question "are you now taking prescription for hypertension" was "yes"; (3) the answer to

the question "have you ever been told that you had high blood pressure" was "yes".

Lipid measurements

Fasting total cholesterol (TC) and triglycerides (TG) were analyzed using enzymatic methods. High-density lipoprotein cholesterol (HDL-C) was determined by the heparin manganese precipitation or direct immunoassay method. LDL-C was calculated using the **Martin-Hopkins equation** as it employs an adjustable factor, the ratio of TG to very low-density lipoprotein cholesterol (VLDL-C), to estimate LDL-C levels (15). Considering the greater inaccuracy of LDL-C estimation at higher TG levels, especially TG > 400 mg/dL, the use of estimating equations in this situation is not currently recommended (16). Therefore, we estimated LDL-C only for participants with TG ≤ 400 mg/dL. RC (mg/dL) was estimated as TC (mg/dL) minus calculated LDL-C (mg/dL) minus HDL-C (mg/dL). LDL-C and RC were also estimated using the Friedewald equation and Sampson-NIH (National Institute of Health) formula, which have been presented in the published paper (17). Apolipoprotein B (apoB) was measured by immuno-turbidimetric assay for 5 consecutive cycles covering the periods 2007-2016.

Concordance/discordance definition

Since there is no recognized physiologically discordant cut-point between different lipid measures, we adopted different thresholds to identify the LDL-C and RC concordant/discordant groups. First, we used the medians as cut-points. The population was divided into 4 groups: (1) low LDL-C/low RC (LDL-C < median, RC < median); (2) low LDL-C/high RC (LDL-C < median, RC > median); (3) high LDL-C/low RC (LDL-C > median, RC < median); (4) high LDL-C/high RC (LDL-C > median, RC > median). Second, we also focused on relevant clinical LDL-C cut-points (70, 100, and 130 mg/dL) according to established guideline recommendations (5, 18, 19). The equivalent population percentile corresponding to these LDL-C values was used to determine the respective RC cut-points.

Covariates

Information about participants age, sex, race/ethnicity, family income-poverty ratio, education level, smoking status, disease status and use of antihypertensive and hypoglycemic medications was collected from household interviews using questionnaires. Body mass index (BMI), waist circumference (WC) and alcohol intake were obtained when participants took physical examinations. Additionally, considering the interconnection between the metabolic abnormalities, we also included glycosylated hemoglobin A1c (HbA1c), fasting blood glucose (FBG), estimated glomerular filtration rate (eGFR), and high-sensitivity C-reactive protein (hs-CRP) in the analysis. Description of laboratory methodology can be found in the laboratory procedures manual for the corresponding cycle. The specific definitions and classifications of covariates were shown in the Supplemental Material.

Statistical analysis

NHANES uses a complex, multi-stage, probability sampling design. Consistent with NHANES analysis recommendations (20), we newly constructed weights for combining survey cycles. New weights were calculated as two-tenths of WTSAF4YR (a fasting weight variable in NHANES) for 1999-2000 and 2001-2002 survey cycles, one-tenths of WTSAF2YR for 2003-2004, 2005-2006, 2007-2008, 2009-2010, 2011-2012, 2013-2014, 2015-2016, 2017-2018 waves.

Characteristics were described depending on whether participants had hypertension, RC levels and LDL-C and RC concordant/discordant groups. Data were presented as mean ± standard error for continuous variables or frequency (weighted percentage) for categorical variables. We used weighted linear regression model (continuous variables) or weighted chi-square test (categorical variables) to compare baseline characteristics. Weighted multivariable logistic regression models adjusting for potential confounders were used to estimate odds ratios (ORs) of hypertension for LDL-C and RC. LDL-C and RC were included in the model as continuous variables (log-transformed) and categorical variables (tertiles), respectively. In the multivariate models, we adjusted for age (continuous), sex (male or female), race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, or other), educational level (less than high school, high school or equivalent, or college or above), family income-poverty ratio (≤ 1.0, 1.1-3.0, or > 3.0), smoking status (never smoker, former smoker, or current smoker), and alcohol drinking (non-drinker, low to moderate drinker, or heavy drinker) in model 1. Model 2 further adjusted for chronic kidney disease (yes or no), diabetes mellitus (yes or no), coronary heart disease (yes or no) in addition to covariates in model 1. In model 3, we further adjusted for eGFR (continuous), FBG (continuous), and HbA1c (continuous). To analyze the association of RC with hypertension independent of LDL-C, we also further adjusted for LDL-C in model 4. In our main analyses, we did not adjust for BMI and WC as they could be mediators on the pathway from lipid disorders to hypertension. The median value was assigned to each tertile as a continuous variable to test for a linear trend. The association of LDL-C and RC concordant/discordant groups with hypertension was also evaluated adjusting for variables as described above. Considering that the nonlinear relationship may affect the rationality of discordant/concordant grouping, we examined LDL-C and RC as nonlinear variables, modeled with 3-knotted restricted cubic spline regression, adjusting all variables in model 3 mentioned above. Before performing regression analyses, we first assessed the collinearity of RC/LDL-C and adjusted covariates. Using variance inflation factor as an assessment tool, covariates with variance inflation factor above 10 were considered as collinear variables, and those with variance inflation factor between 5 and 10 could be excluded as needed. Collinearity test results showed no high collinearity between RC/LDL-C and adjusted covariates (Supplemental Tables S1-S4).

Stratified analyses and interaction analyses were conducted to examine whether the association differed by age quintiles, sex (male or female), obesity status (BMI < 25, 25 ≤ BMI < 30, or BMI ≥ 30 kg/m^2), smoking status (never smoker, former smoker, or current smoker), chronic kidney disease (yes or no), diabetes mellitus (yes or no), survey cycles (1999-2008 or 2009-2018) and hypoglycemic drugs use (yes or no). The *P* values for the product terms between LDL-C and RC discordant/concordant groups and stratification variables were used to evaluate the significance of interactions.

Additional sensitivity analyses were performed to access the robustness of the findings. First, we further adjusted for BMI and WC in addition to model 3, given the possibility that they could be confounders (i.e., affecting lipid levels) rather than mediators (i.e., affected by lipid levels). Collinearity screening results showed that the variance inflation factors for BMI and WC ranged from 5 to 10, so we only further adjusted for BMI (Supplemental Tables S5-S7). Second, considering that differences in HDL-C levels between LDL-C and RC discordant/concordant groups may affect the association results, we further adjusted for HDL-C in addition to model 3. Third, as the levels of TG and RC were correlated, TG was further adjusted for based on model 3. Fourth, to examine whether inflammation had an effect on the observed correlation, we further adjusted for hs-CRP in a subgroup of the study participants (only available in NHANES 2015-2018, N=1,947). We appropriately excluded variables with variance inflation factor greater than 10 to ensure the reliability of the regression results (Supplemental Tables S8-S10). Fifth, LDL-C and RC were recalculated using the Friedewald equation and Sampson-NIH formula to determine the effect of different calculation methods. Sixth, considering the effect of recall bias, we re-conducted the analysis after excluding people who were previously told they had hypertension. Seventh, to rule out a possible effect of antihypertensive drugs on lipids, we performed additional analyses in the population not taking antihypertensive drugs. Finally, given that apoB reflects more accurately the number of atherogenic lipoprotein particles in the blood than LDL-C (21), we explored the association between apoB and RC discordant/concordant subgroups (the medians as cut-points) and hypertension (apoB data only available in NHANES 2007-2016, N=6,854). Data were analyzed from November 2022 to May 2023. All statistical analyses were conducted using R software version 4.2.2 (R foundation for Statistical Computing, Vienna, Austria). All *p* values were two-sided with a significance level of < 0.05.

Results

Of 10,842 participants, 5,048 (weighted proportion, 48.17%) were men; the mean (standard error) age was 42.2 (0.21) years; 41.54% (weighted) individuals had hypertension. The weighted median levels were LDL-C: 118mg/dL, RC: 20mg/dL, apoB: 92mg/dL. Supplemental Table S11 and S12 described the characteristics of the participants according to hypertension status and tertiles of RC levels, which were unevenly distributed among them. A greater number of hypertensive participants were in low LDL-C/high RC group and high LDL-C/high RC group. Compared with individuals in high LDL-C/low RC group, those in low LDL-C/high RC group tended to be younger, women, Mexican American, current smokers and heavy drinkers; were less likely to have higher education levels and family income; had higher BMI, WC, TG and hs-CRP levels but lower HDL-C, non-HDL-C, TC and apoB levels; had higher prevalence of comorbidities and higher rates of antihypertensive and hypoglycemic drugs use. (Table 1).

Distribution of LDL-C and RC

High levels of RC accounted for a relatively large proportion of the participants with increased LDL-C levels (Supplemental Figure S1). All participants with LDL-C below the clinical cut-points had discordantly high levels of RC when TG ≥150mg/dL. At lower LDL-C clinical cut-point, the proportion of discordantly high RC dramatically increased, up to 70.26% (weighted) in those with LDL-C < 70mg/dL (Figure 1).

Association between LDL-C or RC with hypertension

Multivariable restricted cubic spline regression models showed a monotonically increasing relationship between RC or LDL-C and hypertension, even though the nonlinear *p*-value for RC < 0.001 (Figure 2). After adjusting for several potential confounders, we observed a significant association between log RC levels with hypertension (OR, 2.54; 95% CI, 2.17–2.99) in model 3, with unchanged results after adjusting for LDL-C (OR, 2.69; 95% CI, 2.26–3.21) (Table 2). The results were similar when RC was included in the model as categorical variables (tertiles) (Table 2). This association remained marked after including BMI or HDL-C (model 1 and model 2, Supplemental Table S13). Notably, tertiles of RC continued to be associated with hypertension despite adjusting for TG (model 3, Supplemental Table S13). In contrast, the association between tertiles of LDL-C and hypertension was absent after adjusting for BMI, RC or TG (Table 2 and Supplemental Table S13).

Association between LDL-C and RC concordant/discordant groups with hypertension

Compared to the low LDL-C/low RC group, the participants in low LDL-C/high RC group and high LDL-C/high RC group were associated with hypertension (low LDL-C/high RC group: OR, 2.04; 95% CI, 1.72-2.42; high LDL-C/high RC group: OR, 1.81; 95% CI, 1.55-2.10) (Table 3). This increase remained noticeable after adjusting for BMI, HDL-C or TG (model 1-3, Supplemental Table S14). On the other hand, those in the high LDL-C/low RC group had similar hypertension prevalence compared to the low LDL-C/low RC group. We found almost identical results when using different clinical cut-points to define concordance/discordance, except for the clinical cut-point of LDL-C 70 mg/dL and RC 13 mg/dL, given the smaller sample size and fewer number of positive events (Figure 3).

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TABLE 1 Characteristics of participants in different concordant/discordant groups *.

Characteristic	Concordant/discordant of LDL-C and RC				*P* value
	Low LDL-C and low RC (n=3,517)	Low LDL-C and high RC (n=1,853)	High LDL-C and low RC (n=1,558)	High LDL-C and high RC (n=3,914)	
Age, (mean ± SE), years	37.70 ± 0.30	40.75 ± 0.41	44.79 ± 0.44	46.12 ± 0.29	<0.001
Sex					
Male	1951 (57.22)	754 (40.59)	763 (50.47)	1580 (42.23)	<0.001
Female	1566 (42.78)	1099 (59.41)	795 (49.53)	2334 (57.77)	
Race/ethnicity					
Non-Hispanic White	1517 (67.09)	833 (67.48)	670 (67.75)	1889 (72.90)	<0.001
Non-Hispanic Black	888 (13.95)	280 (8.55)	440 (15.73)	495 (6.65)	
Mexican American	519 (7.59)	419 (11.12)	205 (6.01)	922 (9.74)	
Other	593 (11.37)	321 (12.85)	243 (10.50)	608 (10.71)	
Educational level					
Less than high school	209 (3.08)	177 (4.52)	115 (3.65)	484 (5.68)	<0.001
High school or equivalent	1214 (30.12)	707 (34.73)	545 (32.05)	1541 (38.23)	
College or above	2094 (66.80)	969 (60.75)	898 (64.30)	1889 (56.09)	
Family income-poverty ratio					
≤1.0	719 (14.27)	385 (14.47)	256 (9.90)	723 (12.59)	<0.001
1.1-3.0	1383 (34.68)	789 (37.87)	598 (33.79)	1650 (36.02)	
>3.0	1415 (51.05)	679 (47.66)	704 (56.32)	1541 (51.38)	
Smoking status					
Never smoker	1985 (55.81)	872 (48.17)	890 (56.29)	1834 (47.10)	<0.001
Former smoker	714 (21.55)	454 (24.15)	360 (22.71)	1065 (26.32)	
Current smoker	818 (22.64)	527 (27.67)	308 (21.00)	1015 (26.57)	
Alcohol drinking					
Non-drinker	478 (10.61)	348 (14.55)	260 (14.52)	773 (16.46)	<0.001
Low to moderate drinker	1544 (45.44)	590 (33.37)	680 (46.32)	1381 (38.24)	
Heavy drinker	1495 (43.94)	915 (52.08)	618 (39.16)	1760 (45.30)	
BMI	26.29 ± 0.17	29.70 ± 0.21	27.76 ± 0.19	29.82 ± 0.16	<0.001
Waist Circumference	90.43 ± 0.42	100.88 ± 0.52	95.01 ± 0.46	101.70 ± 0.36	<0.001
SBP, mmHg	115.63 ± 0.33	121.23 ± 0.44	118.68 ± 0.44	122.57 ± 0.33	<0.001
DBP, mmHg	68.21 ± 0.26	71.76 ± 0.35	70.83 ± 0.32	73.31 ± 0.24	<0.001
Hypertension	1190 (28.84)	954 (48.72)	680 (38.16)	2139(51.55)	<0.001
Diabetes mellitus	238 (4.27)	274 (11.02)	113 (4.88)	516(9.55)	<0.001
Coronary heart disease	29 (0.45)	37 (1.21)	12 (0.52)	49(0.82)	0.007
Chronic kidney disease	314 (6.95)	277 (11.51)	136 (6.62)	531(10.25)	<0.001
Stroke	41 (0.79)	35 (1.12)	26 (0.86)	82(1.70)	0.002
Antihypertensive drugs use	135 (2.86)	156 (7.23)	84 (4.43)	307 (6.87)	<0.001
Hypoglycemic drugs use	100 (1.63)	135 (5.84)	43 (1.75)	176 (3.01)	<0.001

(Continued)

TABLE 1 Continued

Characteristic	Concordant/discordant of LDL-C and RC				*P* value
	Low LDL-C and low RC (n=3,517)	Low LDL-C and high RC (n=1,853)	High LDL-C and low RC (n=1,558)	High LDL-C and high RC (n=3,914)	
LDL-C, (mean ± SE), mg/dL	89.34 ± 0.48	99.75 ± 0.41	139.12 ± 0.64	150.85 ± 0.54	<0.001
HDL-C, (mean ± SE), mg/dL	60.32 ± 0.42	47.78 ± 0.41	60.12 ± 0.46	48.28 ± 0.28	<0.001
Non-HDL-C, (mean ± SE), mg/dL	104.40 ± 0.51	126.26 ± 0.47	155.86 ± 0.65	180.64 ± 0.61	<0.001
TG, (mean ± SE), mg/dL	66.45 ± 0.41	160.95 ± 1.71	71.56 ± 0.44	163.94 ± 1.26	<0.001
TC, (mean ± SE), mg/dL	164.72 ± 0.59	174.04 ± 0.56	215.97 ± 0.81	228.93 ± 0.68	<0.001
RC, (mean ± SE), mg/dL	15.06 ± 0.06	26.51 ± 0.19	16.74 ± 0.06	29.79 ± 0.17	<0.001
FBG, (mean ± SE), mmol/L	5.35 ± 0.02	5.76 ± 0.05	5.47 ± 0.03	5.79 ± 0.03	<0.001
HbA1c, (mean ± SE), %	5.26 ± 0.01	5.45 ± 0.03	5.40 ± 0.02	5.55 ± 0.02	<0.001
eGFR, (mean ± SE), mL/min/1.73 m^2	103.51 ± 0.48	100.25 ± 0.57	97.21 ± 0.53	94.86 ± 0.39	<0.001
ApoB, mg/dL †	71.32 ± 0.43	83.83 ± 0.48	101.60 ± 0.68	117.00 ± 0.64	<0.001
Hs-CRP, mg/L ‡	3.37 ± 0.33	4.09 ± 0.35	3.37 ± 0.36	3.92 ± 0.33	0.273

apoB, apolipoprotein B; BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; HbA1c, glycosylated hemoglobin A1c; hs-CRP, high-sensitivity C-reactive protein; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; RC, remnant cholesterol; SBP, systolic blood pressure; TG, triglycerides; TC, total cholesterol.
SI conversions: to convert LDL-C, HDL-C, Non-HDL-C, TC, and RC to mmol/L, multiply by 0.02586; to convert TG to mmol/L, multiply by 0.01129.
* The different concordant/discordant groups based on LDL-C 118 mg/dL and RC 20 mg/dL cut-points. All means and SEs for continuous variables and percentages for categorical variables were weighted, with the exception of the number of participants.
† ApoB data only available in 2007-2016.
‡ hs-CRP data only available in 2015-2018.

The association between tertiles of RC and hypertension was slightly weakened after adjusting for hs-CRP (N=1,947, model 4, Supplemental Table S13), but the significance of higher prevalence of hypertension was attenuated among individuals in low LDL-C/high RC group (model 4, Supplemental Table S14).

Results were consistent when analyses were stratified by age quintiles, BMI, self-reported diabetes mellitus, self-reported chronic kidney disease, survey cycles and hypoglycemic drugs use (Figure 4 and Supplemental Table S15). The test for interactions was significant for sex and smoking status (all $p_{\text{interaction}} < 0.05$), with

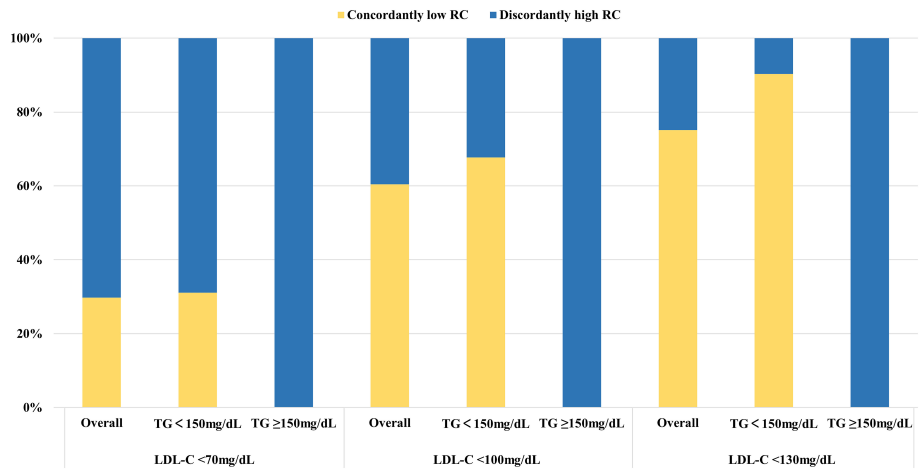


FIGURE 1
Proportions of concordance/discordance among individuals with LDL-C below clinical cut-points in the NHANES 1999 to 2018. Discordantly high RC definition: LDL-C < 70mg/dl, RC > 13mg/dl; LDL-C < 100mg/dl, RC > 17mg/dl; LDL-C < 130mg/dl, RC > 23mg/dl. LDL-C cut-points (70, 100, and 130 mg/dL) were based on established guideline recommendations. The equivalent population percentile corresponding to these LDL-C values was used to determine the respective RC cut-points. Percentages were weighted. LDL-C, low-density lipoprotein cholesterol; RC, remnant cholesterol; TG, triglycerides.

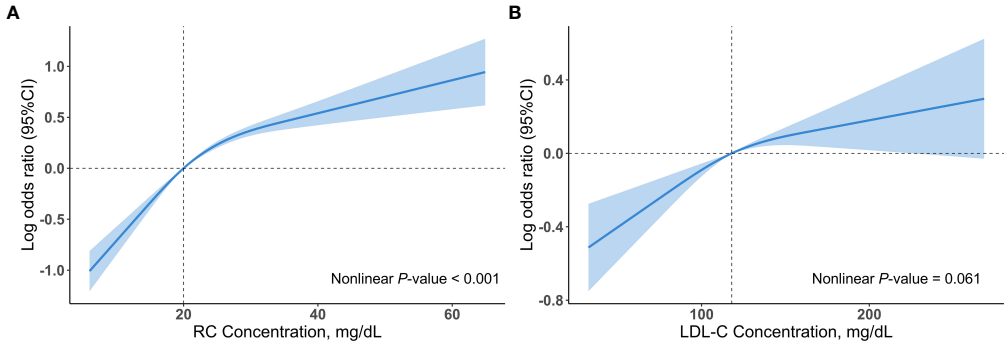


FIGURE 2
Associations (log odds ratios, 95%CIs) of RC and LDL-C concentrations with hypertension using a restricted cubic spline regression model in the NHANES 1999 to 2018. **(A)** Association between RC concentrations and hypertension. **(B)** Association between LDL-C concentrations and hypertension. Results were adjusted for age (continuous), sex (male/female), race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, other), educational level (less than high school, high school or equivalent, college or above), family income-poverty ratio (≤1.0, 1.1-3.0, >3.0), smoking status (never smoker, former smoker, current smoker), alcohol drinking (non-drinker, low to moderate drinker, heavy drinker), chronic kidney disease (yes or no), diabetes mellitus (yes or no), coronary heart disease (yes or no), eGFR (continuous), FBG (continuous), and HbA1c (continuous). All estimates accounted for complex survey design. Restricted cubic spline regression model was conducted with 3 knots. Shadows represent the 95% CIs for the spline model (with respective medians as reference). CI, confidence interval; LDL-C, low-density lipoprotein cholesterol; RC, remnant cholesterol.

low LDL-C/high RC more likely to be related to hypertension in males, never smokers and former smokers. Finally, we observed that our results did not materially change after excluding those previously informed of hypertension (N=322) or those taking antihypertensive drugs (N=682) (Supplemental Tables S16 and S17). Results were consistent when using Sampson-NIH formula or Friedewald equation to recalculate LDL-C and RC (Supplemental Table S18).

TABLE 2 Adjusted ORs (95%CIs) of hypertension according to LDL-C and RC concentrations.

Variables	LDL-C and RC Levels, OR (95%CI)				Per 1 log-transformed increment, mg/dL
	Tertile1	Tertile2	Tertile3	P_{trend}	
LDL-C					
Median (range), mg/dL	86.8 (19.6-104.4)	118.4 (104.5-133.6)	153.1 (133.7-354.1)		
Cases, No./Total No.	1,343/3,613	1,671/3,615	1,949/3,614		4,963/10,842
Model 1 *	Ref	1.20 (1.05-1.37)	1.28 (1.11-1.48)	0.001	1.52 (1.26-1.85)
Model 2 †	Ref	1.23 (1.08-1.40)	1.33 (1.14-1.54)	<0.001	1.58 (1.29-1.93)
Model 3 ‡	Ref	1.22 (1.07-1.40)	1.32 (1.13-1.54)	<0.001	1.57 (1.27-1.94)
Model 4 §	Ref	0.99 (0.86-1.14)	0.89 (0.75-1.07)	0.186	0.86 (0.69-1.09)
RC					
Median (range), mg/dL	14.6 (2.9-17.4)	20.5 (17.5-24.5)	30.7 (24.6-67.5)		
Cases, No./Total No.	1,238/3,615	1,667/3,602	2,508/3,625		4,963/10,842
Model 1 *	Ref	1.49 (1.31-1.69)	2.33 (2.02-2.68)	<0.001	2.74 (2.34-3.20)
Model 2 †	Ref	1.47 (1.28-1.67)	2.22 (1.93-2.56)	<0.001	2.59 (2.21-3.03)
Model 3 ‡	Ref	1.45 (1.27-1.65)	2.18 (1.89-2.52)	<0.001	2.54 (2.17-2.99)
Model 4 ‖	Ref	1.46 (1.27-1.67)	2.21 (1.89-2.58)	<0.001	2.69 (2.26-3.21)

CI, confidence interval; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; RC, remnant cholesterol.
* Model1: adjusted for age (continuous), sex (male/female), race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, other), educational level (less than high school, high school or equivalent, college or above), family income-poverty ratio (≤1.0, 1.1-3.0, >3.0), smoking status (never smoker, former smoker, current smoker), and alcohol drinking (non-drinker, low to moderate drinker, heavy drinker).
† Model 2: further adjusted (from Model 1) for chronic kidney disease (yes or no), and diabetes mellitus (yes or no), coronary heart disease (yes or no).
‡ Model 3: further adjusted (from Model 2) for eGFR (continuous), FBG (continuous), and HbA1c (continuous).
§ Model 4: further adjusted (from Model 3) for log RC (continuous).
‖ Model 4: further adjusted (from Model 3) for log LDL-C (continuous).
All estimates accounted for complex survey design.

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TABLE 3 Adjusted ORs (95%CIs) of hypertension according to different concordant/discordant groups *.

	Concordant/discordant of LDL-C and RC, OR (95%CI)			
	Low LDL-C and low RC	Low LDL-C and high RC	High LDL-C and low RC	High LDL-C and high RC
Cases, No./Total No.	1,190/3,517	954/1,853	680/1,558	2,139/3,914
Model 1 †	ref	2.18 (1.84-2.59)	1.04 (0.87-1.25)	1.85 (1.60-2.14)
Model 2 ‡	ref	2.07 (1.75-2.46)	1.08 (0.89-1.30)	1.83 (1.57-2.12)
Model 3 §	ref	2.04 (1.72-2.42)	1.08 (0.90-1.31)	1.81 (1.55-2.10)

CI, confidence interval; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; RC, remnant cholesterol.
* The different concordant/discordant groups based on LDL-C 118 mg/dL and RC 20 mg/dL cut-points.
† Model1: adjusted for age (continuous), sex (male/female), race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, other), educational level (less than high school, high school or equivalent, college or above), family income-poverty ratio (≤1.0, 1.1-3.0, >3.0), smoking status (never smoker, former smoker, current smoker), and alcohol drinking (non-drinker, low to moderate drinker, heavy drinker).
‡ Model 2: further adjusted (from Model 1) for chronic kidney disease (yes or no), diabetes mellitus (yes or no), and coronary heart disease (yes or no).
§ Model 3: further adjusted (from Model 2) for eGFR (continuous), FBG (continuous), and HbA1c (continuous).
All estimates accounted for complex survey design.

Association between apoB and RC concordant/discordant groups with hypertension

A supplementary analysis (NHANES 2007-2016, N=6,854) showed that among participants with apoB < median (92mg/dL), those with discordant RC ≥ median (20mg/dL) had significantly higher odds of hypertension after adjusting potential confounders (OR, 1.73; 95% CI, 1.38–2.17) (Table 4).

Discussion

In a large and nationally representative sample of general US adults, we found that (i) elevated RC levels were associated with hypertension independent of multiple risk factors, including LDL-C levels and (ii) those with low LDL-C/high RC, but not high LDL-C/low RC, were significantly associated with hypertension independent of HDL-C and TG. Additional analyses showed that RC-associated high prevalence of hypertension may not be related

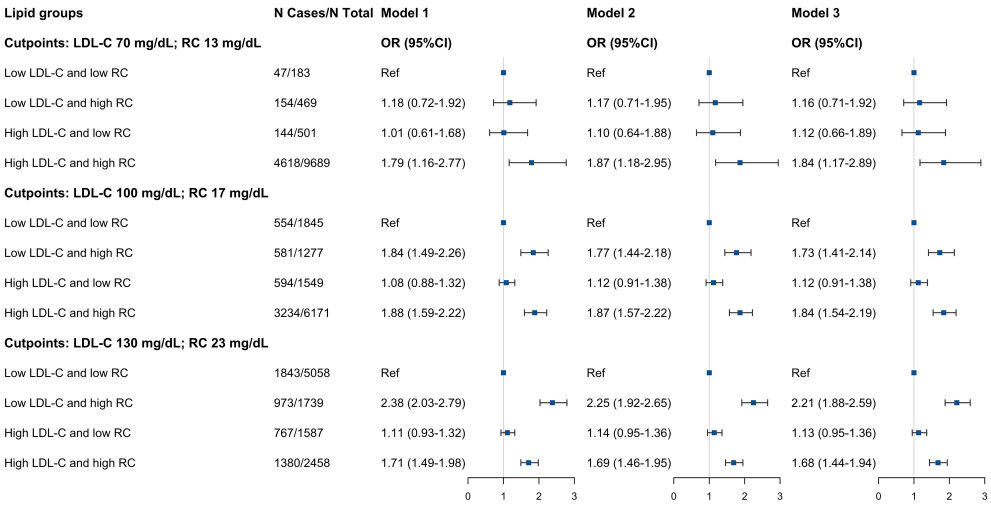


FIGURE 3
Adjusted ORs (95%CIs) of hypertension according to different concordant/discordant groups by LDL-C clinical cut-points (70, 100, and 130 mg/dL) and percentile-equivalents for RC in the NHANES 1999 to 2018. Model 1: adjusted for age (continuous), sex (male/female), race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, other), educational level (less than high school, high school or equivalent, college or above), family income-poverty ratio (≤1.0, 1.1-3.0, >3.0), smoking status (never smoker, former smoker, current smoker), and alcohol drinking (non-drinker, low to moderate drinker, heavy drinker). Model 2: further adjusted (from Model 1) for chronic kidney disease (yes or no), diabetes mellitus (yes or no), and coronary heart disease (yes or no). Model 3: further adjusted (from Model 2) for eGFR (continuous), FBG (continuous), and HbA1c (continuous). Squares represent odds ratios and solid black lines indicate 95% confidence intervals. All estimates accounted for complex survey design. OR, odds ratio; CI, confidence interval; LDL-C, low-density lipoprotein cholesterol; RC, remnant cholesterol.

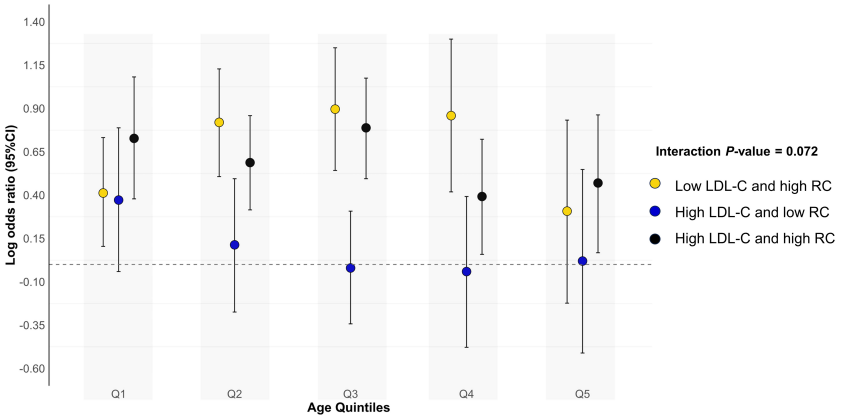


FIGURE 4
Associations (log odds ratios, 95%CIs) of different concordant/discordant groups with hypertension according to age quintiles. Low LDL-C and low RC was used as the reference group. Results were adjusted for age (continuous), sex (male/female), race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, other), educational level (less than high school, high school or equivalent, college or above), family income-poverty ratio (≤1.0, 1.1-3.0, >3.0), smoking status (never smoker, former smoker, current smoker), alcohol drinking (non-drinker, low to moderate drinker, heavy drinker), chronic kidney disease (yes or no), diabetes mellitus (yes or no), coronary heart disease (yes or no), eGFR (continuous), FBG (continuous), and HbA1c (continuous). Circles represent log odds ratios and vertical lines indicate 95% confidence intervals. All estimates accounted for complex survey design. All estimates accounted for complex survey design. CI, confidence interval; LDL-C, low-density lipoprotein cholesterol; RC, remnant cholesterol.

to apoB. Our findings suggest that the association between RC and hypertension beyond LDL-C among the general population. Early identification of RC related risk, especially at normal LDL-C levels, may facilitate the differentiation of individuals at high risk of predisposing to hypertension.

Studies based on Chinese populations have shown that increased RC concentrations was linked to elevated blood pressure and the onset of hypertension (11, 22). Liu et al. (23) found that both RC and TG levels correlated more prominently with arterial stiffness (measured by brachial-ankle pulse wave velocity) than other lipid indices. Another study showed that RC was an independent predictor of endothelial dysfunction (reflected by flow-mediated vasodilation) in the general population (24). These suggest that the close association of RC with hypertension may be linked to poor vascular endothelial function and arterial elasticity. A study by Wu et al. (12) further found that the risk of advancement of arteriosclerosis and atherosclerosis was higher in individuals with high levels of RC, even when LDL-C was in the normal range. Reaffirming the above findings, we demonstrated here in large-scale US general population that RC levels were associated with hypertension in fully adjusted models including LDL-C. We further clarified the position of RC beyond LDL-C in the relationship between cholesterol and hypertension. Considering that hypertension is an important risk factor for ASCVD, our findings may provide evidence for the superior ability of RC to predict ASCVD risk over LDL-C shown in previous studies (6, 7).

Exogenous triglycerides are carried by apoB-48-containing chylomicrons, while hepatic-derived triglycerides are mainly released by apoB-100-containing very low-density lipoproteins (VLDL) particles. These TRLs undergo hydrolysis by lipoprotein lipase and decrease in size, in parallel with a decrease in TG content and an increase in cholesteryl esters in the presence of cholesteryl ester transfer protein, and thus forming small, dense cholesterol-rich remnants (25). One-third of non-fasting plasma cholesterol is

TABLE 4 Adjusted ORs (95%CIs) of hypertension according to different concordant/discordant groups *.

	Concordant/discordant of apoB and RC, OR (95%CI)			
	Low apoB and low RC	Low apoB and high RC	High apoB and low RC	High apoB and high RC
Cases, No./Total No.	773/2,405	406/919	411/945	1,337/2,585
Model 1 †	Ref	1.82 (1.45-2.28)	1.13 (0.93-1.37)	1.95 (1.66-2.27)
Model 2 ‡	Ref	1.76 (1.40-2.22)	1.16 (0.95-1.42)	1.88 (1.61-2.20)
Model 3 §	Ref	1.73 (1.38-2.17)	1.15 (0.94-1.41)	1.83 (1.55-2.15)

apoB, apolipoprotein B; CI, confidence interval; OR, odds ratio; RC, remnant cholesterol.
* The different concordant/discordant groups based on apoB 92 mg/dL and RC 20 mg/dL cut-points in the NHANES 2007 to 2016.
† Model1: adjusted for age (continuous), sex (male/female), race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, other), educational level (less than high school, high school or equivalent, college or above), family income-poverty ratio (≤1.0, 1.1-3.0, >3.0), smoking status (never smoker, former smoker, current smoker), and alcohol drinking (non-drinker, low to moderate drinker, heavy drinker).
‡ Model 2: further adjusted (from Model 1) for chronic kidney disease (yes or no), diabetes mellitus (yes or no), and coronary heart disease (yes or no).
§ Model 3: further adjusted (from Model 2) for eGFR (continuous), FBG (continuous), and HbA1c (continuous).
All estimates accounted for complex survey design.

present in remnant lipoproteins (26). Several reasons may explain the mechanism behind this association found in our study. First, elevated RC may act as a silent promoter of atherosclerotic cardiovascular disease (27). Apart from newly secreted chylomicrons and very large VLDL, most cholesterol-rich remnant particles below 70 nm diameter traverse the endothelium by active transcytosis and remain in the subendothelial layer of the arterial wall, where macrophages phagocytose them and form foam cells, promoting lesion occurrence and progression (28). These remnant particles share similar pathogenic mechanism to that of LDL, but they carry four times more cholesterol molecules per particle than LDL (29). In addition, their larger size compared to LDL, along with their enrichment in apolipoprotein C-III and apolipoprotein E, which increases their affinity for proteoglycans (30, 31), makes them more difficult to return to the arterial lumen. Apolipoprotein E also mediates subendothelial macrophage surface receptor-mediated endocytosis of remnants, which facilitates atherosclerosis (32). Second, increased RC levels may be associated with impaired endothelial function. The mechanism involves induction of apoptosis in endothelial cell through NAD(P)H oxidase-mediated formation of superoxide and production of tumor necrosis factor-α and interleukin 1β, and effects on nitric oxide synthase activity (33, 34). This is corroborated by the decrease of flow-mediated vasodilation indicator and the increase of brachial-ankle pulse wave velocity in previous studies. Third, the causal link between RC and low inflammation exacerbates atherosclerosis and endothelial impairment, whereas the pro-atherosclerotic effect of elevated LDL-C lacks the contribution of inflammation, and this causal association persists even in the absence of diabetes and obesity (35). It may explain the attenuated association of discordantly low LDL-C/high RC with hypertension after adjusting for hs-CRP. Forth, VLDL is catabolized into VLDL remnants, intermediate-density lipoproteins and LDL. Increased levels of VLDL may lead to accumulation of LDL, exerting multiple pathogenic effects.

Remnant particles formation is augmented by overproduction of TRLs or by genetic or physiological triggers that limit lipolysis of lipoprotein lipase, or both (29). In our continuous and tertiles analyses, we showed that increased RC levels were still associated with hypertension even after adjusting for TG. These observations were reproduced in concordant/discordant analyses. These findings suggest that elevated RC levels, regardless of TG levels, may indirectly reflected risk information related to downregulating lipolysis of lipoprotein lipase mechanisms such as decreased apolipoprotein C-II or apolipoprotein A-V activity and increased apolipoprotein C-III or angiopoietin-like protein 3 activity rather than the risk caused by increased remnants from elevated plasma TG levels (36, 37). It may also explain the increased odds of hypertension in those with high RC when apoB was below the threshold. As hypothesized in the study by Quispe et al. (6), RC predicted risk of cardiovascular disease independent of apoB partially explained by RC reflected the activity of key lipid regulatory proteins such as apolipoprotein C-III and angiopoietin-like protein 3, which may be independent of apoB contained on TRLs.

Disparities between the sexes in the stratified analyses may be related to the protection of the vascular endothelium by endogenous estrogen, and the differences in lipid metabolism and the amount and distribution of adipose tissue, with accumulated adipose tissue in different areas may play distinct pathological roles (38). Results stratified by smoking status need to be validated through further research as smoking impairs vascular endothelial function and promotes oxidative stress causing hypertension (39, 40). We speculate that better control of other risk factors not included in our study by current smokers may have contributed to this.

Unfortunately, there is no large-scale clinical study on reducing the risk of ASCVD in RC management. The relevant evidence was mainly found in subgroups of clinical studies or *post hoc* analyses. Previous studies have shown that statins, fibrates, and omega-3 fatty acids can lower RC levels, improve endothelial function, and regulate blood pressure (41–46). However, more evidence is needed on how much reduction in RC levels accompanies the benefits of blood pressure management. Novel therapies that target the metabolic modulation of TRLs may be promising in the future.

Limitations

First, as this study is observational, we cannot rule out the potential of residual confounders. For example, the intake of some medications or lifestyle habits may affect blood pressure, and the consumption of certain supplements such as fish oil may have an effect on RC levels (46). In addition, we cannot draw causal inferences regarding elevated RC levels and hypertension due to the cross-sectional nature of the study design. Second, RC concentrations were not directly measured but estimated, giving rise to possible deviations from the actual levels. However, the calculated RC correlates favorably with the directly measured RC (R^2 0.69) (47) and can be obtained easily for practical application. Third, the association between hypertension and fasting RC levels in our study may be underestimated, considering that postprandial RC levels reflect more information. It remains to be investigated whether postprandial RC levels are also associated with hypertension beyond LDL-C in the US population. Forth, our study was limited to participants with TG ≤400 mg/dL. Derivation of the findings to the overall population will require additional studies to confirm. Fifth, our study included data from those who participated in NHANES from 1999-2018, spanning 20 years. Although subgroup analyses of the 1999-2008 and 2009-2018 survey cycles showed no significant differences in the results, changes in lifestyle, diet, and medication of individuals during different periods may still affect the results of this study.

Conclusions

This study indicated that elevated RC levels were associated with hypertension independent of LDL-C in the general population. Clinical attention should be paid to RC levels in hypertensive individuals, especially in the absence of elevated LDL-C. In addition, this association extended beyond elevated serum TG levels, and lipoproteins other than apoB may be involved. The

present study only illustrates a strong correlation, and the association between RC levels and the prognosis of hypertensive individuals remains to be studied.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: https://wwwn.cdc.gov/nchs/nhanes/Default.aspx.

Ethics statement

The studies involving humans were approved by National Center for Health Statistics (NCHS) ethics review board. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

LS: Conceptualization, Methodology, Writing – original draft. DZ: Conceptualization, Writing – review and editing, Methodology. JJ: Data curation, Methodology, Writing – review and editing. AW: Data curation, Methodology, Writing – review and editing. TD: Data curation, Methodology, Writing – review and editing. XC: Writing – review and editing. YS: Writing – review and editing. ZG: Funding acquisition, Supervision, Writing – review and editing. HX: Funding acquisition, Supervision, Writing – review and editing.

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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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1260764/full#supplementary-material

SUPPLEMENTARY FIGURE 1
The distribution of LDL-C and RC by the concordant/discordant groups across LDL-C 118 mg/dL and RC 20 mg/dL cut-points. **(A)** The box plot of LDL-C and RC concentrations for the different concordant/discordant groups. **(B)** Proportions of LDL-C and RC concordance and discordance. Percentages were weighted. LDL-C, low-density lipoprotein cholesterol; RC, remnant cholesterol.

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EDITED BY
Gabor Czibik,
Queen Mary University of London,
United Kingdom

REVIEWED BY
Jorge Francisco Gomez Cerezo,
Universidad Europeas de Madrid, Spain
Xinpei Wang,
Peking University, China

*CORRESPONDENCE
Fangkun Yang
yangfk@zju.edu.cn
Hanbin Cui
hbcui_nbdyyy@outlook.com
Shuai Yuan
shuai.yuan@ki.se

†These authors have contributed equally to this work

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Apolipoprotein B and interleukin 1 receptor antagonist: reversing the risk of coronary heart disease

Fangkun Yang[1,2,3*†], Ning Huangfu[1,2,3†], Jiaxi Shen[2,3], Pengpeng Su[4], Lujie Zhu[2,3], Hanbin Cui[1,2,3*] and Shuai Yuan[5*]

[1]Department of Cardiology, First Affiliated Hospital of Ningbo University (Ningbo First Hospital), School of Medicine, Ningbo University, Ningbo, China, [2]Key Laboratory of Precision Medicine for Atherosclerotic Diseases of Zhejiang Province, Ningbo, China, [3]Cardiovascular Disease Clinical Medical Research Center of Ningbo, Zhejiang, China, [4]School of Medicine, Wenzhou Medical University, Wenzhou, China, [5]Unit of Cardiovascular and Nutritional Epidemiology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

Aims: Epidemiological evidence for the link of interleukin 1 (IL-1) and its inhibition with cardiovascular diseases (CVDs) remains controversial. We aim to investigate the cardiovascular effects of IL-1 receptor antagonist (IL-1Ra) and underlying mechanisms.

Methods: Genetic variants identified from a genome-wide association study involving 30,931 individuals were used as instrumental variables for the serum IL-1Ra concentrations. Genetic associations with CVDs and cardiometabolic risk factors were obtained from international genetic consortia. Inverse-variance weighted method was utilized to derive effect estimates, while supplementary analyses employing various statistical approaches.

Results: Genetically determined IL-1Ra level was associated with increased risk of coronary heart disease (CHD; OR, 1.07; 95% CI: 1.03-1.17) and myocardial infarction (OR, 1.13; 95% CI: 1.04-1.21). The main results remained consistent in supplementary analyses. Besides, IL-1Ra was associated with circulating levels of various lipoprotein lipids, apolipoproteins and fasting glucose. Interestingly, observed association pattern with CHD was reversed when adjusting for apolipoprotein B (OR, 0.84; 95%CI: 0.71-0.99) and slightly attenuated on accounting for other cardiometabolic risk factors. Appropriate lifestyle intervention was found to lower IL-1Ra concentration and mitigate the heightened CHD risk it posed.

Conclusion: Apolipoprotein B represents the key driver, and a potential target for reversal of the causal link between serum IL-1Ra and increased risk of CHD/MI. The combined therapy involving IL-1 inhibition and lipid-modifying treatment aimed at apolipoprotein B merit further exploration.

KEYWORDS

apolipoprotein B, interleukin 1 receptor antagonist, coronary heart disease, myocardial infarction, lifestyle modification

Introduction

Coronary heart disease (CHD) represents the leading cause of morbidity and mortality worldwide, posing a significant public health concern (1). Recent research has characterized CHD as a chronic inflammatory disease with inflammation-mediated atherosclerosis being a key contributing factor, which sparks interests in the potential benefits of targeting inflammation in cardiovascular diseases (CVDs) (2). Interleukin 1 (IL-1), a prototypical proinflammatory cytokine, plays a pivotal role in the innate immune response by activating the IL-1 receptor and thus leading to the activation of downstream inflammatory mediators. IL-1 pathway has emerged as a promising therapeutic target for rheumatoid arthritis (RA) and CVDs (3). Nevertheless, the question remains whether targeting IL-1 related inflammation could translate into clinical benefit against cardiovascular events.

IL-1 receptor antagonist (IL-1Ra) provides natural inhibition by competing with IL-1α and IL-1β for binding to the receptor (4). Despite its inhibitory role, the epidemiological evidence remains controversial. A meta-analysis comprising six population-based cohorts has revealed that levels of serum IL-1Ra exhibit a positive correlation with the risk of developing CVDs (5). However, a prospective cohort study of over 800 CHD-free individuals found no association between circulating IL-1Ra and the risk of CHD (6). The causality of this association remains shrouded in uncertainty, primarily due to the influence of confounding factors and the potential for reverse causation.

Anakinra is a human recombinant IL1-Ra and a first-line agent for RA, however, its cardiovascular effects remain unclear (7). In a randomized controlled trial (RCT) involving 80 patients with RA, Anakinra was found to significantly improve various vascular function indices as well as myocardial deformation and twisting (8). However, another RCT of 182 patients with non-ST elevation acute coronary syndrome suggested that treatment with Anakinra increased the risk of major adverse cardiovascular events at one year of follow-up (9). Notwithstanding, the duration and statistical power of previous trails have fallen short in evaluating the effect on cardiovascular outcomes.

An alternative strategy focusing on the genetic variants responsible for the inhibition of IL-1 could strengthen the causal inference (10). Utilizing genetic variants randomly assigned at conception as an instrumental variable (IV), it is possible to derive estimates that are less susceptible to environmental confounding factors, measurement error, and reverse causality (11). Our previous study has indicated the potential effect of lifetime exposure to elevated levels of IL-1Ra on an increased CHD risk, but the underlying mechanisms were not fully elucidated (12). Another early Mendelian randomization (MR) study suggested that long-term dual IL-1α/β inhibition increased concentrations of LDL-cholesterol, but not apolipoprotein or glycaemic traits (13). In recent years, the genetic determinants of IL-1Ra and cardiometabolic risk factors have been further well-characterized, which provides a promising tool to assess the causal network. In addition to pharmacological interventions, lifestyle modification represents a crucial aspect of clinical management. An investigation into the interplay between various lifestyle factors and the levels of IL-1Ra could provide insights into the heightened risk of developing CVDs.

Thus, we utilized a genetic approach to mimic the causal effects of serum IL-1Ra on the risks of RA and five major CVDs, including CHD, myocardial infarction (MI), heart failure (HF), atrial fibrillation (AF) and ischemic stroke (IS), as well as circulating lipoprotein lipids, apolipoproteins, glycaemic traits, and blood pressure to gain insights into underlying mechanisms. Furthermore, the causal associations of obesity and lifestyle factors with IL-1Ra concentrations were examined.

Methods

Study design

A comprehensive study was designed to investigate the causal role of IL-1Ra (Figure 1). First, we selected genetic variants as an IV for serum concentrations of IL-1Ra. Second, the combined effects of such genetic variants on RA and circulating concentrations of C-reactive protein (CRP) were assessed to examine whether the genetic IVs were valid and robustly linked with IL-1Ra concentrations. Then, the causal associations of genetically predicted IL-1Ra concentrations with the risk of five major CVDs (CHD, MI, HF, AF and IS) were investigated. Third, the exploratory analysis of IL-1Ra concentrations in relation to main cardiometabolic risk factors were performed to better understand potential mechanisms between long-term pharmacological IL-1 inhibition and CHD/MI risk. Fourth, based on the above analysis, the supplementary analyses were further conducted with the adjustment of risk factor that was closely linked with IL-1Ra concentrations. Fifth, we assessed the effects of common lifestyle factors on the serum concentrations of IL-1Ra and compared the causal effect sizes of lifestyle factors on the risk of CHD with or without the adjustment of IL-1Ra concentrations.

Genetic instrument selection

Genetic variants associated with serum concentrations of IL-1Ra were identified from a genome-wide association study (GWAS) involving 30,931 individuals of European descent across 15 studies (Table 1) (14). The primary study utilized the Olink proximity extension assay cardiovascular I panel to measure 90 cardiovascular-related proteins (14). Single nucleotide polymorphisms (SNPs) for serum concentrations of IL-1Ra were identified from genetic variants in the *IL1RN* gene ± 5 Mb region at the genome-wide significance threshold ($p < 5\times10^{-8}$), followed by linkage disequilibrium pruning based on the European 1000 Genomes Project reference panel ($r^2 < 0.1$; clump distance > 10000 kb) (15). Among SNPs in linkage disequilibrium, the one with the smallest p was retained. Thirteen independent SNPs were selected as a genetic IV with an effect size scaled to one standard-deviations (SD) increase in IL-1Ra concentrations (Supplementary Table 1).

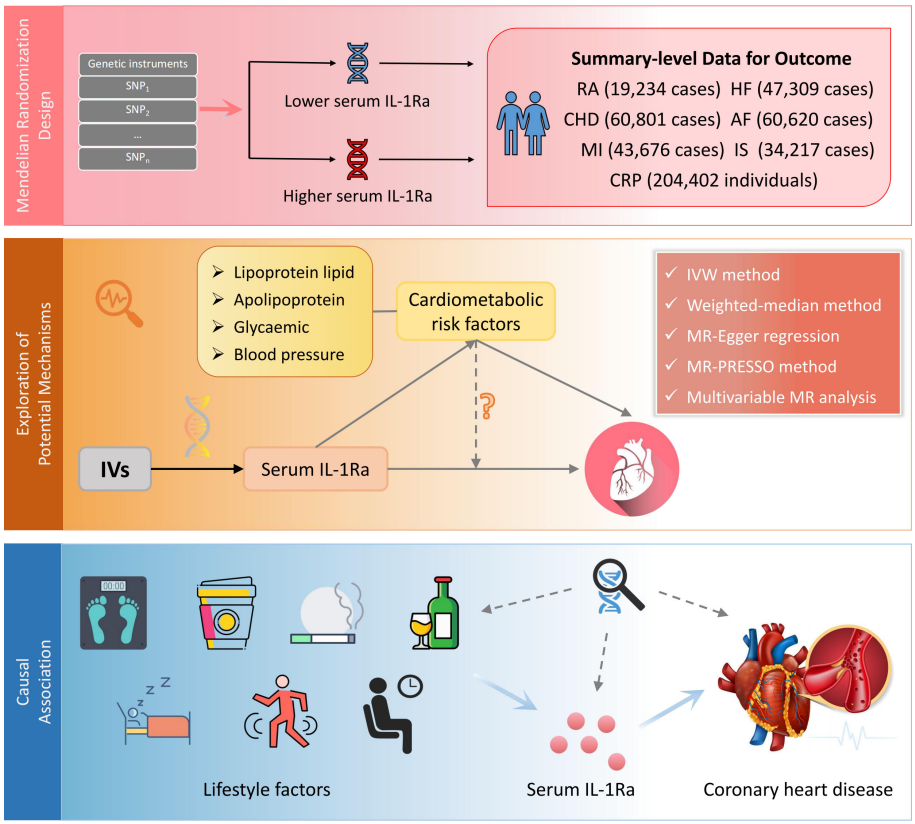


FIGURE 1
Design of the current two-sample Mendelian randomization study. IV, instrumental variable; IL1-Ra, interleukin 1 receptor antagonist; CHD, coronary heart disease; MI, myocardial infarction; HF, heart failure; AF, Atrial fibrillation; IS, ischemic stroke; RA, rheumatoid arthritis; CRP, C-reactive protein.

Data sources

Summary estimates of genetic associations with CHD and MI were extracted from the CARDIoGRAMplusC4D (Coronary ARtery DIsease Genome wide Replication and Meta-analysis (CARDIoGRAM) plus The Coronary Artery Disease (C4D) Genetics) consortium (Table 1) (16). Specifically, the study included ~185,000 participants, comprising 60,801 CHD cases and 123,504 controls, which were from 48 different cohort studies. The CHD cases were defined as acute coronary syndrome, chronic stable angina, coronary stenosis (over 50%) or MI (16). The majority (~80%) were of European ancestry and more than 70% of the total CHD cases had a history of MI (16). Similarly, the genetic associations with RA, CRP, HF, AF and IS were obtained from the corresponding GWASs (17–21). Detailed information was provided in the Table 1.

To explore the potential mechanisms, we obtained genetic associations with 10 main cardiometabolic risk factors, including apolipoprotein A-I, apolipoprotein B, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG); fasting glucose (FG), fasting insulin (FI) and haemoglobin A1c (HbA1c); as well as systolic blood pressure (SBP) and diastolic blood pressure (DBP) (Table 1) (22–24).

Given the significance of lifestyle intervention in clinical management, genetic associations with various lifestyle factors, including obesity (measured by body mass index and waist circumference), smoking initiation, lifetime smoking index, alcohol drinking, alcohol dependence, coffee consumption, caffeine consumption, physical activity (both moderate-to-vigorous and vigorous), sedentary behaviour, sleep duration and insomnia were obtained from corresponding international consortia (Supplementary Table 2) (25–35).

All studies included in the GWAS had obtained ethical approval from the appropriate committees and written informed consent had been obtained from all participants. The data used in the current study were publicly available.

Statistical analysis

The multiplicative random-effects inverse-variance-weighted (IVW) method, as the primary statistical model, was employed to estimate the associations of IL-1Ra concentrations with the risk of RA and CVDs, as well as the circulating concentrations of CRP (36). In addition, some supplementary analyses were conducted to investigate potential horizontal pleiotropy and assess the consistency of primary results, including the maximum likelihood

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TABLE 1 Detailed information on data sources.

Trait	Participants	Ancestry	Adjustments	Unit	PubMed ID
IL-1Ra	30,931 individuals	European	age, sex and 1-10 principal components	SD	33067605
Rheumatoid arthritis	19,234 cases and 61,565 controls	European	top 5-10 principal components	Odds ratio	24390342
C-reactive protein	204,402 individuals	European	age, sex, and population substructure	SD	30388399
Coronary heart disease	60,801 cases and 123,504 controls	Mixed	Not reported	Odds ratio	26343387
Myocardial infarction	43,676 cases and 128,199 controls	Mixed	Not reported	Odds ratio	26343387
Heart failure	47,309 cases and 930,014 controls	European	age, sex, the first 10 principal components	Odds ratio	31919418
Atrial fibrillation	60,620 cases and 970,216 controls	European	sex, age, age (2), and 4 principal components	Odds ratio	30061737
Ischemic stroke	34,217 cases and 404,630 controls	European	age and sex	Odds ratio	29531354
Apolipoprotein A-I	393,193 individuals	European	age, sex, BMI, genotyping chips	SD	32203549
Apolipoprotein B	439,214 individuals	European	age, sex, BMI, genotyping chips	SD	32203549
HDL	403,943 individuals	European	age, sex, BMI, genotyping chips	SD	32203549
LDL	440,546 individuals	European	age, sex, BMI, genotyping chips	SD	32203549
TG	441,016 individuals	European	age, sex, BMI, genotyping chips	SD	32203549
FG	281,416 individuals	European	age, sex and the first 5 principal components	SD	34059833
FI	213,650 individuals	European	age, sex and the first 5 principal components	SD	34059833
HbA1C	215,977 individuals	European	age, sex and the first 5 principal components	SD	34059833
SBP	up to 1,006,863 individuals	European	age, sex, BMI, genotyping chips	SD	30224653
DBP	up to 1,006,863 individuals	European	age, sex, BMI, genotyping chips	SD	30224653

IL1-Ra, interleukin 1 receptor antagonist; HDL, high density lipoprotein; LDL, low density lipoprotein; TG, total triglyceride; FG, fasting glucose; FI, fasting insulin; SBP, systolic blood pressure; DBP, diastolic blood pressure; SD, standard deviation.

method, weighted-median method, MR-Egger regression, and MR Pleiotropy Residual Sum and Outlier (MR-PRESSO) methods (37–39). The weighted median method represents a dependable approach for obtaining consistent causal estimates when up to 50% of the weight arises from the invalid instruments (37). The MR-Egger regression can detect the potential pleiotropy leveraging the embedded intercept test while also provide a corrected estimate accounting for the detected pleiotropy if any. However, it may entail sacrificing statistical power to some extent (38). On the other hand, the MR-PRESSO method can detect potential outliers and generate relatively unbiased causal estimates after removal of outliers (39). Furthermore, the embedded distortion test can be employed to evaluate the disparities between the obtained estimates before and after outlier removal (39). We also undertook the leave-one-out analysis and utilized the scatter plot to depict the associations of genetically predicted IL-1Ra concentrations with CHD/MI to examine whether a single SNP drove the association. Multivariable MR (MVMR) analysis was performed to explore the potential mechanisms and to assess whether the associations of IL-1Ra concentrations with CHD/MI changed after the adjustment for cardiometabolic risk factors (40). The two-step network MR mediation analysis was further conducted to investigate the extent to which any effect of IL-1Ra concentrations on CHD/MI might be mediated through apolipoprotein B (41). And we estimated the proportion mediated by multiplying the effect size of IL-1Ra concentrations on apolipoprotein B by that of apolipoprotein B on CHD/MI. Besides, we further investigated the causal effect of CHD and MI on IL-1Ra concentrations, considering the possibility of reverse causality. When a SNP was unavailable, we employed the SNiPa (http://snipa.helmholtz-muenchen.de/snipa3/) to search for a proxy SNP in linkage disequilibrium (r^2>0.8) with the unavailable SNP based on the European population genotype data. The statistical significance threshold for the association of IL-1Ra concentrations with cardiometabolic risk factors was set at a two-sided p of <0.005 (=0.05/10 tests). Besides, the threshold for the effect of lifestyle on IL-1Ra concentrations was set at a two-sided p of <0.004 (=0.05/13 tests). The statistical analyses were conducted with R software (version 4.2.0) using 'TwoSampleMR', 'MendelianRandomization', and 'MR-PRESSO' packages (39, 42, 43).

Results

The IVW analyses yielded compelling results demonstrating significant associations of genetic predicted higher concentrations of IL-1Ra with decreased risk of RA as well as decreased levels of CRP (Figure 2, Table 2). However, the corresponding risk increased by 9% and 12% per 1-SD increase in serum IL-1Ra for CHD (95% CI: 1.03-1.17; p=6.9×10^{-3}) and MI (95%CI: 1.04-1.21; p=2.2×10^{-3}), respectively (Figure 2). The sensitivity analyses indicated that MR associations of IL-1Ra with CHD/MI risk kept consistent in the

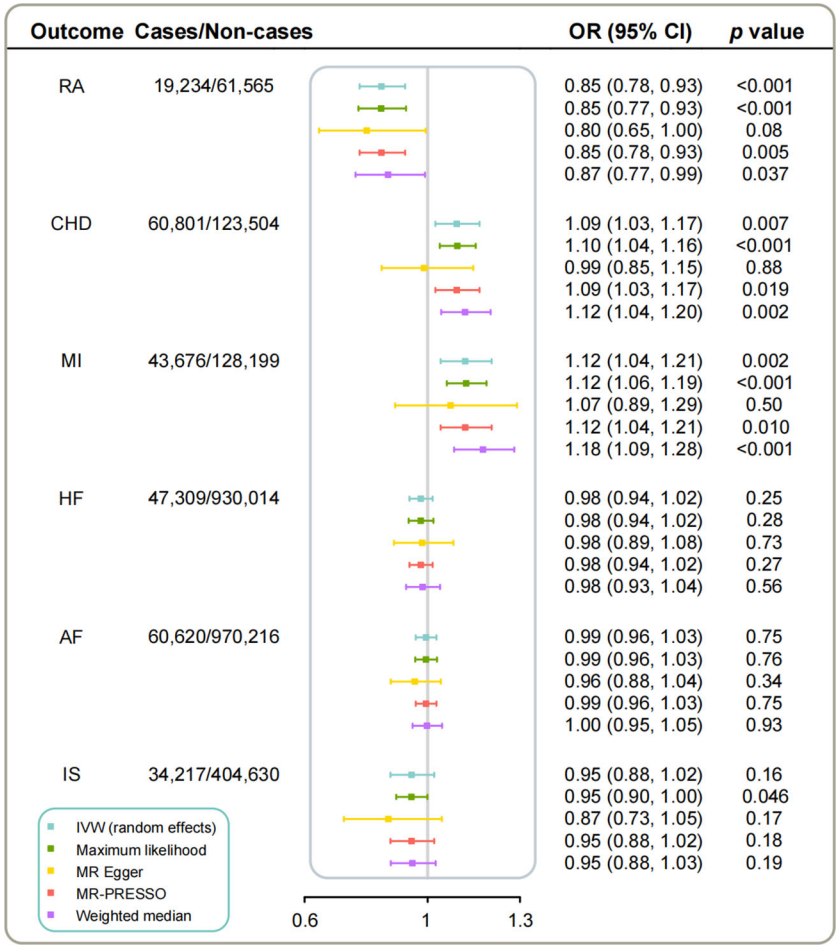


FIGURE 2
The causal associations of genetically predicted IL-1Ra level with RA and CVDs in different statistical models. Odds ratios are scaled per 1 standard deviation increase in the genetically predicted serum IL-1Ra level. IL-1Ra, interleukin 1 receptor antagonist; CHD, coronary heart disease; MI, myocardial infarction; HF, heart failure; AF, Atrial fibrillation; IS, ischemic stroke; RA, rheumatoid arthritis; OR, odds ratio; CI, confidence interval; IVW, inverse variance weighted.

majority of statistical methods, supporting the robustness of our findings, albeit with wider 95% CIs in the MR-Egger regression (Figure 2, Supplementary Table 3). No consistent evidence was found for the causal effect of genetic predicted serum IL-1Ra level on HF, AF or IS (Figure 2). Neither MR-PRESSO nor MR-Egger intercept test detected potential directional pleiotropy. The results of the leave-one-out analysis suggested that the causal association between IL-1Ra concentration and increased risk of CHD/MI was not drastically driven by any individual variant (Supplementary Figures 1–6).

To better understand the potential mechanisms underlying the observed causal associations, we conducted analyses examining the relationship between IL-1Ra and 10 different cardiometabolic risk factors (Table 2). The genetic predisposition towards a 1 SD increase in IL-1Ra concentration was found to be significantly associated with a roughly 0.05 SD increase in apolipoprotein B (effect estimate, 0.049; p=1.9×10^{-23}), LDL (effect estimate, 0.048; p=2.1×10^{-7}) and TG concentration (effect estimate, 0.047; p=4.8×10^{-15}). Additionally, apolipoprotein A-I (effect estimate, 0.034; p=8.7×10^{-7}), HDL (effect estimate, 0.017; p=1.7×10^{-3}) and FG (effect estimate, 0.017; p=3.5×10^{-4}) were also closely linked with IL-1Ra concentration. However, no evidence was found to support the association of IL-1Ra concentration with blood pressure. We then reassessed the association between IL-1Ra and CAD/MI with adjustment for genetically predicted cardiometabolic traits (Figure 3). In the MVMR with adjustment for apolipoprotein A-I, LDL, HDL and FG, the effect estimates were slightly attenuated, compared to the primary results without adjustments. Interestingly, we found that the association pattern between IL-1Ra concentration and the risk of CHD/MI drastically changed on accounting for apolipoprotein B concentrations. The direction of causal effect was reversed. After controlling for apolipoprotein B levels, suggestive evidence was found for the association between genetically predicted higher IL-1Ra concentrations and reduced risk of CHD (OR, 0.84; 95%CI: 0.71-0.99; p=0.04). While the association between IL-1Ra concentrations and MI was not significant (OR, 0.85; 95%CI: 0.71-1.02; p=0.09), with the adjustment for apolipoprotein B. The two-step mediation analysis indicated that 29% (95%CI: 21% to 37%) and 20% (95%CI: 14% to 27%) of the detrimental effect of serum IL-1Ra on CHD and MI was mediated

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TABLE 2 Associations of genetically determined IL-1Ra level with CRP and cardiometabolic risk factors.

	Q	P_Q	IVW			Weighted Median			MR-Egger			
			OR/beta	95% CI	*P*	OR/beta	95% CI	*P*	OR/beta	95% CI	*P*	$P_{pleiotropy}$
CRP	18	0.01	-0.18	-0.22, -0.15	2.5E-24	-0.19	-0.22, -0.15	2.5E-25	-0.22	-0.30, -0.13	2.3E-03	0.39
ApoA1	19	0.07	0.03	0.02, 0.05	8.7E-07	0.04	0.02, 0.05	8.1E-08	0.04	0.01, 0.08	0.03	0.54
ApoB	8	0.69	0.05	0.04, 0.06	1.9E-23	0.05	0.03, 0.06	5.6E-11	0.03	0.00, 0.06	0.05	0.16
HDL	13	0.32	0.02	0.01, 0.03	1.7E-03	0.02	0.01, 0.03	3.1E-03	0.03	0.00, 0.05	0.09	0.50
LDL	11	0.44	0.05	0.04, 0.06	2.1E-17	0.05	0.03, 0.06	1.5E-11	0.03	0.00, 0.05	0.07	0.13
TG	13	0.27	0.05	0.03, 0.06	4.8E-15	0.04	0.03, 0.06	9.1E-09	0.03	0.00, 0.06	0.05	0.30
FG	11	0.54	0.02	0.01, 0.03	3.5E-04	0.01	0.00, 0.02	0.05	0.00	-0.03, 0.02	0.76	0.08
FI	6	0.88	0.01	0.00, 0.02	0.07	0.01	-0.01, 0.02	0.42	0.00	-0.02, 0.03	0.90	0.64
HbA1C	8	0.79	0.00	-0.01, 0.01	0.87	0.00	-0.01, 0.01	0.92	0.00	-0.02, 0.01	0.67	0.69
SBP	27	0.01	-0.19	-0.45, 0.06	0.13	-0.18	-0.40, 0.04	0.10	-0.40	-1.02, 0.22	0.24	0.50
DBP	34	0.00	-0.03	-0.19, 0.13	0.72	0.02	-0.11, 0.16	0.75	-0.25	-0.63, 0.14	0.24	0.25

CRP, C-reactive protein; ApoA1, Apolipoprotein A-I; ApoB, Apolipoprotein B; HDL, high density lipoprotein; LDL, low density lipoprotein; TG, total triglyceride; FG, fasting glucose; FI, fasting insulin; SBP, systolic blood pressure; DBP, diastolic blood pressure; OR, odds ratio; CI, confidence interval. P_Q, the *p* for Cochrane's Q; $P_{pleiotropy}$, the *p* of MR-Egger intercept test.

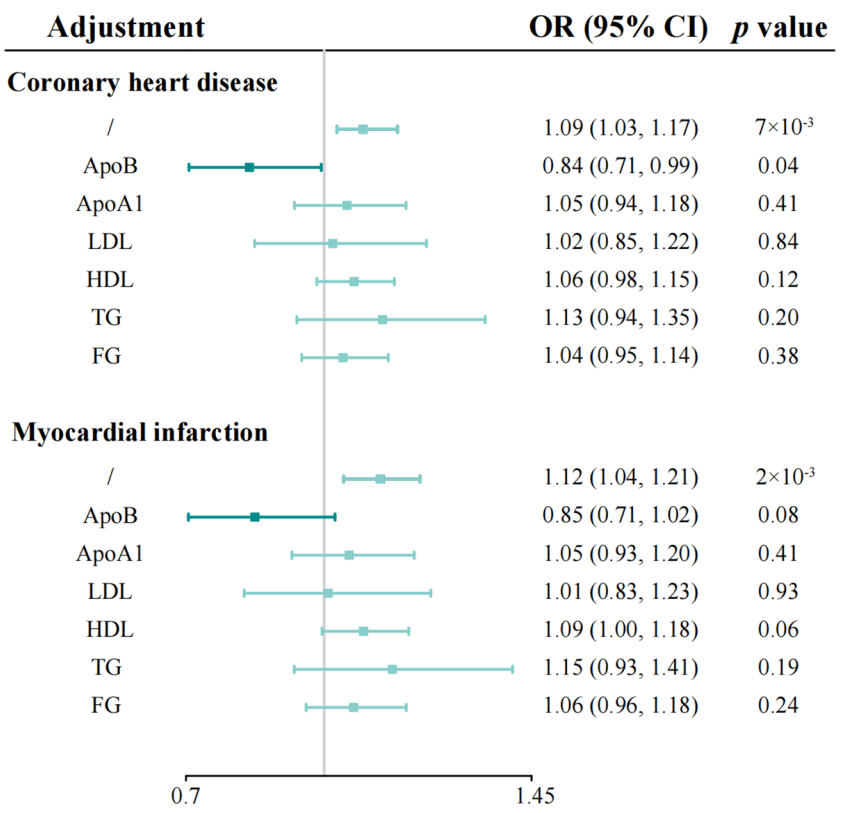


FIGURE 3
Multivariable Mendelian randomization analyses of genetically predicted IL-1Ra level with coronary heart disease and myocardial infarction on adjusting for cardiometabolic risk factors. Odds ratios are scaled per 1 standard deviation increase in the genetically predicted serum IL-1Ra level. IL-1Ra, interleukin 1 receptor antagonist; OR, odds ratio; CI, confidence interval.

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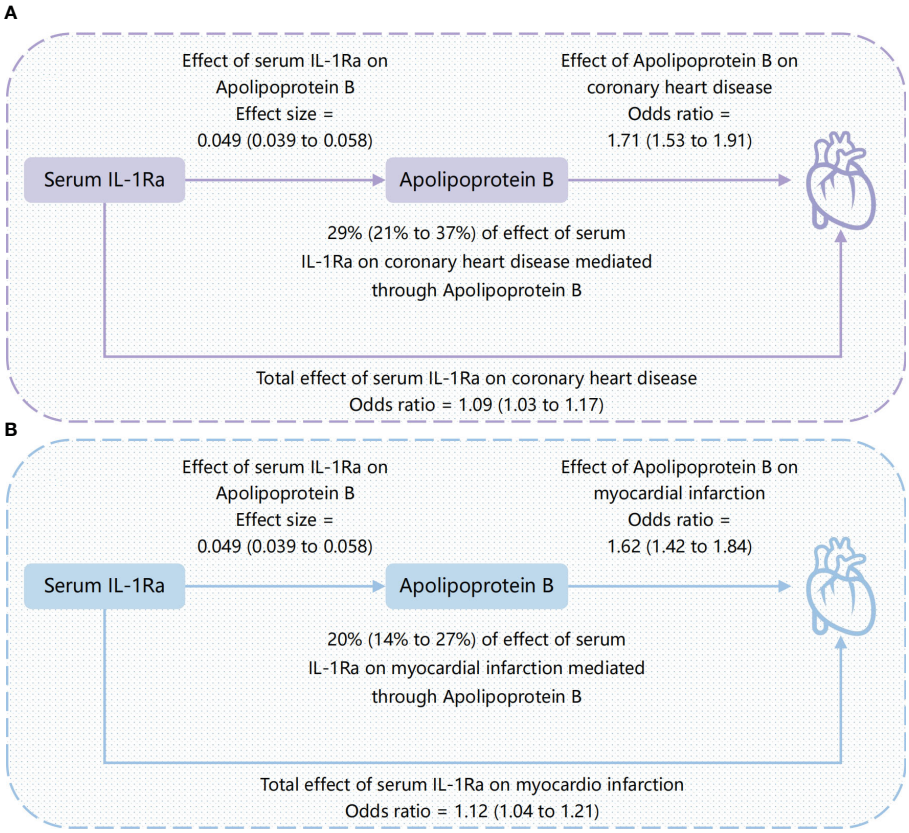


FIGURE 4
Causal directed acyclic graph showing the total effect of serum IL-1Ra on coronary heart disease and myocardial infarction, and the effect mediated by apolipoprotein B. **(A)** the mediating role of apolipoprotein B between serum IL-1Ra and coronary heart disease; **(B)** the mediating role of apolipoprotein B between serum IL-1Ra and myocardial infarction. The presented causal effect estimates with the corresponding 95% CI (shown in parenthesis) are scaled per one standard deviation increase in serum IL-1Ra. IL-1Ra, interleukin 1 receptor antagonist; CI, confidence interval.

through apolipoprotein B concentrations, respectively (Figure 4). Reverse-direction analyses were also performed to assess the potential reverse causality, and we found no significant evidence for the reverse causal effect of CHD or MI on the serum IL-1Ra levels (Supplementary Tables 4–7, Supplementary Figures 7–12).

We further investigated the associations of obesity and 11 lifestyle factors on serum IL-1Ra concentrations (Supplementary Figure 13). Body mass index, waist circumference, sedentary behaviour, and insomnia were positively associated with increased serum concentrations of IL-1Ra. On the other hand, suggestive evidence was found for the inverse association between sleep duration and IL-1Ra concentrations. No significant associations were detected for other studied lifestyle factors with IL-1Ra concentrations. In the mediation analysis, we found that adjusting for IL-1Ra concentrations led to a 47.8% attenuation of the causal effect estimate of sleep duration on the risk of CHD, and a 15.6% and 13.1% attenuation for smoking index and sedentary behaviour, respectively (Supplementary Figure 14).

Discussion

The current study employed a comprehensive framework of MR analyses based on large-scale human genetic data to investigate the causal associations of long-term IL-1 inhibition with the cardiometabolic profile. We found a significant positive association between serum IL-1Ra concentration and the risk of developing CHD and MI, as well as circulating levels of lipoprotein lipids, apolipoproteins and FG. Notably, the observed association between IL-1Ra and CHD/MI was primarily driven by apolipoprotein B. No evidence was found for the link of serum IL-1Ra with HF, AF or IS. Obesity and several unhealthy lifestyle factors, like sedentary behaviour and insomnia were associated with increased concentrations of IL-1Ra. Mediation analysis suggested that IL-1Ra might mediate half of effects of a short sleep duration on CHD risk. These findings on the other side imply that in addition to drug treatment, appropriate lifestyle intervention helps to lower IL-1Ra concentration and consequently mitigate the risk of CHD.

Our findings were in line with a meta-analysis of six population-based cohort studies, involving over 20,000 participants, which revealed that the risk of CVD increased by about 11% (95% CI, 1.06-1.17), for every 1 SD increase in serum IL-1RA (5). Furthermore, the current study revealed the long-term and stable effect of lifetime exposure to elevated serum IL-1RA level. This observation was in agreement with our previous research, which demonstrated a causal association between IL-1RA concentration and CHD risk (12). Additionally, an early MR study suggested that long-term dual inhibition of IL-1α/β elevated LDL-cholesterol

concentration, but not apolipoprotein or glycaemic traits (13). Nevertheless, our study provided consistent evidence for the causal effects of serum IL-1RA levels on apolipoprotein and FG. More importantly, we discovered that apolipoprotein B played a key role in mediating and potentially reversing the relationship between the IL-1RA and the risk of developing CHD, which may pave the way for new approaches to preventing and treating this condition.

The role of IL-1RA in incidence and development of CHD is not fully understood and remains controversial. On one hand, IL-1RA is known to bind to the IL-1 receptor, thereby preventing the binding of both IL-1α and IL-1β, and it has been proposed that inhibiting the IL-1 pathway may prevent the development of various CVDs, including atherosclerosis, MI, and heart failure (7, 44). On the other hand, it has been indicated that IL-1 can directly affect lipid metabolism by suppressing the activity of lipoprotein lipase (45). Observational studies revealed a positive and significant correlation between IL-1RA and several molecules related to lipid metabolism, including cholesterol, triglycerides, and apolipoproteins B, all of which are known risk factors for CHD (5, 45). Apolipoprotein B has been postulated as a key factor in the development of atherosclerosis, likely through the "response to retention" hypothesis (46). Lipoprotein particles, particularly those containing apolipoprotein B, become trapped in the innermost layer of arterial walls. The size and composition of apolipoprotein B particles, as well as the number of particles present in the bloodstream, would influence the tendency to be trapped in the arterial wall and contribute to the development of atherosclerosis (46). Genetic evidence also suggested that apolipoprotein B was the predominant lipids trait and may play a dominant role in the development of CHD (22). The effect estimates of other blood lipids were substantially attenuated, when adjusting for apolipoprotein B (22).

Besides, the IL-1 receptor is widely distributed across human cells and plays crucial roles in numerous physiological processes, including host defence, wound healing, and autoimmunity, to name a few (4). Both IL-1α and IL-1β have been shown to have potentially beneficial cardiovascular effects, which might be hindered by the presence of IL-1Ra. Interestingly, an investigation conducted on an IL-1 receptor-deficient mouse indicated the cardioprotective effects of IL-1 signalling in advanced atherosclerosis (47). IL-1α, expressed by various cell types, not only mediates inflammation-related functions but also serves as an autocrine growth factor. IL-1β has also been shown to promote atheroprotective changes in advanced atherosclerotic lesions, such as facilitating outward remodelling and aiding in the formation and maintenance of the fibrous cap rich in smooth muscle cells and collagen (48).

The link between lifestyle and the IL-1 pathway in CVDs is compelling. Emerging evidence suggests that an unhealthy lifestyle, such as a diet high in fat and calories, physical inactivity, sleep deficit and chronic stress, can markedly upregulate IL-1 production, triggering a cascade of pro-inflammatory responses (49, 50). Sleep deprivation has been found to upregulate the expression and production of IL-1β in circulation and peripheral tissues across a variety of species, including humans, mice, rats, rabbits, cats, and monkeys, which underscores the critical role of sleep in regulating immune responses and inflammatory signaling pathways (51). Growing evidence suggests that regular physical activity exerts an overall anti-inflammatory effect by stimulating the release of muscle-derived myokines that enhance the production of IL-1Ra and IL-10, reducing dysfunction in adipose tissue and enhancing oxygen delivery (50). Cigarette smoking has also been linked to a diverse array of changes in immune and inflammatory markers, including IL-1RA, IL-1β and CRP, particularly among older individuals with a history of prolonged smoking (52).

The present study employed a comprehensive MR approach to investigate the causal relationship and underlying mechanism between IL-Ra and CHD. The study design enabled unbiased estimation of causal effects from observational data and sensitivity analyses. Moreover, the potential interaction between various lifestyle factor and serum IL-Ra was further investigated. However, certain limitations should be acknowledged. First, the possibility of horizontal pleiotropy cannot be entirely ruled out, although this was not detected in MR-Egger intercept test and MR-PRESSO analysis. Second, the current study was restricted to individuals of European ancestry. Thus, the generalizability of the results to other populations may be limited. Besides, the research population of current study was not exclusively of European descent. Third, the magnitude and duration of inhibition may differ between genetic and pharmacological IL-1 inhibition, and the findings need to be further investigated. Furthermore, the current study was limited to the use of summary-level data, and therefore, an investigation into the potential differences in the association patterns between sexes was not possible due to the lack of available sex-specific data.

Conclusion

In summary, this study yields compelling evidence for the causal link between genetically predicted serum IL-1Ra and elevated risk of CHD/MI, with apolipoprotein B as the key driver, and a potential target for reversal. The potential cardiovascular benefits of a combined therapy involving IL-1 inhibition and lipid-modifying treatment aimed at apolipoprotein B merit further exploration, especially in individuals received IL-1Ra treatment, such as patients with RA. IL-1 pathway appeared to mediate the associations between unhealthy lifestyle factors and CHD risk, providing potential therapeutic targets for individuals with an unhealthy lifestyle to reduce CHD risk.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Files, further inquiries can be directed to the corresponding author/s.

Ethics statement

Ethical approval was not required for the studies involving humans because all studies included in the GWAS had obtained ethical approval from the appropriate committees and written informed consent had been obtained from all participants. The data used in the current study were publicly available. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

FY: Conceptualization, Investigation, Methodology, Software, Writing – original draft. NH: Conceptualization, Investigation, Software, Supervision, Writing – original draft. JS: Resources, Validation, Visualization, Writing – original draft. PS: Data curation, Formal Analysis, Validation, Writing – review & editing. LZ: Data curation, Resources, Validation, Writing – review & editing. HC: Funding acquisition, Resources, Supervision, Writing – review & editing. SY: Conceptualization, Investigation, Methodology, Software, Supervision, Writing – original draft.

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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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1278273/full#supplementary-material

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EDITED BY
Gabor Czibik,
Queen Mary University of London,
United Kingdom

REVIEWED BY
Hotimah Masdan Salim,
Nahdlatul Ulama University of Surabaya,
Indonesia
Aditya Yashwant Sarode,
Columbia University, United States

*CORRESPONDENCE
Tianbiao Zhou
zhoutb@aliyun.com

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Cardiovascular outcomes and safety of SGLT2 inhibitors in chronic kidney disease patients

Xiutian Chen, Jiali Wang, Yongda Lin, Kaijin Yao, Yina Xie and Tianbiao Zhou*

Department of Nephrology, The Second Affiliated Hospital of Shantou University Medical College, Shantou, China

Background: Sodium–glucose co-transporter 2 (SGLT2) inhibitors provide cardiovascular protection for patients with heart failure (HF) and type 2 diabetes mellitus (T2DM). However, there is little evidence of their application in patients with chronic kidney disease (CKD). Furthermore, there are inconsistent results from studies on their uses. Therefore, to explore the cardiovascular protective effect of SGLT2 inhibitors in the CKD patient population, we conducted a systematic review and meta-analysis to evaluate the cardiovascular effectiveness and safety of SGLT2 inhibitors in this patient population.

Method: We searched the PubMed® (National Library of Medicine, Bethesda, MD, USA) and Web of Science™ (Clarivate™, Philadelphia, PA, USA) databases for randomized controlled trials (RCTs) of SGLT2 inhibitors in CKD patients and built the database starting in January 2023. In accordance with our inclusion and exclusion criteria, the literature was screened, the quality of the literature was evaluated, and the data were extracted. RevMan 5.3 (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark) and Stata® 17.0 (StataCorp LP, College Station, TX, USA) were used for the statistical analyses. Hazard ratios (HRs), odds ratios (ORs), and corresponding 95% confidence intervals (CIs) were used for the analysis of the outcome indicators.

Results: Thirteen RCTs were included. In CKD patients, SGLT2 inhibitors reduced the risk of cardiovascular death (CVD) or hospitalization for heart failure (HHF) by 28%, CVD by 16%. and HHF by 35%. They also reduced the risk of all-cause death by 14% without increasing the risk of serious adverse effects (SAEs) and urinary tract infections (UTIs). However, they increased the risk of reproductive tract infections (RTIs).

Conclusion: SGLT2 inhibitors have a cardiovascular protective effect on patients with CKD, which in turn can significantly reduce the risk of CVD, HHF, and all-cause death without increasing the risk of SAEs and UTIs but increasing the risk of RTIs.

KEYWORDS

sodium-glucose cotransporter 2(SGLT2) inhibitors, chronic kidney disease (CKD), randomized controlled trial (RCT), meta-analysis, cardiovascular

Introduction

Diabetes is a chronic metabolic disease, and type 2 diabetes mellitus (T2DM) is the most common. According to statistics, in 2021, the prevalence of diabetes among people aged 20 to 79 worldwide was estimated to be 10.5% (536.6 million people). This is expected to rise to 12.2% (783.2 million people) by 2045. The prevalence of diabetes is similar between men and women, with the highest prevalence occurring in those between the ages of 75 and 79. The 2021 global diabetes-related health expenditure was about 966 billion US dollars. It is estimated that the expenditure will reach 105.4 billion US dollars by 2045. At present, more than 500 million people in the world suffer from diabetes, which means that more than 10.5% of the world's adults suffer from this disease (1). The number of diabetic patients will continue to increase rapidly in the future.

Chronic kidney disease (CKD) is defined as having a low glomerular filtration rate or high proteinuria, which affects 15%–20% of adults worldwide. Diabetes is the main cause of CKD, while hyperglycemia and CKD are the main risk factors for cardiovascular disease (CVD) and total mortality. CKD increases the risk of various adverse consequences, but CVD is particularly important because it is the main cause of death in this clinical population. CKD is associated with a variety of CVD results, including coronary heart disease, stroke, peripheral arterial disease, arrhythmia, heart failure, and venous thrombosis. It is worth noting that CKD is particularly related to serious CVD outcomes, such as cardiovascular mortality and heart failure (2).

Sodium–glucose co-transporter 2 (SGLT2) is the dominant transporter in sodium–glucose co-transporters that mediate the process of renal reabsorption of glucose. SGLT2, mainly distributed in the S1 segment of the renal proximal tubule, is a transporter with low affinity and high transport capacity, and its main physiological function is to complete the reabsorption of 90% of the glucose in the glomerular filtration fluid in the renal proximal tubule (3, 4). SGLT2 inhibitors are a class of antihyperglycemic drugs approved for the treatment of T2DM. These drugs block the reabsorption of glucose in the kidneys by inhibiting SGLT2, thus increasing urinary glucose excretion, promoting urination, lowering blood sugar levels, and improving blood sugar control in an insulin-independent way. SGLT2 inhibitors can block glucose reabsorption in proximal renal tubules, and they have many beneficial effects, including reducing body weight and serum uric acid, lowering blood pressure levels, and weakening glomerular hyperfiltration, which may be related to urinary sodium excretion with diabetes. In addition to lowering blood pressure, SGLT2 inhibitors have other protective properties that may be related to the cardiovascular outcomes of diabetic nephropathy.

Abbreviations: SGLT, sodium–glucose co-transporters; HF, heart failure; T2DM, type 2 diabetes mellitus; CKD, chronic kidney disease; RCT, randomized controlled trial; HR, hazard ratio; OR, odds ratio; CIs, confidence intervals; CVD, cardiovascular death; HHF, hospitalization for heart failure; SAE, serious adverse effect; UTI, urinary tract infection; RTI, reproductive tract infection; eGFR, estimated glomerular filtration rate; MD, mean difference; SMD, standardized mean difference.

Due to the high level of expression of SGLT2, sodium–glucose reabsorption in proximal renal tubules is increased, which leads to a decrease in sodium ions reaching the dense spots of the distal renal tubules, in turn leading to the dilation of afferent arterioles. Therefore, the glomerulus presents high perfusion, high internal pressure, and high filtration. SGLT2 inhibitors can reduce renal hyperfiltration, activate glomerular feedback contraction into glomerular arterioles, and reduce intraglomerular pressure through the mechanism of increasing sodium secretion. SGLT2 inhibition has also been proven to prevent renal hyperfiltration by lowering blood pressure levels and glomerular size and inhibiting renal growth factors (5). SGLT2 inhibitors can reduce the increase in systolic blood pressure in the treatment of T2DM. Moreover, the effects of dapagliflozin and canagliflozin can stabilize the changes in patients' levels of triglycerides. This shows that SGLT2 inhibitors can not only lower blood sugar levels but also improve blood pressure and blood lipid levels through the lowering of blood sugar levels (6). It has been shown that SGLT2 inhibitors combined with an angiotensin-converting enzyme inhibitor upregulate the renin–angiotensin system effect in nephropathy, therefore suggesting that blood pressure changes may be influenced by SGLT2 inhibitors (7, 8).

Many studies have shown that the use of SGLT2 inhibitors can reduce the risk of severe cardiac and renal prognosis, such as reducing the risk of vascular death and hospitalization for heart failure (HHF) (9–11). However, experimental subgroups have analyzed the cardiovascular protective effect of SGLT2 inhibitors on CKD patients and found that the results are inconsistent (12–15).

In summary, the prevalence of CKD is high, and diabetes is the most common cause, which often occurs together with hypertension. SGLT2 inhibitors have a cardiovascular protective effect in patients with diabetes and heart failure. However, clinical trial results are inconsistent regarding the level of cardiovascular protection of SGLT2 inhibitors for patients with CKD. Therefore, this study will comprehensively discuss the cardiovascular benefits and safety of SGLT2 inhibitors for patients with CKD, so as to provide evidence for the clinical application of SGLT2 inhibitors.

Materials and methods

Search strategy

We searched the PubMed® (National Library of Medicine, Bethesda, MD, USA) and Web of Science™ (Clarivate™, Philadelphia, PA, USA) databases; the search range was from the establishment of each database to January 2023. The keywords were diabetes neuropathies, randomized controlled trial, and sodium-glucose co-transporter-2 inhibitor. The recall rate was improved by searching American clinical research centers and reading the related references included in the literature.

Inclusion and exclusion criteria

Inclusion criteria were as follows: (1) a randomized controlled trial (RCT), which was randomized, double-blind, and controlled;

(2) some of the patients met the diagnostic criteria for CKD of an estimated glomerular filtration rate (eGFR) of < 60 mL/min/1.73m^2; (3) the experimental group was given SGLT2 inhibitors and the control group was given placebo; (4) effectiveness indicators of the incidence of cardiovascular death (CVD), heart failure hospitalisation (HFF), or all-cause deaths; and (5) safety indicators of the incidence of serious adverse effects (SAEs), reproductive tract infections (RTIs), and urinary tract infections (UTIs).

Exclusion criteria were as follows: (1) no complete test design and defects in the test design; (2) being a Phase II clinical study; (3) the repeated publication of research; and (4) no such validity index at the end of the experiment.

Outcome measures

After selecting the effect amount, two researchers independently extracted the input data and then exchanged and compared the data after completion. After data reconciliation, a meta-analysis was performed using Stata 17.0 (STATA.MP; StataCorp LP, College Station, TX, USA) and RevMan 5.3 (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark), which measured data using 95% confidence intervals (CIs) and mean differences (MDs). Enumeration was carried out using the hazard ratio (HR) and the odds ratio (OR) and their corresponding 95% CIs. The I^2 and p-values were taken as heterogeneity test indexes. When the I^2 value was ≤ 50% and the p-value was ≥0.1, the heterogeneity between studies was considered to be low, and the fixed-effects model was used. Conversely, when the I^2 value was > 50% and the p-value was < 0.1, some heterogeneity between studies was considered, and the random-effects model was adopted in accordance with the Cochrane Manual. This was followed by an analysis of the source of heterogeneity.

Quality assessment

The Cochrane risk offset assessment tool was used for the quality assessment.

Statistical analysis

Our selection of statistical indicators is as follows. The effect quantity that reasonably reflected the overall data was selected in accordance with the data type. The standardized mean difference (SMD) was selected for continuous variable data, and the HR and OR were selected for two-category variable data. For the heterogeneity analysis, after selecting the effect amount, two researchers independently extracted the input data and exchanged and compared the data after completion. After data reconciliation, a meta-analysis was performed using Stata 17.0 and RevMan 5.3, which measured data using 95% CIs and MDs. The I^2 and p-values were taken as heterogeneity test indexes. Subgroup analyses were planned for patients with CKD who had diabetes. For the sensitivity analysis, the included studies were excluded one by one in the Stata 17.0 software to assess the stability of the results. In the published offset evaluation, Begg's and Peter's tests could be used for quantitative evaluation. A p-value < 0.05 was defined as the publication offset. When there was a publication offset, the cause was found, and the resulting stability was assessed using the shear-complement method.

Results and analysis

Literature retrieval results

Each database was searched from the establishment of each database to January 2023 with the search terms DIABETIC NEPHROPATHIES, RANDOMIZED CONTROLLED TRIAL, and SODIUM-GLUCOSE CO-TRANSPORTER 2 INHIBITOR. Keyword retrieval was preferred in all databases, and PubMed retrieval was taken as an example. The search expressions were: ((("Diabetic Nephropathies" [MeSH]) OR "Renal Insufficiency, Chronic" [MeSH])) AND "Randomized Controlled Trial" [Publication Type]) AND (sodium-glucose cotransporter 2 inhibitor OR SGLT 2 inhibitor OR canagliflozin OR dapagliflozin OR empagliflozin OR ertugliflozin OR ipragliflozin OR luseogliflozin OR tofogliflozin OR Sotagliflozin). This improved the recall rate by retrieving relevant references from the American Clinical Research Center and reading the included articles. PubMed retrieved 71 published articles, and Web of Science retrieved 73 articles. Three articles were retrieved from other sources, totaling 147 articles. Use EndNote (Clarvate) or manually delete 65 duplicate articles, and delete 11 unrelated articles based on their titles. Based on the abstracts and full text of other articles, we excluded three study designs, 17 secondary or repeated studies, 37 non targeted study data, and two pharmacokinetic studies. Finally, 12 articles (10, 12–22) were included in the present study. The specific literature screening flow chart is shown in Figure 1.

Basic characteristics and quality evaluation of the inclusion study

All 12 studies were randomized, controlled, double-blind trials and a Cochrane risk assessment was used to evaluate the risk quality. There were 12 pieces of literature corresponding to 13 RCTs, and each of the studies completely described the method of random distribution and the hidden scheme of distribution. All 12 studies reported the number of patients and their reasons for being lost to follow-up, dropping out, or quitting. The longest follow-up time was 8 years, and the shortest follow-up time was 26 months. The baseline level for the eGFR was reported. See Figure 2 for the research risk assessment and Table 1 for the general features.

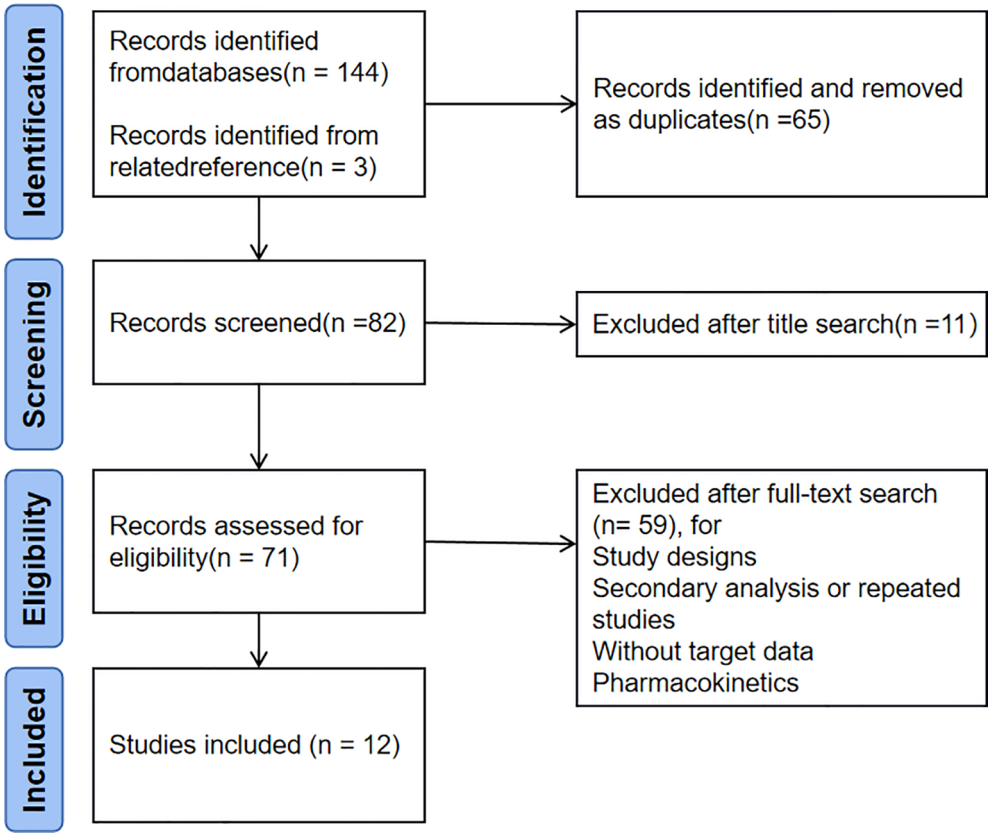


FIGURE 1
Flow diagram of the selection process.

Specific data analysis

Risks of cardiovascular death or hospitalization for heart failure

There were 12 studies included in this (CKD) analysis: the scope of one study (20) was 25–75 mL/min/1.73m^2, and seven (10, 15, 17–19, 21, 22) of the 12 studies had diabetes as a complication. The other group (10, 12–15, 17, 21) (with an eGFR ≥ 60 mL/min/1.73m^2) was included in this study, and all the people in this group had diabetes as a complication. There was heterogeneity among studies for the CKD groups (I^2 = 45.4%; p = 0.033). The random-effects model was used to analyze the HR values in the 12 studies. Compared with the placebo group, SGLT2 inhibitors reduced the risk of CVD or HHF (HR = 0.72, 95% CI 0.66 to 0.78; p = 0.000), and the difference was statistically significant (see Figure 3).

Excluding an eGFR of 25–75 mL/min/1.73m^2, there was no heterogeneity among the studies of the CKD groups (I^2 = 33%; p = 0.127), and the fixed-effects model (HR = 0.749, 95% CI 0.705 to 0.795; p = 0.00) was used. A subgroup analysis of the patients with diabetes showed that there was heterogeneity among the studies (I^2 = 46.8%, p = 0.068; HR = 0.71, 95% CI 0.63 to 0.79, p = 0.000), and there was no obvious additional benefit compared with the mixed population.

In the eGFR ≥ 60 mL/min/1.73m^2 group, there was no heterogeneity among the studies (I^2 = 15.7%; p = 0.302). Compared with the placebo group, the SGLT2 inhibitor group reduced the risk of CVD or HHF (HR = 0.82, 95% CI 0.77 to 0.89; p = 0.000} with a statistical difference. The diabetes subgroup analysis showed that there was no heterogeneity among the studies (I^2 = 0.0%; p = 0.697), and the effect value HR was combined with the fixed-effects model, with a statistical difference (HR = 0.88, 95% CI 0.80 to 0.97; p = 0.008]. In summary, in the diabetes subgroup analysis, the SGLT2 inhibitor group reduced the risk of CVD and HHF.

Risks of cardiovascular death

Among the 12 studies included, five (10, 16–18, 22) described CVD in the CKD population. The heterogeneity of CVD risk among the 12 studies was very low (I^2 = 13.3%; p = 0.314). The combined analysis of HR values by using the fixed-effects model showed that the risk of CVD in the SGLT2 inhibitor group had a value of HR equal to 0.86 (95% CI 0.81 to 0.92; p=0.000) compared with the placebo group, which was statistically significant. The heterogeneity of the CKD group was very low (I^2 = 0%; p = 0.925), and the analyzed HR was 0.84 (95% CI 0.74 to 0.95; p = 0.006], which was still statistically significant, as shown in Figure 4. In summary, the SGLT2 inhibitor group reduced the risk of CVD in patients with or without CKD.

Risks of hospitalization for heart failure

Ten (10, 12–15, 17–19, 21, 22) of the 12 studies included HHF data, and three (10, 17, 18) of the studies described HHF in the CKD population. The heterogeneity among the studies was very low (I^2 = 0%; p = 0.993). If the fixed-effects model was used for the

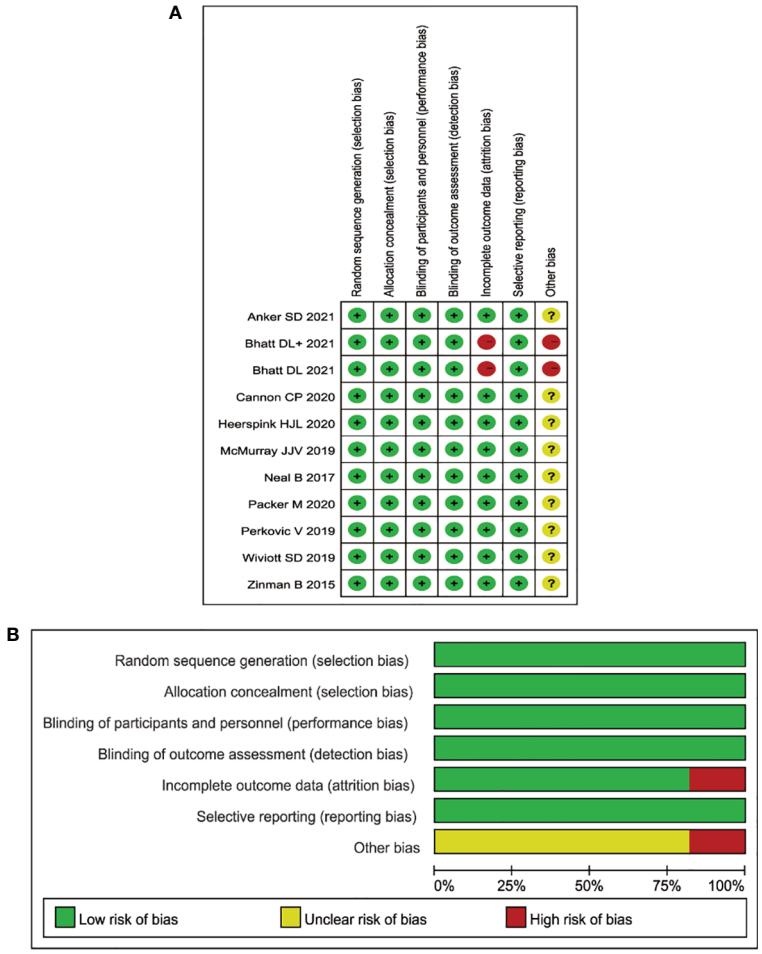


FIGURE 2
(A) Aggregate risk-of-bias graph for each experimental animal study. **(B)** Risk-of-bias summary.

combination analysis of HR values, the risk of HHF in the SGLT2 inhibitor group, compared with that in the placebo group, had a value of HR equal to 0.68 (95% CI 0.64 to 0.73; p = 0.000), which was statistically significant. In the hospitalization risk study for heart failure in patients with CKD, the heterogeneity was very low (I^2 = 0%; p = 0.856), and the analyzed HR was 0.65 (95% CI 0.56 to 0.74; p = 0.000), which was still statistically significant. In summary, SGLT2 inhibitors reduced the risk of HHF in patients with or without CKD.

Risk of all-cause death

Five (10, 12, 16, 17, 20) of the 12 included studies described all-cause death in the CKD population. There was significant heterogeneity among the studies (I^2 = 41.6%; p = 0.064). When the HR values were combined and analyzed using the random-effects model, compared with the placebo group, the risk of all-cause death with SGLT2 inhibitors had a value of HR equal to 0.87 (95% CI 0.82 to 0.93; p = 0.0000), which had statistical significance. In the all-cause death risk study for CKD patients, the heterogeneity was very low (I^2 = 28.2%; p = 0.234), and the analyzed HR was 0.86 (95% CI 0.78 to 0.95; p = 0.003), which was statistically significant. In summary, SGLT2 inhibitors reduced the risk of all-cause death in patients with or without CKD.

Serious adverse events

Ten (10, 12–15, 17–20, 22) of the 12 studies contained data for SAEs, and four (10, 17, 18, 20) of them described SAEs in the CKD population. A total of 67,407 patients were included, including 36,259 in the SGLT2 inhibitor group and 31,148 in the placebo group. The heterogeneity among studies was extremely low (I^2 = 0%, p = 0.98). The fixed-effects model was applied to merge the effect values' OR using the Mantel–Haenszel (M–H) method. Compared with the placebo group, the incidence of SAEs in the SGLT2 inhibitor group was relatively low (OR = 0.88, 95% CI 0.85 to 0.91; p < 0.0001), which was statistically different, in which the risk of SAEs in CKD patients was very low (I^2 = 0%; p = 0.58).

Reproductive tract infections

Nine (10, 12, 14–20) of the 12 studies included data on RTIs, and five (10, 16–18, 20) of the studies described RTIs in the CKD population. A total of 62,244 patients were included, including 32,499 cases in the SGLT2 inhibitor group and 29,745 cases in the placebo group. There was low heterogeneity among studies

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TABLE 1 Basic characteristics of inclusion study.

Study	Study Design	Sample Size	CKD	Follow-up time	Follow-up Time	eGFR (ml/min/ 1.73m^2)	LVEF%
Anker SD 2021 (12)	RCT	5988	YES +NO	Empagliflozin(n=2997) Placebo(n=2991)	Up to 1403 days	eGFR>40	T:54.3±8.8 C:54.3±8.8
Bhatt DL 2021 (17)	RCT	1222	YES	Sotagliflozin(n=608) Placebo(n=614)	Up to 21.6 months	eGFR≥30	T:35 (28–47) C:35 (28–45)
Bhatt DL+ 2021 (18)	RCT	10584	YES	Sotagliflozin(n=5292) Placebo(n=5292)	Up to 30 months	25≤eGFR≤60	NA
Cannon CP 2020 (19)	RCT	8246	YES +NO	Ertugliflozin(n=5499) Placebo(n=2747)	Up to 6 years	eGFR≥30	NA
Heerspink HJL 2020 (20)	RCT	4304	YES	Dapagliflozin(n=2152) Placebo(n=2152)	Up to 38.2 months	25≤eGFR≤75	NA
McMurray JJV 2019 (13)	RCT	4744	YES +NO	Dapagliflozin(n=2373) Placebo(n=2371)	Up to 27.8 months	eGFR≥30	T:31.2±6.7 C:30.9±6.9
Neal B 2017 (21)	RCT	10142	YES +NO	Canagliflozin(n=5795) Placebo(n=4347)	Up to 8 years or about 3 years	eGFR>30	NA
Packer M 2020 (14)	RCT	3730	YES +NO	Empagliflozin(n=1863) Placebo(n=1867)	Up to 1040 days	eGFR≥20	T:27.7±6.0 C:27.2±6.1
Perkovich V 2019 (10)	RCT	4401	YES	Canagliflozin(n=2202) Placebo(n=2199)	Up to 4.6 years	eGFR≥30	NA
The EMPA-KIDNEY Collaborative Group 2022 (16)	RCT	6609	YES	Empagliflozin(n=3304) Placebo(n=3305)	Up to 26 months	20≤eGFR≤90	NA
Wiviott SD 2019 (15)	RCT	17160	YES +NO	Dapagliflozin(n=8582) Placebo(n=8578)	up to 5.2 years	eGFR ≥60	T:85.4±15.8 C:85.1±16.0
Zinman B 2015 (22)	RCT	7028	YES +NO	Empagliflozin(n=4691) Placebo(n=2337)	Up to 4.6 years	eGFR≥30	NA

+The author of this study is the same as the author of the previous study (the same below).
CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; LVEF, left ventricular ejection fraction; NA, not assessed; RCT, randomized controlled trial.

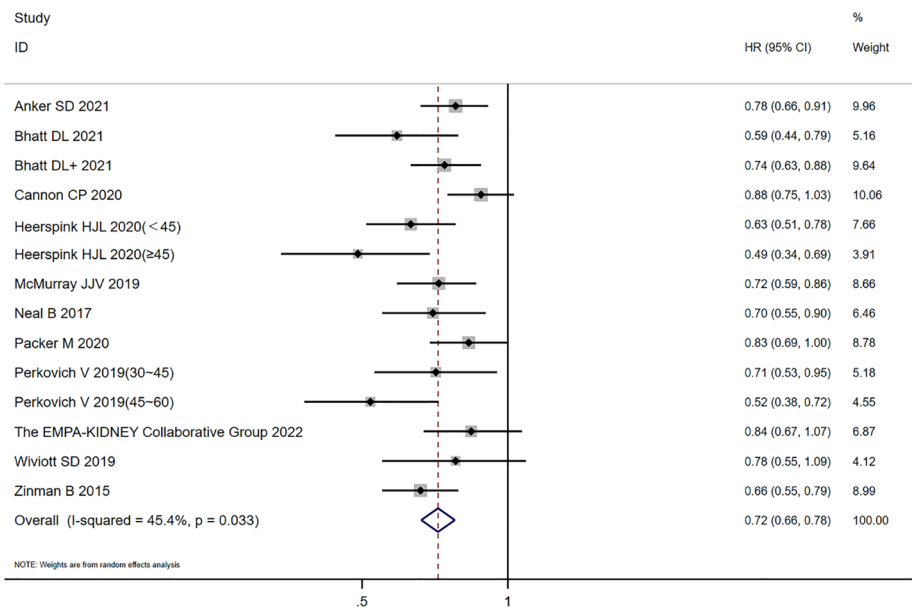


FIGURE 3
Forest plot of CVD or HHF risk comparisons between SGLT2 inhibitors and placebo in the CKD group. The "+" denotes different studies by the same author and the eGFR subgroups of the same study are shown in parentheses.

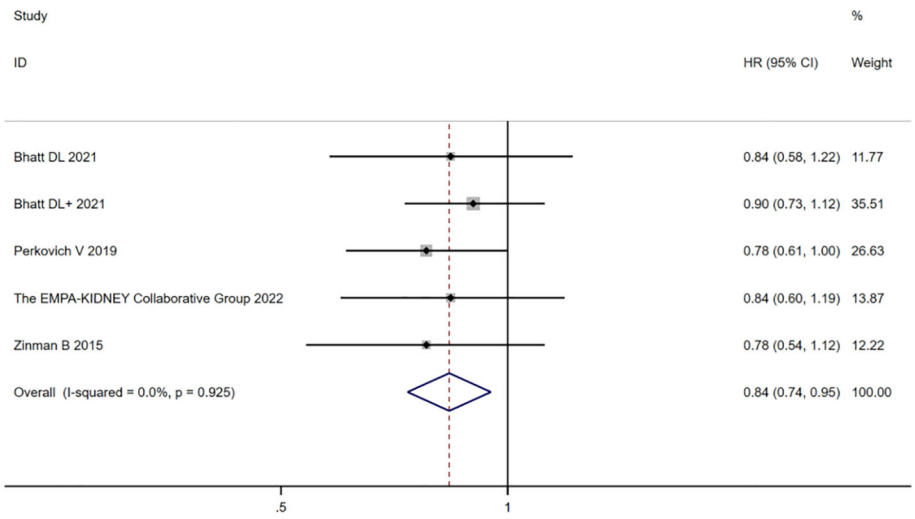


FIGURE 4
Forest plot of the risk of cardiovascular death in the CKD group, SGLT2 inhibitors, and placebo. The "+" denotes different studies by the same author and the eGFR subgroups of the same study are shown in parentheses.

(I^2 = 31.3%; p = 0.22). The fixed-effects model was used, and the M–H method was used to combine the effect values' OR. The SGLT2 inhibitor group had a higher incidence of RTIs than the placebo group (OR = 3.56, 95% CI 2.96 to 4.27; $p < 0.0001$), which was statistically different. In the study on the risk of RTIs in patients with CKD, the heterogeneity was very low (I^2 = 0%; p = 0.80), and the analyzed OR was 3.06 (95% CI 2.29 to 4.10; $p < 0.0001$), which was still statistically significant. That is, the SGLT2 inhibitors increased the risk of RTIs in patients with or without CKD.

Urinary tract infections

Eleven (10, 12–20, 22) of the 12 studies contain data on UTIs, and five (10, 16–18, 20) of them describe UTIs in the CKD population. A total of 74,016 patients were included, including 39,563 cases in the SGLT2 inhibitor group and 34,453 cases in the placebo group. The heterogeneity among studies was extremely low (I^2 = 20.7%; p = 0.26). The fixed-effects model was applied, and the M–H method was selected to merge the effect values' OR. Compared with the placebo group, the incidence of UTIs in the SGLT2 inhibitor group had an OR equal to 1.10 (95% CI 1.03 to 1.18; p = 0.004), and there was no statistical difference. Among them, the risk of UTIs in CKD patients was very low (I^2 = 0%; p = 0.87), and the OR was 1.06 (95% CI 0.96 to 1.17; p = 0.22), which was not statistically different. In summary, the SGLT2 inhibitors have no effect on the risk of UTIs in patients with or without CKD.

Publication bias analysis

More than 10 studies were included in the outcome index. In addition, the funnel chart was drawn using Stata 17.0. As shown in Figure 5, a CVD/HHF risk funnel diagram of patients in the CKD group was drawn. The other groups contained fewer than 10 articles, and the publication deviation was evaluated by Begg's test or Peter's test with Stata 17.0. Apart from the Egger test of the CVD/HHF results in the CKD group (p = 0.013 < 0.05), which indicated that there was significant bias, no significant publication bias was observed in any results ($p > 0.05$). Therefore, the results of CVD or HHF in the CKD group were subjected to shear supplementary correction to obtain no additional studies, and the results still had a value of HR equal to 0.72 (95% CI 0.66 to 0.78; p = 0.000). However, after deleting the studies with an eGFR range of ≥ 25 mL/min/1.73m^2 to ≤ 75 mL/min/1.73m^2, the Egger test has no significant publication bias.

Sensitivity analysis

STATA 17.0 was used to analyze the sensitivity of the outcome indicators. After all the studies of the outcome indicators were eliminated one by one, the combined effect amount did not change significantly, suggesting that the cardiovascular outcome indicators and adverse reaction indicators were stable.

Discussion

CKD is a chronic progressive disease that is related to many CVDs other than heart disease, including stroke, peripheral arterial disease, aortic aneurysm, and venous thrombosis (23–26). In the cohort study, people with low eGFR or CKD but no elevated urine albumin-to-creatinine ratio had a higher independent relative death risk. Similar results were observed for cardiovascular-specific mortality (27). CKD is usually defined as low eGFR or increased proteinuria. CKD affects 10% to 16% of the adult population in Asia, Australia, Europe, and the USA (28). It increases the risk of various adverse consequences. The 2020 Global Burden of Disease study identified CKD as one of the top 10 causes of poor prognosis

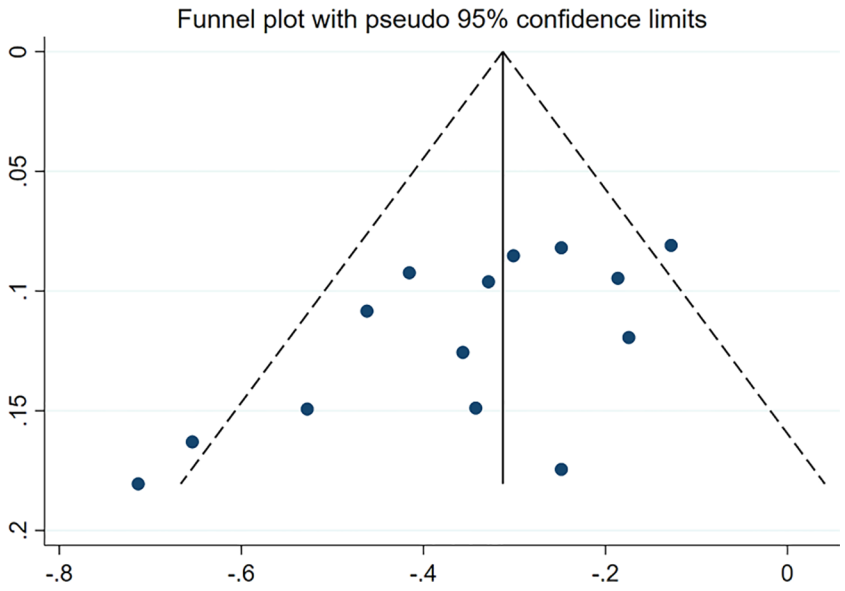


FIGURE 5
CVD/HHF funnel diagram of the CKD group.

in the world (29). Among the main adverse consequences related to CKD, CVD is one of the most important diseases because it is one of the main causes of death in this clinical population.

A lot of evidence shows that CKD is related to many CVD results other than heart disease, including coronary heart disease (30), stroke (31), heart failure (32), peripheral arterial disease (24), abdominal aortic aneurysm (26), and venous thrombosis (33). In several studies, CKD has been strongly correlated with sporadic or generalized atrial fibrillation (34, 35). It is reported that CKD is related to sudden cardiac death (36, 37). Patients with CKD often have atherosclerotic dyslipidemia, which is characterized by an increase in triglyceride and low-density lipoprotein cholesterol levels and a decrease in high-density lipoprotein cholesterol levels (38).

The results of this study show that SGLT2 inhibitors can reduce the risk of CVD in CKD patients, especially in reducing the risk of HHF. At the same time, SGLT2 inhibitors also reduced the risk of CVD or HHF in the non-CKD group. The benefits in a mixed population and a diabetic population were also compared, and the results showed that there was no obvious difference between the two groups. At present, the molecular mechanism of improving cardiovascular and renal function is unclear. Studies have shown that SGLT2 inhibitors can improve urinary protein and delay the progress of CKD mainly by inhibiting the SGLT2 receptor in renal tubules, inhibiting the reabsorption of sodium ions and glucose, contracting into the glomerular arteriole through the tubuloglomerular balance, and reducing the pressure on the glomerulus (39).

SGLT2 inhibitors have been shown to provide remarkable benefits in the clinical study of CKD patients. Many studies have shown that SGLT2 inhibitors can reduce the risk of serious cardiac and renal outcomes in patients (10, 11, 16, 18), improve the patient's cardiac and renal outcomes, and reduce the number of hospitalizations (9, 10), thereby reducing the medical expenses in this respect. SGLT2 inhibitors can also reduce the incidence of hyperkalemia in CKD patients (40).

SGLT2 inhibitors may play their role by controlling the energy metabolism pathway *in vivo* through urine glucose excretion and by promoting cell apoptosis, resisting autophagy, upregulating cell repair mechanisms, inducing cell anti-stress abilities, reducing vascular inflammation and arterial stiffness, inhibiting the reabsorption of sodium, and reducing the load of body fluids. At the same time, the decrease in urinary protein, systolic blood pressure, and diastolic blood pressure levels is also related to the decrease in cardiovascular risk.

CKD often needs comprehensive treatment, often combined with angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, beta-blockers, and other drugs. Because SGLT2 inhibitors contract into the bulbar arteriole through tubuloglomerular feedback, the combination of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers may increase the risk of acute renal injury. A retrospective analysis of a clinical study found that SGLT2 inhibitors combined with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers still had the function of protecting renal function, compared with the group without SGLT2 inhibitors, and did not increase the incidence of acute renal adverse events in CKD patients (41). SGLT2 inhibitors combined with angiotensin-converting enzyme inhibitors can up-regulate the renin–angiotensin system in CKD patients (7, 8). Because SGLT2 inhibitors can lower blood sugar levels, a meta-analysis of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers combined with SGLT2 inhibitors in the treatment of diabetes found that combined therapy increased the risk of hypoglycemia (42). Therefore, attention should be paid to the possibility of hypoglycemia during treatment.

In this study, SGLT2 inhibitors did not increase the incidence of SAEs or UTIs, but they did increase the incidence of genital infections. Multiple studies share similar perspectives with this study. This study has shown that SGLT2 inhibitors exposure is not associated with an increased risk of UTIs (43). No difference was found in UTI incidence when comparing SGLT2 inhibitors with placebo in patients (44). Another study has shown that SGLT2is does not have an increased risk of genitourinary infections compared with metformin (45). A retrospective study found that SGLT2 inhibitors medications were not commonly initiated in the 6 months prior to the occurrence of a UTI (46). The results of another study are consistent with our conclusion about genital infections with SGLT2 inhibitors, and most of the reported infections responded to standard treatment (47). This study also found that, apart from SGLT2 inhibitors, factors including personal hygiene, menopause, and circumcision might have a possible role in reported events of genital infection among T2DM patients on SGLT2 inhibitors therapy. Similar to the results of another safety analysis study of SGLT2 inhibitors, the results of this study showed that the drug has a higher risk of non-spinal fracture, lower limb amputation, genital infection, diabetic ketoacidosis, hypovolemia, hypoglycemia, and severe UTIs, with similar risks; however, SGLT2 inhibitors have a lower risk of acute kidney injury (48). Furthermore, another study found that the use of SGLT2 inhibitors would lead to a slight increase in the rate of fungal UTIs (49). It is inconsistent with the conclusion of this study that SGLT2 inhibitors will not increase the probability of UTIs.

Studies have shown that the conclusions are different with the use of different types of SGLT2 inhibitors. Compared with placebo and other active treatments, canagliflozin, dapagliflozin, and empagliflozin are associated with significantly increased risks of genital infections. Only dapagliflozin has a dose–response relationship with UTIs and genital infections (50). This may be related to the early decline of the eGFR when SGLT2 inhibitor treatment started; however, the follow-up data showed that the eGFR returned to near the baseline, and the decline of the eGFR was no different from that of the placebo (51), or the slowdown of the decline of the eGFR compared with the control group (20, 52). Some studies have also found that SGLT2 inhibitors have statistical significance in alleviating the decline slope of the eGFR (53, 54). This is because the amount of liquid and NaCl delivered to the distal renal tubule increases, and the glomerular filtration rate is reduced by tubuloglomerular feedback (55). When combined with loop diuretics, SGLT2 inhibitors resulted in a significant increase in 24-hour urine volume, while urinary sodium levels did not increase. The possible sodium benefits of SGLT2 inhibitors may be short-lived and only appear in the early stages (56). It is consistent with the changing trend of the eGFR.

Conclusion

SGLT2 inhibitors can protect the cardiovascular system of CKD patients and reduce the risk of CVD or HHF. Compared with placebo, the risk of CVD, HHF, and all-cause death are all reduced, and the use of SGLT2 inhibitors does not increase the incidence of SAEs or UTIs but may increase the incidence of genital infections.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

TZ contributed to the conception and design of the study. XC and JW were responsible for the collection of data, performing the statistical analysis, and manuscript preparation. YL, KY, and YX were responsible for checking the data. XC was responsible for drafting the manuscript. All authors contributed to the article and approved the submitted version.

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Figures are created with BioRender.com.

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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EDITED BY
Gabor Czibik,
Queen Mary University of London,
United Kingdom

REVIEWED BY
Ahmed Y. Azzam,
October 6 University, Egypt
I-Shiang Tzeng,
National Taipei University, Taiwan

*CORRESPONDENCE
Xin Hu
huxingxxy@gmail.com
Manni Wang
wangmanni@scu.edu.cn

†These authors have contributed equally to this work

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The obesity paradox in intracerebral hemorrhage: a systematic review and meta-analysis

Zexu Wang[1†], Jie Wang[2†], Jiayan Wang[1], Yinghua Liao[1], Xin Hu[1*] and Manni Wang[3*]

[1]Department of Neurosurgery, West China Hospital, Sichuan University, Chengdu, Sichuan, China, [2]Department of Hematology, West China Hospital, Sichuan University, Chengdu, Sichuan, China, [3]Department of Biotherapy, Cancer Center, West China Hospital, Sichuan University, Chengdu, Sichuan, China

Background: Intracerebral hemorrhage (ICH) has a mortality rate which can reach 30–40%. Compared with other diseases, obesity is often associated with lower mortality; this is referred to as the 'obesity paradox'. Herein, we aimed to summarize the studies of the relations between obesity and mortality after ICH.

Method: For this systematic review and meta-analysis (PROSPERO registry CRD42023426835), we conducted searches for relevant articles in both PubMed and Embase. Non-English language literature, irrelevant literature, and non-human trials were excluded. All included publications were then qualitatively described and summarized. Articles for which quantitative analyses were possible were evaluated using Cochrane's Review Manager.

Results: Ten studies were included. Qualitative analysis revealed that each of the 10 studies showed varying degrees of a protective effect of obesity, which was statistically significant in 8 of them. Six studies were included in the quantitative meta-analysis, which showed that obesity was significantly associated with lower short-term (0.69 [0.67, 0.73], p<0.00001) and long-term (0.62 [0.53, 0.73], p<0.00001) mortality. (Data identified as (OR [95%CI], p)).

Conclusion: Obesity is likely associated with lower post-ICH mortality, reflecting the obesity paradox in this disease. These findings support the need for large-scale trials using standardized obesity classification methods.

Systematic review registration: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42023426835, identifier CRD42023426835.

KEYWORDS

obesity, intracerebral hemorrhage, obesity paradox, mortality, stroke

Abbreviations: BMI, body mass index; CI, confidence interval; eLOS, extended length of stay; HR, hazard ratio; ICH, intracerebral hemorrhage; ICU, intensive care unit; IS, ischemic stroke; mRS, modified Rankin Scale; NIS, National Inpatient Sample; NOS, Newcastle-Ottawa Scale; OR, odds ratio.

1 Introduction

Intracerebral hemorrhage (ICH), a nontraumatic hemorrhage caused by blood vessel ruptures in the brain parenchyma, accounts for 20–30% of all strokes and 30–40% of deaths during the acute phase (1). ICH has an overall incidence of 24.6 per 100.000 patient years, and is higher in Asian populations (2).

Obesity is a well-established risk factor for increased morbidity and mortality among the general population (3, 4). According to previous studies, obesity is a strong predictor of a number of diseases, including diabetes, cardiovascular disease, hypertension, etc. (5, 6) However, a theoretical 'obesity paradox' indicates that obese patients may have a better outcome than non-obese patients among patients with the same disease. The obesity paradox was first identified in those with cardiovascular disease (7, 8). In a multivariate analysis of 1203 patients with advanced heart failure, high BMI was a strong predictor of good prognosis (7). Research on the obesity paradox can guide us in the nutritional management of our patients and may have a positive effect on improved outcomes. With the global increase in obesity (9, 10) and the discovery of an obesity paradox in a variety of diseases (11), the importance of in-depth study of this phenomenon has become increasingly important.

Past studies of the relations between stroke and obesity have demonstrated that the latter is independently associated with favorable functional recovery at 3-month follow-up (12). Although several ICH studies exist (13–20), their results have been contradictory. Some have found significant protective effects, while others demonstrated an association with higher mortality (14). Therefore, a summary and analysis of past study results on the relations between outcomes after ICH and obesity is warranted.

Herein, we aimed to systematically review the literature addressing a possible obesity paradox in patients with ICH, and to combine currently available data using meta-analysis to estimate the role of the obesity in ICH.

2 Methods

2.1 Study design

This systematic review and meta-analysis were conducted and registered according to PROSPERO (CRD42023426835).

2.2 Search strategy and process

Briefly, the search was based on both keywords and index terms using two databases: PubMed and Embase. The summary search strategy was "cerebral hemorrhage" and "BMI/obesity". The first screening was carried out independently by two reviewers, each based on the title and abstract, with any disagreements resolved by a third reviewer. The second screening was done by reading the full texts in consultation between the two reviewers.

2.3 Study selection and data extraction

We included articles that contained data related to BMI, data related to ICH outcomes. Exclusion criteria include: 1. Case reports, case series, letters, commentaries, book chapters, animal studies, and descriptive studies without calculated risk estimates (hazard ratio [HR], odds ratio [OR], or relative risk); 2. Non-English articles; 3. Studies without statistical calculations and data analyses; 4. Studies without separate data related to patients with ICH, including those that classified ICH as stroke for statistical purposes.

The first author, country, year of article publication, total number of patients with ICH, gender ratio, mean age, follow-up time, obesity classification criteria, and baseline outcome indicators were extracted.

2.4 Quality assessment

The included literature was evaluated using the Newcastle-Ottawa Scale (NOS). Comparability on the most important factors was defined (i.e., hypertension, hyperlipidemia, hyperglycemia). A median follow-up time >1 month was considered sufficiently long. Missed follow-up at rates <20% were considered adequate. One point was added if the evaluation indicator was explicitly stated in the text. No points were added if they were not explicitly stated or could be inferred as absent. A total score >8 was considered to indicate a high quality study.

2.5 Obesity definition

The body mass index (BMI) is an internationally applied tool used to screen weight for height and serves as a health indicator. The BMI formula = (weight ÷ height^2), where weight is in kilograms and height is in meters. Due to differences in BMI classifications, we standardized the definition of obesity in quantitative analyses herein.

In addition to obese versus nonobese dichotomization, we also adopted BMI as a more detailed obesity classification criterion. For Eastern populations, using the WHO BMI classification, we redefined 'overweight' or 'obese' as obese and 'underweight' and 'normal weight' as nonobese. For Western populations, BMI >30 was considered obese. If no specific classification was mentioned in the article, BMI was disregarded and the authors' obesity classification was used.

2.6 Statistical analysis

We categorized the studies included in the meta-analysis into long- and short-term mortality comparisons according to their follow-up times. Short-term mortality was defined as in-hospital mortality and 30-day mortality; later mortality was defined as long-term mortality.

Statistical analyses were performed using pooled OR with 95% confidence intervals (95%CI), and dichotomous variables were calculated using the Mantel-Haenszel method and fixed/random effects models. The Mantel-Haenszel model for determining linear relationships between ordered categorical and dichotomous variables is applicable to this study. Heterogeneity of the included studies was tested using the Cochrane I2 test, with a threshold of $p<0.10$ indicating the presence of heterogeneity. If there was no heterogeneity, a fixed-effects model was applied. Otherwise, a random-effects model was used. For analyses using the random

effects model, if the combined term is less than 5 (including 5), we will use the Hartung-Knapp adjusted approach for correction (21, 22). A Z-test was performed for the overall effect, and $p<0.05$ was considered statistically significant. In this study, the Z-test was able to show that the mean mortality rate of obese ICH patients differed from that of non-obese ICH patients. A sensitivity analysis will be conducted to determine the stability of the findings using a literature-by-literature exclusion approach. Data analyses were conducted using Review Manager 5.4.1. and R 4.2.1.

3 Results

3.1 Search results and study characteristics

The study selection process is summarized in Figure 1, constructed based on the PRISMA statement, and the research includes 10 studies in the end. A final ten studies, representing a cumulative 567,766 patients with ICH, were included.

Each of the eight included studies had a sample size >1,000 patients with ICH, which is considered relatively large. Follow-up durations of the included literature varied from in-hospital to over 10 years, with multiple follow-up periods present within some studies. In addition to mortality, other quantitative prognostic outcomes included the modified Rankin Scale (mRS), special disposition rate, and prolonged discharge rate. Of the eight included papers, two did not provide specific case data, so the final meta-analysis was performed on six papers.

Study characteristics varied considerably among studies (Table 1).

3.1.1 Study 1

In this Japanese study, Oki et al. (18) investigated the relations between BMI and stroke mortality (including cerebral infarction and ICH) among 9,526 men and women aged 30 years or older who were randomly selected throughout Japan in 1980. These participants were followed for 19 years. HR and 95%CI were examined by Cox's proportional hazards regression models, including BMI levels.

3.1.2 Study 2

To examine the association between BMI and functional outcome, Dangayach et al. (13) analyzed 202 patients admitted to the neurological intensive care unit (ICU) who were prospectively

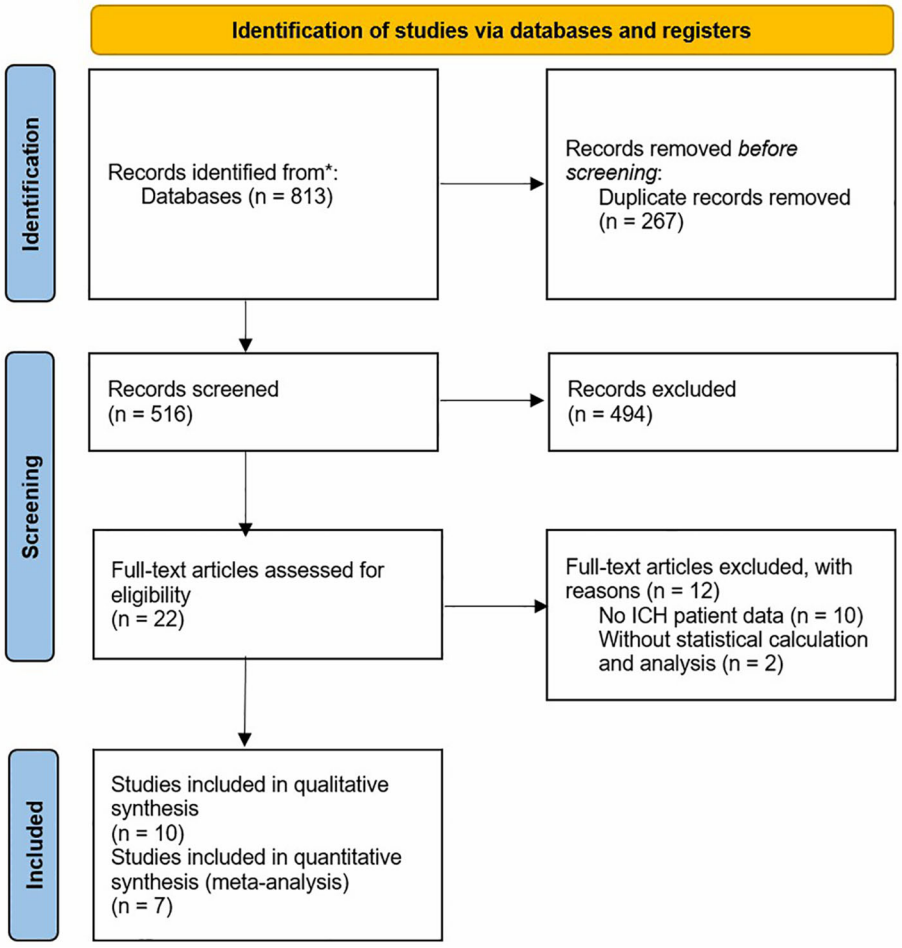


FIGURE 1
Flow diagram of study selection process.

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TABLE 1 Study characteristics.

First Author	Country	Year	ICH Cases	Mean Age	% male	Follow-up Time	Obesity Categories (kg/m^2)	Outcomes
Western population								
V. Javalkar	US	2020	47,700	68	52	In-hospital	NR	In-hospital mortality: 24%
H. Hoffman	US	2020	123,415	NR	49.6	In-hospital	BMI 30–39.9 BMI ≥ 40	Overall mortality: 28.1% Non-routine discharge disposition: 81.1% Tracheostomy: 4.5% Gastrostomy: 8.5% Median LOS: 5.0d (interquartile range 2.0–9.0) Mean (± standard deviation) Inflation-adjusted hospital charge: $67,229 ± 106,479
H. Hoffman	US	2020	751	61 (median)	54.2	30-day	BMI 30–39.9 BMI ≥ 40	30d mortality: 23.3% Non-routine discharge: 85.1% eLOS: 21.2%
S. R. Persaud	US	2019	99,212	68.78	NR	In-hospital	BMI < 30 BMI 30–39.9 BMI ≥ 40	In-hospital Mortality: 25.2% Non-routine discharge disposition*: 74.9% Extended hospitalization: 37.8% Tracheostomy/gastrostomy: 10.0% VP shunt: 0.9%
N. S. Dangayach	US	2018	202	61	57	90-day	BMI < 25 BMI ≥ 25	90d mortality: 41% mRS score 4–6 (moderate–severe disability or death): 66%
Asian population								
W. Sun	China	2016	1,572	61.1	59.8	3-month 12-month	BMI <18.5 BMI 18.5–23 BMI 23–25 BMI ≥25	12m mortality: 26.2% 3m death or high dependency: 54.0% 12m death or high dependency: 51.4%
B. J. Kim	Republic of Korea	2011	1,356	59.7	55.2	30-day 33.6-month (mean)	BMI < 18.5 BMI 18.5–23.0 BMI 23.0–24.9 BMI 25.0–29.9 BMI > 30.0	30d mortality: 7.2% Long-term mortality: 26.9%
I. Oki	Japan	2006	9,526	50.6	43.8	17.3-year (mean)	BMI < 18.5 BMI 18.5–23.0 BMI 23.0–24.9 BMI 25.0–29.9 BMI > 30.1	Overall mortality: 0.04%
Z. Cao	China	2021	82,789	NR	62.4	In-hospital	BMI < 18.5	In-hospital mortality: 2.3% Non-routine disposition: 8.5%
							BMI 18.5–22.9	
							BMI 23.0–24.9	
							BMI > 25.0	
N. Yoshida	Japan	2022	201,243	NR	NR	In-hospital	BMI < 18.5	Overall population outcome NR
							BMI 18.5–23.0	
							BMI 23.0–24.9	
							BMI 25.0–30	
							BMI > 30	

BMI, body mass index; NR, not reported; LOS, length of stay; Elos, extended length of stay; US, United States; and VP, ventriculoperitoneal. *shown in the text as routine discharge disposition. #High dependence defined as mRS score 3–5.

enrolled in the Columbia University ICH Outcomes Project. Patients were divided into two groups: overweight (BMI ≥25 kg/m^2) and not overweight (BMI <25 kg/m^2). BMI was calculated using data collected at the time of the initial emergency department or in-hospital evaluation. Exact mortality was not mentioned.

3.1.3 Study 3

Javalkar et al. (16) analyzed 47,700 patients with ICH in the United States (US) National Inpatient Sample (NIS) database from 2012–2015. BMI classification standards were not reported.

3.1.4 Study 4

Sun et al. (20) used ChinaQUEST, a hospital-based register, to analyze 1,571 patients with ICH. BMI categories were underweight (BMI <18.5 kg/m^2), normal weight (BMI 18.5–23 kg/m^2), overweight (BMI 23–25 kg/m^2), and obese (BMI ≥25 kg/m^2).

3.1.5 Study 5

In their study using the NIS, Persaud et al. (19) analyzed the 99,212 patients from 2007–2014. BMI categories were nonobese (BMI <30 kg/m^2), obese (BMI ≥30 and ≤40 kg/m^2), and morbid obese (BMI >40 kg/m^2).

3.1.6 Study 6

Hoffman et al. (15) analyzed data from 123,415 patients with ICH in the NIS from 2002–2011 to examine the effects of obesity on outcomes after spontaneous ICH. The BMI categories were the same used in study 5.

3.1.7 Study 7

This South Korean study analyzed 1,604 patients with ICH. Kim et al. (17) analyzed the association between obesity and 30-day and long-term mortality risks.

3.1.8 Study 8

Data for this study were from craniotomies for evacuation in supratentorial ICH from 2006–2017, and included 751 patients. Hoffman et al. (14) analyzed 30-day mortality, non-routine discharge disposition, and extended length of stay (eLOS, defined as the top quartile for the cohort).

3.1.9 Study 9

Cao et al. (23) evaluated the associations between BMI and in-hospital mortality, complications, and discharge disposition in ICH using data derived from the China Stroke Center Alliance, a national, hospital-based, multicenter, voluntary, quality assessment and improvement initiative performed in China. A total of 82,789 patients were involved from August 2015–July 2019.

3.1.10 Study 10

Yoshida et al. (24) analyzed 5,020,464 records from the national Japanese Registry of All Cardiac and Vascular Diseases-Diagnosis Procedure Combination dataset over from 2012–2019. They evaluated BMI trends and its impact on in-hospital mortality rates within six acute cardiovascular disease types: acute heart failure, acute myocardial infarction, acute aortic dissection, ischemic stroke (IS), ICH, and subarachnoid hemorrhage.

3.2 Risk-of-bias and quality of studies

Based on the NOS scale, we evaluated each article in three broad areas: selection, comparability, and outcome. The results of this evaluation are in Table 2. No study scored >7 points. It is worth noting that the included studies did better on both selection and outcomes, while scoring lower on comparability of both important and other factors, which may have led to higher heterogeneity in our meta-analysis.

3.3 Qualitative synthesis

3.3.1 Short-term mortality

As described above, we identified in-hospital and 30-d mortality as 'short-term mortality'. Six studies analyzed in-hospital mortality (studies 3, 5, 6, 9, and 10) and two analyzed 30-day mortality (studies 7 and 8).

In studies 3 (OR 0.64, 95%CI 0.59–0.69), 5 (OR 0.69, 95%CI 0.62–0.76), and 6 (OR 0.62, 95%CI 0.56–0.69), an association was found between obesity and lower mortality, while the protective effect of morbidly obesity was weaker than that of obesity. In study 8, despite the trend toward a protective effect of obesity, statistical significance was not found (OR 0.624, 95%CI 0.381–1.022); interestingly, in their analysis of morbid obesity, excessive BMI (>40) was associated with higher mortality (OR 2.175, 95%CI 1.034–4.578). In study 9, underweight (OR 2.057, 95%CI 1.193–3.550) was associated with higher mortality rates. In study 10, overweight (OR 0.87, 95%CI 0.4–0.90) and obese I (OR 0.93, 95%CI 0.90–0.97) were protective, while underweight was associated with higher mortality (OR 1.26, 95%CI 1.22–1.31).

There were no statistically significant results reported in study 7.

3.3.2 Long-term mortality

In study 1, compared with the reference group (BMI 25.0–29.9), the only protective effect was in the group with BMI 25.0–29.9 (HR 0.83, 95%CI 0.36–1.91). In the group with BMI ≥30.0, this became a harmful mortality factor. However, this was not a statistically significant effect.

Study 4 analyzed 12-month mortality with Cox proportional hazard models. Both the unadjusted HR and the adjusted HR (0.75, 95%CI 0.59–0.95 and HR 0.71, 95%CI 0.56–0.91, respectively) showed significant effects of obesity on lower mortality. In addition, the investigators analyzed for mortality or high

TABLE 2 Quality evaluation.

Study	Selection				Comparability		Outcome			Score
	Representativeness	Selection of non-exposed	Ascertainment of non-exposed	No sudden death from ICH	Comparability on most important factors	Comparability on other risk factors	Assessment of outcome	Sufficient follow-up duration	Follow-up adequacy	
Oki et al.	√	√	√	√	N/A	N/A	√	√	√	7
Kim et al.	√	√	√	—	—	—	√	√	√	6
Sun et al.	√	√	√	√	—	—	√	√	√	7
Dangayach et al.	√	√	√	—	√	—	√	√	—	6
Persaud et al.	√	√	√	—	√	—	√	N/A	√	6
Hoffman et al.	√	√	√	√	√	—	√	√	√	5
Hoffman et al.	√	√	√	—	—	—	√	N/A	√	5
Javalkar et al.	√	√	√	—	—	—	√	N/A	√	5
Cao et al.	√	√	√	√	—	—	√	—	√	6
Yoshida et al	√	√	√	√	—	—	√	—	√	6

√ represents reported, N/A not applicable: unknown risk, —: unmentioned.

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dependence within 3- and 12-month follow-up. All results showed a protective effect of obesity, to varying degrees.

In study 7, overweight (HR 0.69, 95%CI 0.49–0.96) and obese (HR 0.61, 95%CI 0.43–0.88) were protective factors. Underweight was also significantly associated with higher mortality (HR 1.64, 95%CI 1.11–2.40).

In addition, Dangayach use the mRS score to evaluate ICH outcomes (13), dividing patients into those with an mRS score 0–3 or mRS score 4–6. Higher BMI (BMI ≥25 kg/m^2) was associated with better outcomes (adjusted OR 2.05, 95%CI 1.03–4.06, p=0.04). In-hospital mortality data were also collected.

Table 3 shows mortality risk findings for each study.

3.4 Meta-analysis

Seven studies were included in the meta-analysis, among which four included short-term mortality data and three reported long-

TABLE 3 ICH studies reporting associations between BMI and mortality.

First author	Follow-up	Analyses by sex	BMI categories (kg/m^2)	Risk of mortality (OR(95%CI))	
				Univariate analysis	Multivariate analysis
Western population					
V. Javalkar	In-hospital	—	NR	—	0.64 (0.59–0.69)
H. Hoffman	In-hospital	—	Nonobese (<30)	—	REF
		—	Obese (30 to <40)	—	0.62 (0.56–0.69)
		—	Morbidly obese (≥40)	—	0.76 (0.66–0.88)
H. Hoffman	30-day	—	Nonobese (<30)	—	REF
		—	Obese (30 to <40)	—	0.624(0.381 – 1.022)
		—	Morbidly obese (≥40)	—	2.175(1.034 – 4.578)
S. R. Persaud	In-hospital	—	Nonobese (<30)	REF	REF
		—	Obese (30 to <40)	0.60 (0.55–0.65)	0.69 (0.62–0.76)
		—	Morbidly obese (≥40)	0.77 (0.70–0.86)	0.85 (0.74–0.97)
N. S. Dangayach	90-day	—	<25	REF	—
		—	≥25	0.70 (0.39, 1.26)	—
Asian population					
W. Sun	12-month	—	Underweight (<18.5)	0.97 (0.67–1.41)	0.78 (0.54–1.15)
		—	Normal weight (18.5 to <24)	REF	REF
		—	Overweight (24 to <28)	0.8 (0.62–1.03)	0.83 (0.63–1.08)
		—	Obese (≥28)	0.75 (0.59–0.95)	0.71 (0.56–0.91)
B. J. Kim	30-day	—	Underweight (<18.5)	—	0.75 (0.26–2.11)
		—	Normal weight (18.5 to <23)	—	REF
		—	Overweight (23 to <25)	—	0.86 (0.47–1.56)
		—	Obese (≥25)	—	0.89 (0.47–1.68)
	Long-term	—	Underweight (<18.5)	—	1.64 (1.11–2.40)
		—	Normal weight (18.5 to <23)	—	REF
		—	Overweight (23 to <25)	—	0.69 (0.49–0.96)
		—	Obese (≥25)	—	0.61 (0.43–0.88)
I. Oki	17.3-year	Both	<18.5	—	1.23 (0.46, 3.29)
			18.5 to <23.0	—	1.26 (0.67, 2.37)
			23.0 to <25.0	—	REF

(Continued)

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TABLE 3 Continued

First author	Follow-up	Analyses by sex	BMI categories (kg/m^2)	Risk of mortality (OR(95%CI))	
				Univariate analysis	Multivariate analysis
			25.0 to <30.0	—	0.83 (0.36, 1.91)
			≥30.0	—	2.31 (0.65, 8.26)
		Men	<18.5	—	1.00 (0.24, 4.12)
			18.5 to <23.0	—	1.37 (0.55, 3.40)
			23.0 to <25.0	—	REF
			25.0 to <30.0	—	1.36 (0.44, 4.23)
			≥30.0	—	6.61 (0.79, 55.65)
		Women	<18.5	—	1.55 (0.39, 6.24)
			18.5 to <23.0	—	1.19 (0.48, 2.95)
			23.0 to <25.0	—	REF
			25.0 to <30.0	—	0.50 (0.15, 1.72)
			≥30.0	—	1.47 (0.30, 7.25)
Z. Cao	In-hospital	—	Underweight (<18.5)	1.493 (1.216–1.833)	2.057 (1.193–3.550)
		—	Normal weight (18.5 to <23)	REF	Reference
		—	Overweight (23 to <25)	0.861 (0.768–0.966)	0.887 (0.647–1.216)
		—	Obese (≥25)	1.032 (0.926–1.151)	1.141 (0.846–1.539)
N. Yoshida	In-hospital	—	Underweight (<18.5)	—	1.26 (1.22–1.31)
		—	Normal weight (18.5 to <23)	—	REF
		—	Overweight (23 to <25)	—	0.87 (0.40–0.90)
		—	Obese I (25.0 to <30)	—	0.93 (0.90–0.97)
		—	Obese II (>30)	—	1.00 (0.94–1.07)

NR, not reported; REF, reference.

term mortality data. Their quantitative results are expressed as (OR [95%CI], p). In the analysis of heterogeneity, we found heterogeneity in short-term mortality data, (p<0.00001) and used a random-effects model. Long-term mortality data were not heterogeneous, (p=0.01) and fixed-effects models were used. These analyses are presented in two forest plots (Figures 2, 3).

In the pooled analysis, obesity was associated with significantly lower short-term (0.73 [0.62, 0.88], p=0.0006) and long-term (0.62 [0.53, 0.73], p<0.00001) mortalities. Due to the small number of studies included in the analysis of short-term mortality and the use of a random-effects model, we calculated the results using the Hartung-Knapp adjusted approach. The result obtained was 0.73 [0.59; 0.91], p=0.0183, indicating that obesity is still significantly associated with lower short-term mortality.

In the sensitivity analysis, we excluded the included studies one by one and combined the remaining studies. The finding that

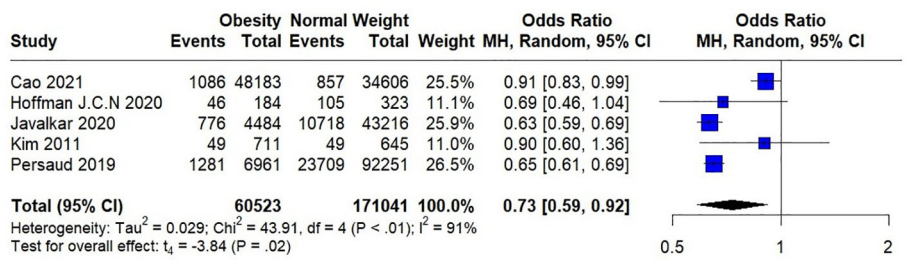


FIGURE 2
Forest plot of effect of obesity versus normal weight on short-term mortality.

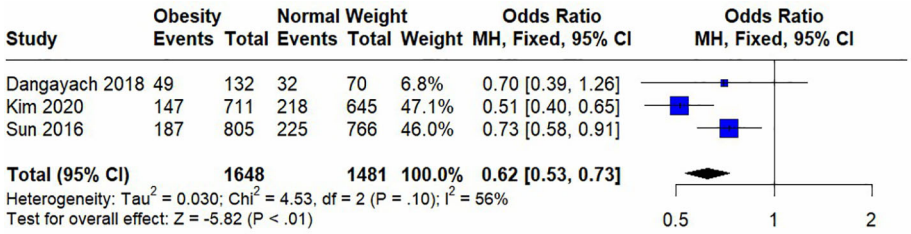


FIGURE 3
Forest plot of effect of obesity versus normal weight on long-term mortality.

obesity was associated with lower mortality remained unchanged in both the short-term and long-term mortality analyses, demonstrating the stability of the conclusions from the Meta-analysis. (Figures 4, 5). Meanwhile, we found that heterogeneity in the analysis of short-term mortality was mainly caused by Study 9, which is consistent with the conjecture in the quality evaluation. Because of the stability of the results, we decided to keep this article in the quantitative analysis.

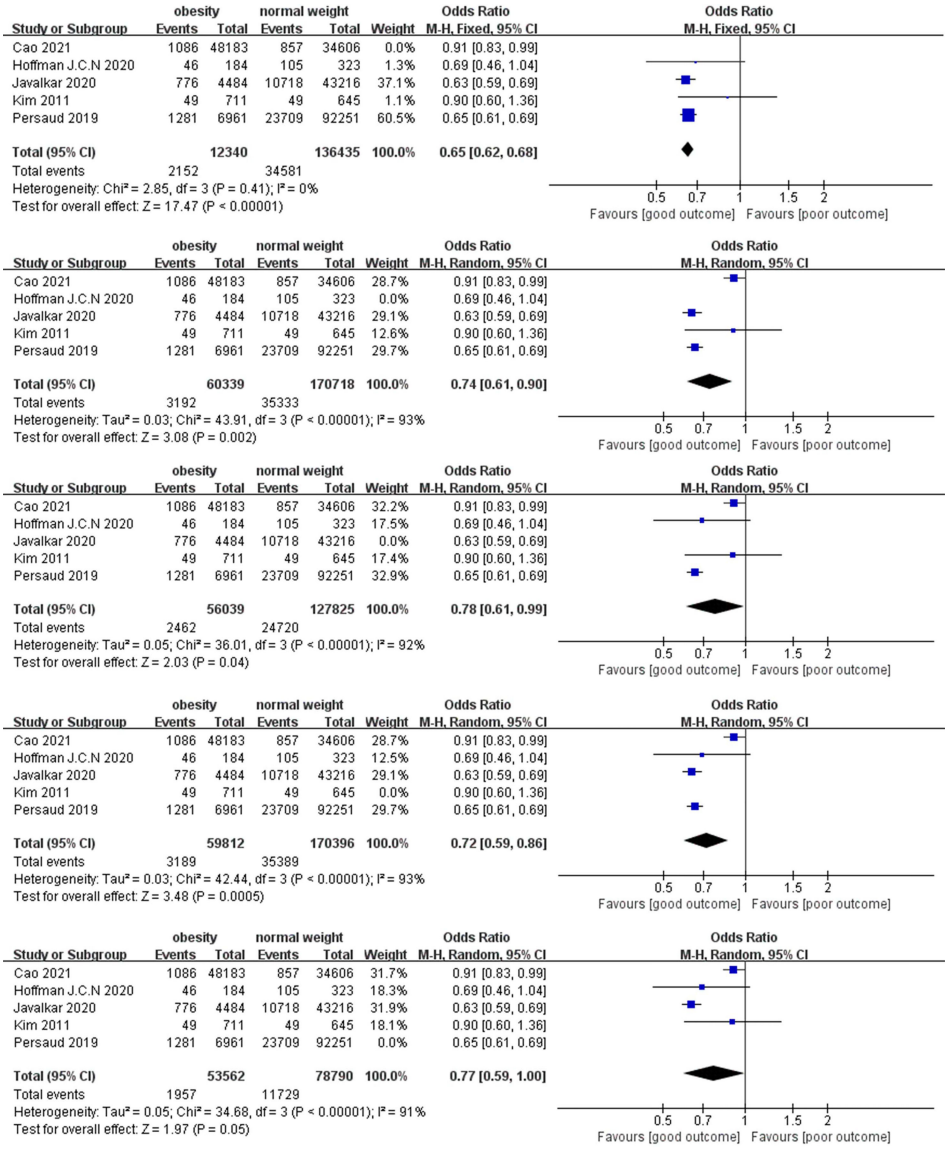


FIGURE 4
Sensitive analysis on short-term mortality.

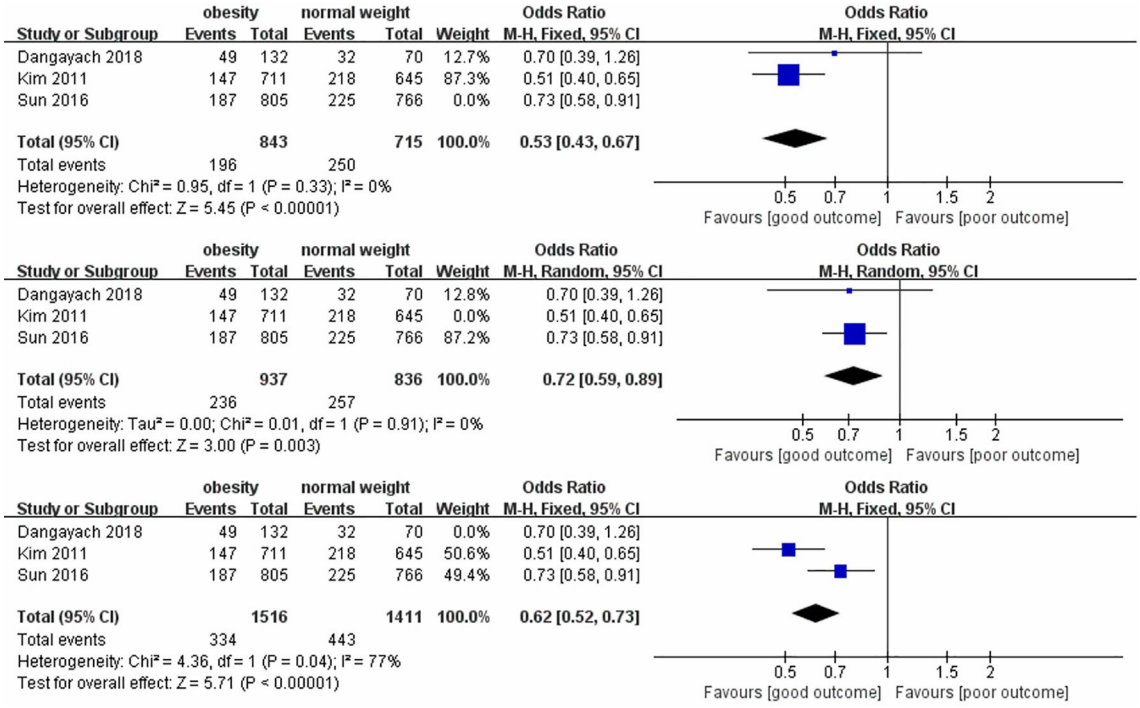


FIGURE 5
Sensitive analysis on long-term mortality.

4 Discussion

All the studies included herein showed a protective tendency of obesity, or higher BMI, among patients with ICH against mortality, and with statistically significant findings (13, 16, 17, 19, 20). In our meta-analysis, statistically significant results were found.

Though the protective effect of obesity in ICH can be explained by physical mechanisms, this cannot be fully examined in the included studies due to methodological shortcomings. Patients with obesity have an increased metabolic reserve of adipose or muscle mass, which may help patients tolerate inflammatory events during treatment (17, 25). In addition to mortality, one study (13) also used the mRS to analyze the relations between obesity and outcome among patients with ICH, showing positive associations. Another potential mechanism of the obesity paradox is the resistance to unintentional weight loss from ICH, though weight loss is more likely in hemorrhagic stroke than in IS. In addition, unintentional weight loss at 3-month follow-up may be a more clinically relevant outcome (26).

Simultaneously, previous studies question the protective effect of morbid obesity (using the Western BMI categories). Several studies have shown a weakened protective effect in this case (15, 19) and, though not statistically significant, morbid obesity has been related to higher mortality (14). The reason for this inconsistency in the obesity paradox may be the higher complication rates with morbid obesity. For example, there is a relation between obesity and insulin resistance (27), and diabetes mellitus may contribute to poorer outcomes in ICH (28, 29).

In addition to the obesity findings, an effect of underweight was also noted in several studies. Some researchers contend that underweight is associated with worst outcomes from ischemic and hemorrhagic stroke (17, 30, 31). Yet when Sun et al. assessed these previous results, they found methodological inadequacies that called these conclusions into question (20).

This review was limited by the inclusion of too few articles, emphasizing the need for broader analyses. BMI categorization discrepancies also pose challenges to quantitative reviews. Herein, this problem was solved by using dichotomy variation, meaning that all data from patients with obesity were pooled despite BMI definition differences. In addition, whether BMI is a proper way to examine obesity among patients with ICH is unknown. Some authors have suggested that the interaction between cardiorespiratory fitness and BMI may play a more important role in understanding the obesity paradox (25). Ethnic differences in ICH mortality are also noteworthy. Sun W et al. noted that mortality among their cohort was obviously lower than that previously reported in Western patients with ICH (32). Thus, these results should be generalized to specific ethnic groups with great caution.

5 Conclusion

Quantitative meta-analysis showed that obesity is associated with lower post-ICH mortality. Qualitative analysis of each included article indicated similar trends toward the emergence of

an obesity paradox, although more definitive statistical results are needed. Future research should assess the relations between morbid obesity and outcomes, include more standardized, empirical obesity measurements, and report data collection procedures more clearly. Such measures are encouraged, in part, due to the increasing numbers of patients with obesity.

Data availability statement

The original contributions presented in the study are included in the article/supplementary materials. Further inquiries can be directed to the corresponding authors.

Author contributions

ZW: Conceptualization, Data curation, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review and editing. JieW: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Software, Writing – original draft, Writing – review and editing. JiaW: Conceptualization, Investigation, Methodology, Software, Writing – original draft. YL: Conceptualization, Investigation, Methodology, Supervision, Writing – review and editing. XH: Conceptualization, Investigation, Writing – review and editing, Methodology. MW: Conceptualization, Investigation, Writing – review and editing, Data curation.

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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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EDITED BY
Daigo Sawaki,
Jichi Medical University, Japan

REVIEWED BY
Reinhold Gottfried Erben,
University of Veterinary Medicine Vienna, Austria
Marion Hofmann Bowman,
University of Michigan, United States

*CORRESPONDENCE
Hideki Fujii
fhideki@med.kobe-u.ac.jp

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Exploring the implications of blocking renin-angiotensin-aldosterone system and fibroblast growth factor 23 in early left ventricular hypertrophy without chronic kidney disease

Kentaro Watanabe, Hideki Fujii*, Kohei Okamoto, Keiji Kono, Shunsuke Goto and Shinichi Nishi

Division of Nephrology and Kidney Center, Kobe University Graduate School of Medicine, Kobe, Japan

Background: Whether fibroblast growth factor 23 (FGF23) directly induces left ventricular hypertrophy (LVH) remains controversial. Recent studies showed an association between FGF23 and the renin-angiotensin-aldosterone system (RAAS). The aim of this study was to investigate changes in FGF23 levels and RAAS parameters and their influences on LVH.

Methods: In the first experiment, male C57BL/6J mice were divided into sham and transverse aortic constriction (TAC) groups. The TAC group underwent TAC at 8 weeks of age. At 1, 2, 3, and 4 weeks after TAC, the mice were sacrificed, and blood and urine samples were obtained. Cardiac expressions of FGF23 and RAAS-related factors were evaluated, and cardiac histological analyses were performed. In the second experiment, the sham and TAC groups were treated with vehicle, angiotensin-converting enzyme (ACE) inhibitor, or FGF receptor 4 (FGFR4) inhibitor and then evaluated in the same way as in the first experiment.

Results: In the early stage of LVH without chronic kidney disease, serum FGF23 levels did not change but cardiac FGF23 expression significantly increased along with LVH progression. Moreover, serum aldosterone and cardiac ACE levels were significantly elevated, and cardiac ACE2 levels were significantly decreased. ACE inhibitor did not change serum FGF23 levels but significantly decreased cardiac FGF23 levels with improvements in LVH and RAAS-related factors, while FGFR4 inhibitor did not change the values.

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Conclusions: Not serum FGF23 but cardiac FGF23 levels and RAAS parameters significantly changed in the early stage of LVH without chronic kidney disease. RAAS blockade might be more crucial than FGF23 blockade for preventing LVH progression in this condition.

KEYWORDS

fibroblast growth factor 23, renin-angiotensin-aldosterone system, left ventricular hypertrophy (LVH), aldosterone (Ald), angiotensin-converting enzyme 2 (ACE2)

Introduction

Cardiovascular disease (CVD) is a serious problem affecting the prognosis of patients with chronic kidney disease (CKD) (1). left ventricular hypertrophy (LVH) is the most common cardiac abnormality and is an independent risk factor for CVD mortality in patients with CKD (2, 3). Many risk factors, such as hemodynamic overload, uremic toxins, anemia, and activation of the renin-angiotensin-aldosterone system (RAAS), are involved in LVH in patients with CKD (4, 5). In addition to these factors, CKD-mineral bone disorder (CKD-MBD) plays an important role in LVH progression in patients with CKD. Although fibroblast growth factor 23 (FGF23) is a phosphaturic hormone, recent experimental and clinical studies have reported that FGF23 is related to CVD progression, and CVD and all-cause mortality (6). Serum FGF23 levels have been reported to be highly elevated particularly at the advanced stage of CKD (7).

Previous clinical and experimental studies showed that serum FGF23 levels were significantly associated with LVH in patients with CKD (8–12), whereas other previous clinical and experimental studies revealed that there was no significant relationship between FGF23 and LVH (13–15). Thus, the influence of FGF23 on LVH progression remains controversial (16). In addition, several recent studies showed an association between FGF23 and RAAS in LVH (17). The detailed changes in FGF23 levels and RAAS parameters, and their relationships during LVH progression remain unclear. In the present study, we firstly investigated the changes in serum and intracardiac FGF23 levels and RAAS parameters in the early stage of LVH progression, and secondly assessed the influences of FGF23 and RAAS blockade on their changes and LVH, using a pressure-overload induced LVH model mouse without CKD.

Materials and methods

Animals

Male C57BL/6 mice were obtained from Japan SLC, Inc. (Shizuoka, Japan). The mice were housed under light- and temperature-controlled environments and were fed a standard diet with water available ad libitum.

We created a pressure-overload-induced LVH model by transverse aortic constriction (TAC), using male C57BL/6 mice. At 8 weeks of age, the mice underwent TAC or sham operation. They were anesthetized with a combination of 0.3 mg/kg of medetomidine (Domitor; Nippon Zenyaku Kogyo Co., Ltd., Fukushima, Japan), 4.0 mg/kg of midazolam (Dormicum; Astellas Pharma Inc., Tokyo, Japan), and 5.0 mg/kg of butorphanol (Vetorphale; Meiji Seika Pharma, Ltd., Tokyo, Japan) via intraperitoneal administration and then intubated and attached to an experimental animal ventilator (MK-AT210D; Muromachi Kikai Co., Ltd., Tokyo, Japan). The ventilator settings were as follows: breathing rate, 120 breaths per minute; tidal volume, 0.3 mL. After disinfection of the skin with 70% alcohol and thoracic incision, through the second intercostal space, the thymus was separated and the transverse aorta was exposed. A 5-0 silk suture was tied around the transverse aorta with a 27-gauge needle (Terumo Co., Ltd., Tokyo, Japan) to produce a constriction after the removal of the needle. The muscles and skin were closed, and after spontaneous breathing appeared, the mice were extubated and moved into a cage placed on a pad that was maintained at 37°C. In the sham group, the surgical procedure is identical to that in the TAC group, with the exception of the ligation of the transverse aorta.

In the first experiment, to investigate the time-course changes in FGF23 levels, RAAS parameters, and other parameters, mice were sacrificed, and their samples were evaluated at 1, 2, 3, and 4 weeks after sham operation (Sham group) or TAC operation (TAC group) (Figure 1A).

In the second experiment, mice were randomly assigned to the following six groups: (i) sham-operated mice treated with vehicle (S + V group, n = 5); (ii) sham-operated mice treated with enalapril maleate (S + E group, n = 6); (iii) sham-operated mice treated with H3B-6527, which is a highly selective fibroblast growth factor receptor 4 inhibitor (FGFR4i) (S + F, n = 6); (iv) TAC mice treated with vehicle (T + V group, n = 5); (v) TAC mice treated with enalapril maleate (T + E group, n = 6); and (vi) TAC mice treated with H3B-6527 (T + F group, n = 6). The treatment with vehicle, enalapril maleate, or H3B-6527 was started at postoperative day 1 and continued until sacrifice at 12 weeks of age (Figure 1B). Enalapril maleate was dissolved in drinking water at a concentration of 0.15 g/L and administered at liberty in the drinking water (estimated dose of 10-15 mg/kg body weight). The

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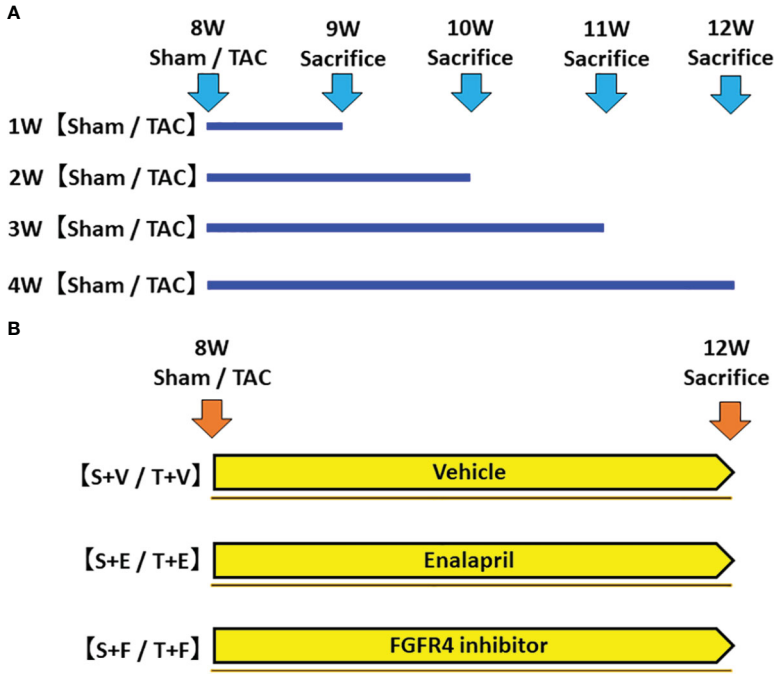


FIGURE 1
Protocol of the experiments. **(A)** The first experiment: Evaluation of changes in FGF23 levels and RAAS parameters during the development of LVH. We created a LVH model by TAC and evaluated these parameters at 1, 2, 3, and 4 weeks post-operation. **(B)** The second experiment: Effects of FGF23 and RAAS blockade on LVH. We created a LVH model by TAC and administered ACEi or FGFR4i post-operation. The animals were sacrificed, and their samples were evaluated 4 weeks after the operation and treatment. FGF23, fibroblast growth factor 23; RAAS, renin-angiotensin-aldosterone system; LVH, left ventricular hypertrophy; TAC, transverse aortic constriction; ACEi, angiotensin converting enzyme inhibitor; FGFR4i, fibroblast growth factor receptor 4 inhibitor.

dose was decided by referring to previous studies (18, 19). H3B-6527 was prepared by dissolving stock solution (3 mM) in dimethyl sulfoxide (DMSO) and then diluting using phosphate-buffered saline. It was administered intraperitoneally daily (estimated dose of 10 mg/kg body weight). The dose was decided by referring to a previous study (20). H3B-6527 is a covalent inhibitor that targets the distinctive hinge cysteine in the FGFR4 kinase domain, selectively inhibiting FGFR4 while sparing other FGFRs. This compound plays a blocking role (21). Vehicle treatment involved daily intraperitoneal injections of DMSO.

All animal procedures were approved by the Institutional Animal Care and Use Committee at Kobe University Graduate School of Medicine (permit number: P210618) and were in strict accordance with the recommendations stipulated by the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All efforts were made to minimize suffering, and the evaluations were reported in accordance with the ARRIVE guidelines (22).

Biochemical analysis

Urine was collected from each mouse, held in an individual metabolic cage (Tecniplast, Exton, PA, USA), over a 24-h period. Blood samples were collected from the left ventricle before sacrificing under anesthesia. Serum samples were centrifuged for 5 minutes at 860 G and were stored at −80°C until analysis. Urine samples were also stored at −80°C for subsequent analysis.

Serum urea nitrogen and creatinine levels were measured using a Fuji Dri-Chem 3500 system (FUJIFILM Japan, Tokyo, Japan). Serum calcium and phosphorus levels were measured using Calcium E-Test Wako and Phospha C-Test Wako (FUJIFILM Wako Pure Chemical Industries, Osaka, Japan), respectively. Serum intact parathyroid hormone (iPTH), intact fibroblast growth factor 23 (iFGF23), and aldosterone levels were measured using ELISA (enzyme-linked immunosorbent assay) kits (mouse PTH 1-84 ELISA kit: Immutopics International, San Clemente, CA, USA; FGF23 ELISA kit: KAINOS laboratories, Inc., Tokyo, Japan; Aldosterone ELISA kit: Abcam, Cambridge, UK).

Blood pressure measurement

Systolic blood pressure was measured by tail-cuff plethysmography (MK-2000; Muromachi Kikai Co., Ltd., Tokyo, Japan). To reduce the possibility of stress artifacts, mice were allowed to acclimatize to the environment for at least 15 minutes, and the mean of 10 measurements was then calculated.

Echocardiographic measurements

Mice were mildly anesthetized with 1.5%-2% isoflurane mixed with 0.5-1.0 L/min 100% O_2, and then, echocardiography was

performed using a commercially available echocardiography system (F37; Hitachi Aloka Medical, Ltd., Tokyo, Japan). A two-dimensional short-axis view of the left ventricle was obtained at the papillary muscle level.

Histological analysis

A transverse section of the heart was embedded in paraffin, sectioned, and stained with hematoxylin and eosin for histological analysis. Cross-sectional areas or diameters of myocardial fibers were analyzed using image analysis software (LUMINA VISION version 3.7.4.2; Mitani Corp., Tokyo, Japan). Immunohistochemical staining was performed for angiotensin-converting enzyme (ACE), angiotensin-converting enzyme 2 (ACE2), angiotensin II, and FGF23 using anti-ACE antibody (Abcam), anti-ACE2 antibody (R&D Systems, Minneapolis, MN, USA), anti-angiotensin II antibody (Novus Biologicals, Littleton, CO, USA), and anti-FGF23 antibody (R&D Systems). Areas with positive immunohistochemical staining areas were analyzed using image analysis software (LUMINA VISION version 3.7.4.2).

RNA extraction and real–time polymerase chain reaction

Obtained heart samples were stored at -80°C until later analysis. For bone samples, the right femur was extracted, and the proximal metaphysis was isolated from the diaphysis. After flushing the bone with phosphate-buffered saline to eliminate the bone marrow, it was promptly frozen in liquid nitrogen. Subsequently, the right proximal metaphysis and diaphysis of the femur, devoid of bone marrow, were finely powdered using a mortar and pestle under liquid nitrogen in RNase-free conditions. Total RNA was extracted from the samples using RNAiso Plus (Takara Bio Inc., Shiga, Japan) and a high-salt solution for precipitation for bone (Takara Bio Inc., Shiga, Japan). The extracted RNA serves as the template for cDNA synthesis with the ReveTra ACE qPCR RT kit (TOYOBO Co., Ltd., Osaka, Japan) according to the manufacturer's instructions. Real-time polymerase chain reaction (RT-PCR) was performed on the ABI 7500 system (Thermo Fisher Scientific, Waltham, MA, USA) using the SYBR Green Assay with Thunderbird SYBR qPCR Mix (TOYOBO). We analyzed the results using the 7500 System SDS Software Version 2.0.1 (Thermo Fisher Scientific). The mRNA expression levels of target genes were normalized to the expression level of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The primer sequences used in this study were as follows: atrial natriuretic peptide (ANP): forward 5'-AGGCAGTCGATTCTGCTTGA, reverse 5'-CGTGATAGATGAAGGCAGGAAG; brain natriuretic peptide (BNP): forward 5'-TAGCCAGTCTCCAGAGCAATTC, reverse 5'-TTGGTCCTTCAAGAGCTGTCTC; FGF23: forward 5'-ACAAGGACACCTAAACCGAACAC, reverse 5'-AGCTACTGACTGGTCCTATCACAGAA; angiotensinogen (AGT): forward 5'-TCTCTTTACCCCTGCCCTCT, reverse 5'-GAAACCTCTCATCGTTCCTTG; ACE: forward 5'-CCCTAGAGAAAATCGCCTTCTTG, reverse 5'-CGAAGATACCACCAGTCGAAGTT; ACE2: forward 5'-TGGGCAAACTCTATGCTG, reverse 5'-TTCATTGGCTCCGTTTCTTA; angiotensin II type 1 receptor (AT1R): forward 5'-CCATTGTCCACCCGATGAAG, reverse 5'-TGCAGGTGACTTTGGCCAC; GAPDH: forward 5'- GCAAAGTGGAGATTGTTGCCA, reverse 5'- AATTTGCCGTGAGTGGAGTCA.

Statistical analysis

Values are presented as mean ± standard deviation (SD). Differences in the data between the corresponding T and S groups were analyzed using the Student's t-test. Differences in the data among the groups treated with vehicle, enalapril maleate, or H3B-6527 were analyzed using one-way analysis of variance followed by the Holm method. Pearson's correlation coefficient or Spearman's rank correlation coefficient was used to analyze relationships between variables as appropriate. A two-tailed *p*-value of <0.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics version 24.0 (IBM Corp., Armonk, NY, USA).

Results

Changes in animal characteristics and biochemical data during LVH progression

As for the first study, the characteristics and biochemical data of the mice at sacrifice are shown in Table 1. Heart weight increased in a time-dependent manner and was greater in the TAC group than in the Sham group. There were no significant differences in systolic blood pressure, and serum creatinine, calcium, and phosphorus levels at all weeks. Serum aldosterone levels were significantly higher in the TAC group than in the Sham group; however, serum iFGF23 levels did not change in both group during the study period (Figures 2A, B). As expected, echocardiographic findings showed that LVH gradually progressed in the TAC mice (Table 2).

Changes in the cardiac expressions of FGF23 and RAAS- and LVH-related factors during LVH progression

As shown in Figures 3A–D, cardiac mRNA expressions of FGF23, ANP, BNP, and ACE gradually increased with the development of LVH. Although cardiac mRNA expressions of AGT and AT1R did not significantly differ between the study groups, the expression of ACE2 was significantly lower in the TAC group than in the Sham group at 4 weeks after surgery (Figures 3E–G). Furthermore, the mRNA expression of FGF23 was significantly and extremely lower in the heart than that in the bone at 4 weeks after TAC (Figure 4).

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TABLE 1 Animal characteristics at each week after TAC in experiment 1.

	1 wk		2 wk		3 wk		4 wk	
	Sham (n = 5)	TAC (n = 5)	Sham (n = 5)	TAC (n = 5)	Sham (n = 5)	TAC (n = 5)	Sham (n = 5)	TAC (n = 5)
BW (g)	24.3 ± 0.5	24.2 ± 1.2	26.1 ± 2.0	25.6 ± 1.1	24.8 ± 1.0	26.1 ± 0.7	25.6 ± 1.5	26.4 ± 1.8
HW (mg)	98.1 ± 4.7	117.4 ± 11.2[a]	103 ± 6.9	136.9 ± 6.1[be]	98.4 ± 5.8	160.8 ± 13.4[ce]	101.7 ± 5.5	178.3 ± 17.8[de]
RHW (g/100g BW)	0.41 ± 0.02	0.49 ± 0.03[a]	0.40 ± 0.01	0.54 ± 0.04[be]	0.40 ± 0.01	0.62 ± 0.04[ce]	0.40 ± 0.01	0.68 ± 0.05[de]
SBP (mmHg)	93 ± 2	96 ± 4	93 ± 6	92 ± 6	94 ± 4	96 ± 6	93 ± 4	93 ± 8
HR (bpm)	471 ± 95	464 ± 44	513 ± 69	431 ± 31	426 ± 54	450 ± 34	521 ± 60	456 ± 51
sCr (mg/dL)	0.30 ± 0.07	0.22 ± 0.08	0.34 ± 0.11	0.26 ± 0.05	0.32 ± 0.11	0.28 ± 0.04	0.26 ± 0.09	0.20 ± 0.07
sCa (mg/dL)	8.34 ± 0.89	8.03 ± 0.36	7.95 ± 0.55	8.54 ± 0.62	7.92 ± 0.49	7.08 ± 0.71	7.91 ± 0.6	7.78 ± 1.06
sP (mg/dL)	6.92 ± 0.79	7.34 ± 0.35	6.77 ± 1.16	7.76 ± 0.5	7.62 ± 0.72	7.4 ± 0.91	7.17 ± 0.76	7.71 ± 1.14

[a] $p < 0.05$ versus sham group at 1 week; [b] $p < 0.05$ versus sham group at 2 weeks; [c] $p < 0.05$ versus sham group at 3 weeks; [d] $p < 0.05$ versus sham group at 4 weeks; [e] $p < 0.05$ versus TAC group at 1 week.

TAC, transverse aortic constriction; BW, body weight; HW, heart weight; RHW, relative heart weight; SBP, systolic blood pressure; HR, heart rate; sCr, serum creatinine level; sCa, serum calcium level; sP, serum phosphate level.

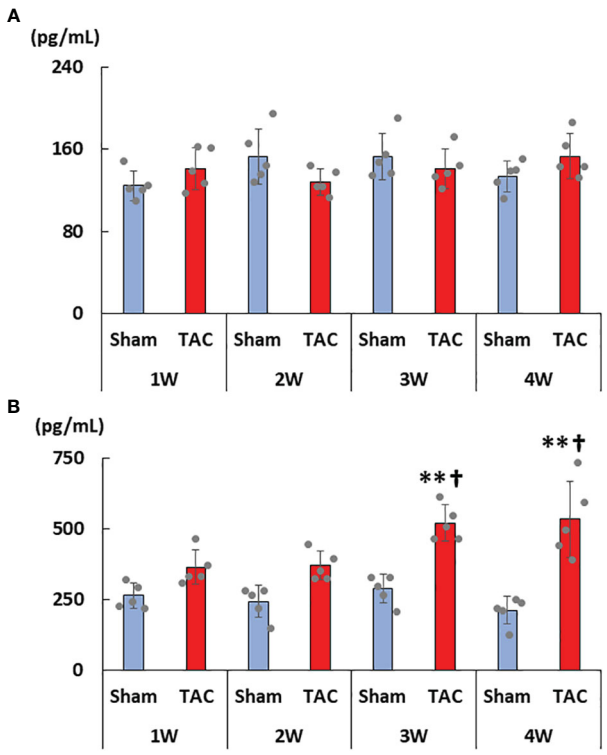


FIGURE 2
Changes in serum FGF23 and aldosterone levels. **(A)** Serum intact FGF23 levels. **(B)** Serum aldosterone levels. FGF23, fibroblast growth factor 23; TAC, transverse aortic constriction. Values are expressed as means ± SD. The blue bars represent values in the sham group and the red bars represent values in the TAC group at 1, 2, 3, and 4 weeks post-operation. * $p < 0.05$ and ** $p < 0.01$ versus the corresponding group. † $p < 0.05$ and ‡ $p < 0.01$ versus TAC at 1 week.

Effects of RAAS and FGF23 inhibition on LVH

To examine the influence of the RAAS and FGF23 on LVH, we additionally studied the effect of blocking the RAAS and FGF receptor 4 (FGFR4) on LVH in TAC mice, using enalapril and H3B-6527, which is an FGFR4i.

At 12 weeks of age, the characteristics and biochemical data of the mice treated with vehicle, enalapril, or FGFR4i are shown in Table 3. Systolic blood pressure, and serum creatinine, calcium, phosphorus, iPTH, and iFGF23 levels did not significantly differ among the groups. Serum aldosterone levels were elevated in TAC mice and decreased on enalapril treatment. Conversely, FGFR4i treatment did not decrease the levels (Table 3).

Heart weight gain was suppressed in TAC mice treated with enalapril; however, there was no change in TAC mice treated with FGFR4i. On echocardiography, LVH in TAC mice was ameliorated by enalapril treatment but not FGFR4i treatment (Table 4). Histological analysis indicated that the cardiomyocyte width and area decreased with enalapril treatment but not with FGFR4i treatment (Figure 5).

As shown in Figures 6A–D, cardiac mRNA expressions of FGF23, ANP, BNP, and ACE increased in the T + V and T + F groups but decreased in the T + E group. Although the mRNA expressions of AGT and AT1R did not significantly differ among the groups, the mRNA expression of cardiac ACE2 was significantly higher in the T + E group than in the T + V and T + F groups (Figures 6E–G). Immunohistochemical staining analysis revealed that FGF23, ACE, and angiotensin II protein expressions increased in the T + V group and decreased in the T + E group (Figures 7A–C). In addition, the expression of ACE2 decreased in the T + V group and increased in the T + E group (Figure 7D). However, these expressions in the T + F group were similar to those in the T + V group (Figures 7A–D).

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TABLE 2 Echocardiographic findings at each week after TAC in experiment 1.

	1 wk		2 wk		3 wk		4 wk	
	Sham (n = 5)	TAC (n = 5)	Sham (n = 5)	TAC (n = 5)	Sham (n = 5)	TAC (n = 5)	Sham (n = 5)	TAC (n = 5)
EF (%)	63.4 ± 0.7	61.5 ± 1.7	64.5 ± 1.6	63.2 ± 4.3	62.7 ± 1.6	62 ± 2.8	63.4 ± 2.1	62.7 ± 2.5
FS (%)	29.4 ± 0.4	28.2 ± 1.1	30.2 ± 1.0	29.4 ± 2.8	29.0 ± 1.0	28.6 ± 1.8	29.5 ± 1.5	29.0 ± 1.6
LVM (mg)	89.8 ± 13.6	121.6 ± 13.2[a]	83.7 ± 12.1	133.5 ± 7.5[b]	87.2 ± 7.9	172.8 ± 11.9[cf]	86.5 ± 15.2	200.7 ± 36[df]
LVAW (mm)	0.83 ± 0.08	0.99 ± 0.06[a]	0.73 ± 0.04	1.07 ± 0.04[b]	0.76 ± 0.08	1.19 ± 0.01[cf]	0.73 ± 0.06	1.28 ± 0.12[df]
LVPW (mm)	0.87 ± 0.10	0.97 ± 0.06	0.72 ± 0.10	1.04 ± 0.03[b]	0.72 ± 0.10	1.10 ± 0.05[c]	0.73 ± 0.10	1.15 ± 0.08[df]
LVDd (mm)	3.21 ± 0.28	3.40 ± 0.20	3.52 ± 0.20	3.38 ± 0.18	3.56 ± 0.34	3.68 ± 0.20	3.57 ± 0.09	3.83 ± 0.40
LVDs (mm)	2.25 ± 0.20	2.44 ± 0.18	2.46 ± 0.17	2.40 ± 0.19	2.53 ± 0.26	2.63 ± 0.15	2.52 ± 0.06	2.71 ± 0.33

[a] $p < 0.05$ versus sham group at 1 week; [b] $p < 0.05$ versus sham group at 2 weeks; [c] $p < 0.05$ versus sham group at 3 weeks; [d] $p < 0.05$ versus sham group at 4 weeks; [e] $p < 0.05$ versus TAC group at 1 week

AC, transverse aortic constriction; EF, ejection fraction; FS, functional shortening; LVM, left ventricular mass; LVAW, left ventricular anterior wall thickness; LVPW, left ventricular posterior wall thickness; LVDd, diastolic left ventricular diameter; LVDs, systolic left ventricular diameter.

Discussion

The present study demonstrated that in the early stage of LVH without CKD, (i) not serum FGF23 but cardiac FGF23 levels significantly increased during LVH progression; (ii) cardiac FGF23 expression was lower in the heart than in the bone in the TAC group; (iii) serum aldosterone levels and cardiac ACE expression significantly increased and cardiac ACE2 expression significantly decreased in the TAC group; and (iv) ACEi could prevent LVH progression but FGF4Ri could not.

In previous experimental studies, it was reported that the RAAS induced FGF23 production. *In vitro* studies showed that aldosterone and angiotensin II upregulated the expression of FGF23 in osteoblastic cells and neonatal rat ventricular myocytes and mineralocorticoid receptor blockers reversed this effect (23, 24). Moreover, an *in vivo* study showed that FGF23 expression was significantly upregulated in mouse hearts in response to angiotensin II infusion (25). In the present study, serum FGF23 levels did not increase during LVH progression in the absence of CKD; however, serum aldosterone levels increased. By contrast, intracardiac FGF23 expression significantly increased. Our recent study also found that there was a significant relationship between the expression of intracardiac FGF23 and the expression of intracardiac RAAS-related factors (26). Uninephrectomy-induced RAAS activation was reported to cause AMP-activated protein kinase (AMPK) inhibition in the remnant kidney of rats (27). Moreover, the

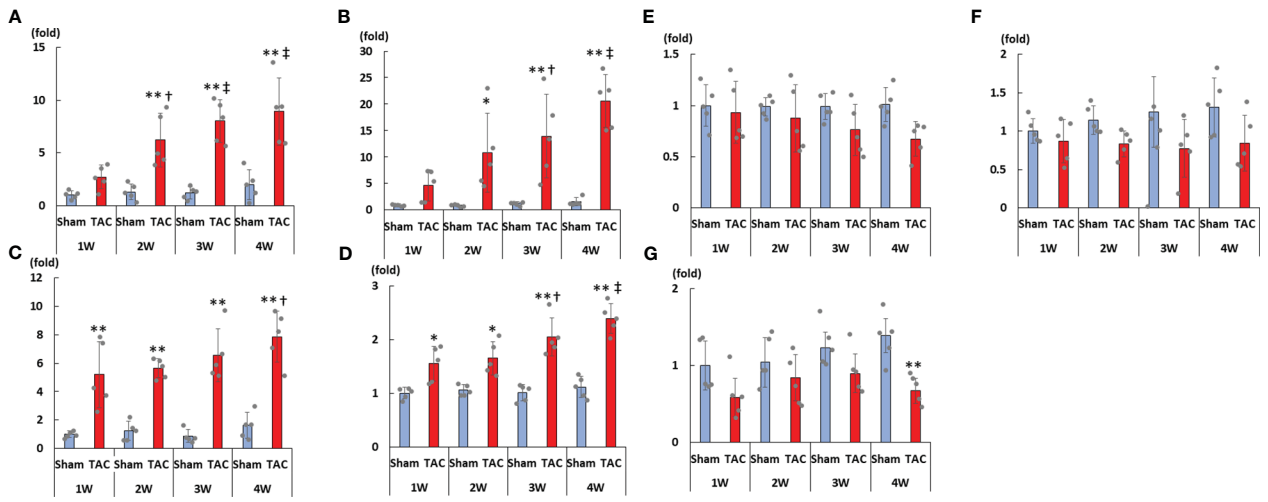


FIGURE 3
mRNA expressions of LVH-related factors in the heart tissue. **(A)** FGF23. **(B)** ANP. **(C)** BNP. **(D)** ACE. **(E)** AGT. **(F)** AT1R. **(G)** ACE2. LVH, left ventricular hypertrophy; FGF23, fibroblast growth factor 23; TAC, transverse aortic constriction; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; ACE, angiotensin converting enzyme; AGT, angiotensinogen; AT1R, angiotensin II type 1 receptor; ACE2, angiotensin-converting enzyme 2. Values are expressed as means ± SD. The blue bars represent values in the sham group and the red bars represent values in the TAC group at 1, 2, 3, and 4 weeks post-operation. * $p < 0.05$ and ** $p < 0.01$ versus the corresponding group. † $p < 0.05$ and ‡ $p < 0.01$ versus TAC at 1 week.

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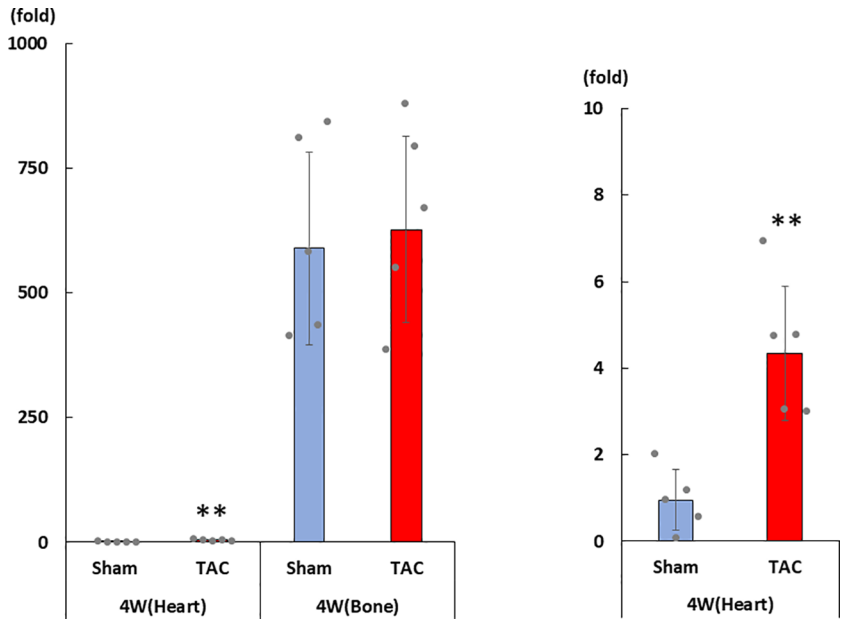


FIGURE 4
mRNA expression of FGF23 in the heart and the bone at 4 weeks after TAC. FGF23, fibroblast growth factor 23; TAC, transverse aortic constriction. Values are expressed as means ± SD. The blue bars represent values in the sham group and the red bars represent values in the TAC group at 1, 2, 3, and 4 weeks post-operation. * $p < 0.05$ and ** $p < 0.01$ versus the corresponding group.

AMPK activator down-regulated FGF23 transcription and AMPKa1-knockout mice had high serum FGF23 levels (28). Considering these results, the RAAS seems to regulate FGF23 production. Conversely, previous studies reported that FGF23 increased ATII production in neonatal rat ventricular myocytes (22, 23). Additionally, ATII receptor antagonists significantly attenuated FGF23-induced cellular hypertrophy. Therefore, it is speculated that FGF23-mediated activation of the RAAS in the heart might promote LVH (29, 30). In FGF23 knockout mice, chronic FGF23 administration decreased the renal expression of the ace2 gene but short-term FGF23 administration did not (31). Our study showed that ACEi reduced intracardiac FGF23 expression and upregulated intracardiac ACE2 expression. Although these findings indicate that there are interactions between FGF23 and the RAAS, a causal relationship between them remains unclear. Considering that serum FGF23 levels did not increase during LVH progression and not FGFR4i but ACEi prevented RAAS activation in the present study, we speculate that local RAAS activation may induce the increase in cardiac FGF23 levels.

Previous studies reported that FGF23 was associated with LVH. In an experimental study in mice, direct injection of FGF23 into the heart or systemic administration of a high-dose recombinant

TABLE 3 Animal characteristics after each treatment for 4 weeks in experiment 2.

	S + V (n = 5)	S + E (n = 5)	S + F (n = 5)	T + V (n = 5)	T + E (n = 5)	T + F (n = 5)
BW (g)	25.8 ± 1.6	26.2 ± 1.1	25 ± 0.8	25.3 ± 1.8	26.1 ± 1.6	25.5 ± 1.3
HW (mg)	102 ± 10	97 ± 6	99 ± 2	142 ± 15	119 ± 10**†	142 ± 18
RHW (g/100g BW)	0.39 ± 0.02	0.37 ± 0.01	0.40 ± 0.01	0.56 ± 0.05**	0.46 ± 0.03**†	0.56 ± 0.06**
SBP (mmHg)	96 ± 11	92 ± 8	93 ± 3	87 ± 7	89 ± 10	85 ± 9
HR (bpm)	542 ± 31	542 ± 31	551 ± 35	495 ± 50	519 ± 66	535 ± 33
sCr (mg/dL)	0.22 ± 0.06	0.17 ± 0.04	0.2 ± 0.03	0.23 ± 0.06	0.19 ± 0.04	0.19 ± 0.04
sCa (mg/dL)	7.7 ± 0.3	7.2 ± 1.1	7.6 ± 0.2	7.7 ± 0.5	8.2 ± 0.4	7.7 ± 0.1
sP (mg/dL)	8.3 ± 0.5	9.2 ± 1.1	9.1 ± 0.7	9.1 ± 0.7	9.8 ± 0.9	9.1 ± 0.7
iFGF23 (pg/mL)	111.1 ± 17.3	106.8 ± 14.2	149.7 ± 37.9	110.5 ± 24.1	104.8 ± 7.9	163.5 ± 49.2
Ald (pg/mL)	205.9 ± 30.4	224.5 ± 83.7	168.8 ± 33.8	396.4 ± 84.9**	158.8 ± 35.9‡	340 ± 53.6**

* $p < 0.05$ versus S + V group; ** $p < 0.01$ versus S + V group; † $p < 0.05$ vs T + V group; ‡ $p < 0.01$ versus T + V group.
BW, body weight; HW, heart weight; RHW, relative heart weight; SBP, systolic blood pressure; HR, heart rate; sCr, serum creatinine level; sCa, serum calcium level, sP, serum phosphorus level; iFGF23, serum intact fibroblast growth factor 23 level; Ald, serum aldosterone level.

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TABLE 4 Echocardiographic findings after each treatment for 4 weeks in experiment 2.

	S + V (n = 5)	S + E (n = 5)	S + F (n = 5)	T + V (n = 5)	T + E (n = 5)	T + F (n = 5)
EF (%)	65.0 ± 1.2	65.5 ± 1.9	65.4 ± 0.8	64.2 ± 1.6	65.1 ± 1.0	63.4 ± 1.1
FS (%)	30.5 ± 0.9	30.8 ± 1.3	30.8 ± 0.5	29.9 ± 1.1	29.5 ± 2.8	29.3 ± 0.7
LVM (mg)	88.4 ± 18.2	75.8 ± 12.7	80.7 ± 11.9	147.2 ± 21.0**	112.9 ± 18.0†	137 ± 10.7**
LVAW (mm)	0.82 ± 0.09	0.75 ± 0.04	0.76 ± 0.09	1.29 ± 0.1**	1.07 ± 0.08†	1.22 ± 0.08**
LVPW (mm)	0.7 ± 0.05	0.65 ± 0.05	0.65 ± 0.05	1.07 ± 0.07**	0.9 ± 0.07‡	1.05 ± 0.05**
LVDd (mm)	2.42 ± 0.21	2.36 ± 0.19	2.47 ± 0.06	2.29 ± 0.28	2.24 ± 0.16	2.2 ± 0.11
LVDs (mm)	3.48 ± 0.28	3.4 ± 0.24	3.52 ± 0.09	3.2 ± 0.29	3.22 ± 0.27	3.18 ± 0.11
EF (%)	65.0 ± 1.2	65.5 ± 1.9	65.4 ± 0.8	64.2 ± 1.6	65.1 ± 1.0	63.4 ± 1.1

* $p < 0.05$ versus S + V group; ** $p < 0.01$ versus S + V group; † $p < 0.05$ vs T + V group; ‡ $p < 0.01$ versus T + V group
TAC, transverse aortic constriction; EF, ejection fraction; FS, functional shortening; LVM, left ventricular mass; LVAW, left ventricular anterior wall thickness; LVPW, left ventricular posterior wall thickness; LVDd, diastolic left ventricular diameter; LVDs, systolic left ventricular diameter.

FGF23 induced LVH (11). In animal models and patients with CKD, FGF23 was reported to induce LVH via FGFR4 activation without klotho (12, 32). As previous experimental studies were conducted using specific animal models such as kl/kl mice, FGFR4$^{-/-}$ mice, and, knock-in mice with an FGFR4 gain-of-function mutation, it remains unclear whether FGF23 directly induces LVH in clinical settings. In contrast, other previous studies reported that FGF23 was not associated with LVH. Patients with FGF23-related hypophosphatemic diseases did not show LVH, and no significant correlations between LVH and serum FGF23 levels were observed (33, 34). Even in animal studies, X-linked hypophosphatemia model mice, which showed high serum FGF23 levels and hyperphosphatemia without kidney dysfunction, did not manifest LVH (15, 35). LVH was induced by TAC in mice with global genetic ablation of FGF23 (36) and neutralization of FGF23 by treatment with FGF23-Ab did not improve LVH in CKD rats fed

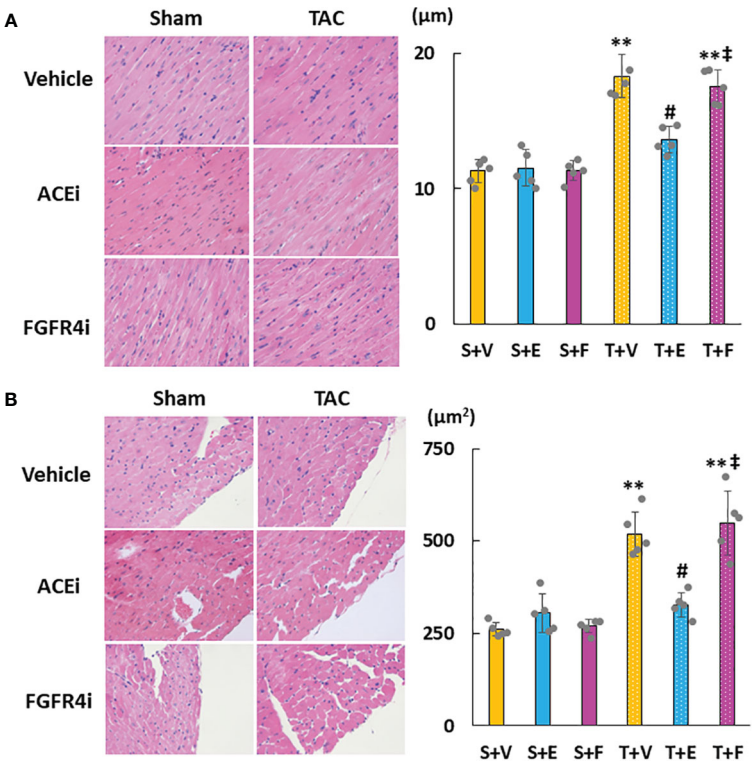


FIGURE 5
Evaluation of cardiac morphology. **(A)** Diameter of myocardial fiber. **(B)** Cross-sectional area of cardiomyocytes. Values are expressed as means ± SD. A yellow solid bar represents a value in the sham + vehicle (S+V) group, a blue solid bar represents values in the sham + enalapril (S+E) group, and a pink solid bar represents values in the sham + FGFR4i (S+F) group. A yellow dotted bar represents values in the TAC + vehicle (T+V) group, a blue dotted bar represents values in the TAC + enalapril (T+E) group, and a pink dotted bar represents values in the TAC + FGFR4i (T+F) group at 4 weeks after operation and following treatment. * $p < 0.05$ and ** $p < 0.01$. † $p < 0.05$ and ‡ $p < 0.01$ versus S + V. # $p < 0.05$ and ## $p < 0.01$ versus T + V.

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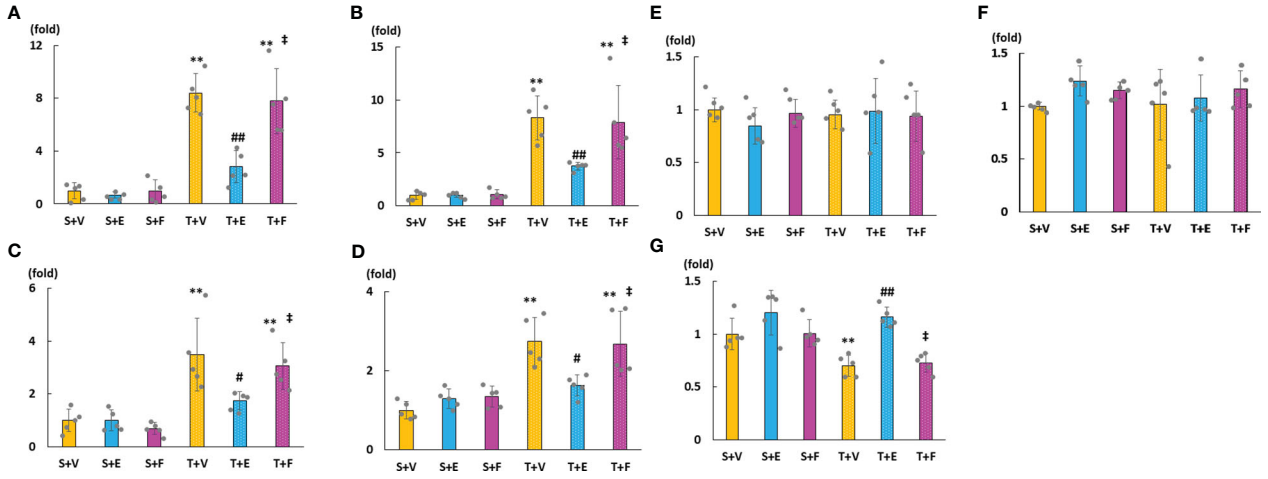


FIGURE 6
mRNA expressions of LVH-related factors in heart tissue. **(A)** FGF23. **(B)** ANP. **(C)** BNP. **(D)** ACE. **(E)** AGT. **(F)** AT1R. **(G)** ACE2. FGF23, fibroblast growth factor 23; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; ACE, angiotensin converting enzyme; AGT, angiotensinogen; AT1R, angiotensin II type 1 receptor; ACE2, angiotensin-converting enzyme 2. Values are expressed as means ± SD. A yellow solid bar represents values in the sham + vehicle (S+V) group, a blue solid bar represents values in the sham + enalapril (S+E) group, and a pink solid bar represents values in the sham + FGFR4i (S+F) group. A yellow dotted bar represents values in the TAC + vehicle (T+V) group, a blue doted bar represents values in the TAC + enalapril (T+E) group, and a pink dotted bar represents values in the TAC + FGFR4i (T+F) group at 4 weeks after operation and following treatment. * $p < 0.05$ and ** $p < 0.01$ versus the corresponding group. † $p < 0.05$ and ‡ $p < 0.01$ versus S + V. # $p < 0.05$ and ## $p < 0.01$ versus T + V.

a high-phosphate diet and resulted in increased serum phosphate levels and aortic calcification associated with an increased risk of mortality (14). Conditional knockout mice for osteoblast/osteocyte-specific disruption of the FGF23 gene with CKD induced by adenine showed more severe LVH, although the elevation of serum FGF23 levels was suppressed compared to that in mice with only CKD, and therefore, increased bone FGF23 is necessary rather than harmful to protect against the cardiorenal consequences of elevated tissue phosphate. Moreover, the development of LVH in CKD is multifactorial and not solely dependent on markedly elevated

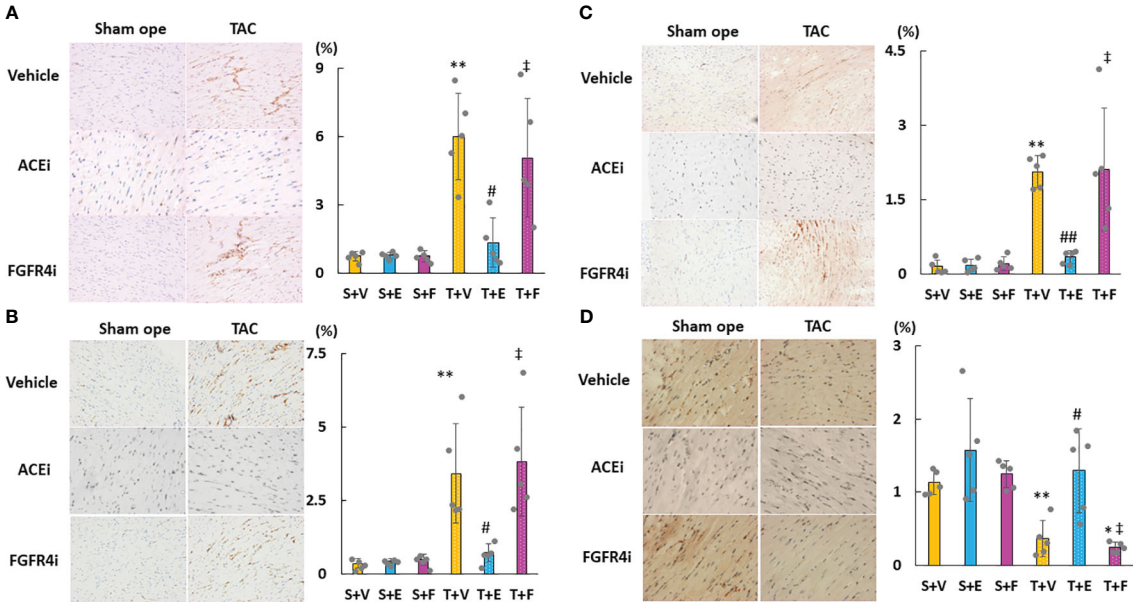


FIGURE 7
Evaluation of the protein expressions of LVH-related factors in heart tissue using immunohistochemistry. **(A)** FGF23. **(B)** ACE. **(C)** Angiotensin II. **(D)** ACE2. LVH, left ventricular hypertrophy; FGF23, fibroblast growth factor 23; ACE, angiotensin-converting enzyme; AT1R, angiotensin II type 1 receptor; ACE2, angiotensin-converting enzyme 2. Values are expressed as means ± SD. A yellow solid bar represents values in the sham + vehicle (S+V) group, a blue solid bar represents values in the sham + enalapril (S+E) group, and a pink solid bar represents values in the sham + FGFR4i (S+F) group. A yellow dotted bar represents values in the TAC + vehicle (T+V) group, a blue dotted bar represents values in the TAC + enalapril (T+E) group, and a pink dotted bar represents values in the TAC + FGFR4i (T+F) group at 4 weeks after operation and following treatment. * $p < 0.05$ and ** $p < 0.01$ versus the corresponding group. † $p < 0.05$ and ‡ $p < 0.01$ versus S + V. # $p < 0.05$ and ## $p < 0.01$ versus T + V.

serum FGF23 levels (37). *In vitro* application of phosphate alone increased the expression of LVH-related markers (15) and treatment with phosphate binder improved hyperphosphatemia and LVH, although serum FGF23 levels remained higher in CKD mice (38). These findings suggest that a high serum FGF23 level alone does not always cause LVH, and serum phosphate levels are also important for LVH. The heart produced FGF23 in animal models of pathological conditions, including heart failure, LVH, and myocardial infarction (15, 39–41). In these studies, serum FGF23 levels were also elevated, probably due to kidney dysfunction followed by heart failure. The present study showed that not serum FGF23 but cardiac FGF23 expression increased and was significantly correlated with the RAAS in the early stage of LVH induced by overload pressure without obvious kidney dysfunction. Our study also revealed that not FGFR4i but ACEi ameliorated LVH and RAAS parameters. Taking these results into account, we speculate that the activation of RAAS might induce LVH along with an increase in cardiac FGF23 levels.

In TAC mice, previous studies reported that activation of the RAAS plays a key role in the LVH progression (42, 43). Similarly, in the present study, activated RAAS parameters were significantly correlated with LVH parameters (data not shown), and ACEi ameliorated LVH. Our previous study also reported that more severe LVH was induced in TAC mice than in CKD rats although serum FGF23 levels were comparable between the two groups (26). In CKD rats, suppression of the RAAS by the administration of a vitamin D receptor activator prevented LVH though serum FGF23 levels remained high (44). In the present study, serum aldosterone levels increased twofold in the TAC group although serum FGF23 levels did not significantly change. These results suggest that the main factor that contributed to LVH was the RAAS rather than FGF23 in TAC mice.

Our study has several limitations. First, we showed changes in FGF23 levels and RAAS parameters only in the early stage of LVH without CKD. If these variables were observed for a much longer time or in the presence of CKD, the findings might differ from the results of the present study. Second, although we inhibited the FGF23 signal using FGF4Ri in the present study, we could not examine the pathophysiological changes associated with the inhibition of the heart-specific FGF23 signal. Third, the influence of inhibiting or activating ACE2 on cardiac FGF23 remains unclear in our study. Fourth, utilizing the tail-cuff method for blood pressure measurement has the potential to induce stress-induced changes in blood pressure due to animal restraint, despite being a nonoperative and cost-effective alternative compared to the telemetry method. Further studies are needed in the near future to clarify these issues.

Conclusions

We speculate that the RAAS activation may be more important than FGF23 signaling in the early stage of pressure-overload-induced LVH. A further study is needed to clarify how FGF23 and the RAAS interact with each other in detail and whether FGF23 contributes to LVH in various situations.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

All animal procedures were approved by the Institutional Animal Care and Use Committee at Kobe University School of Medicine (permit number: P160707-R1) and were in strict accordance with the recommendations stipulated by the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

Author contributions

HF: Conceptualization, Supervision, Writing – review & editing, Writing – original draft. KW: Investigation, Writing – original draft. KO: Investigation, Writing – review & editing. KK: Data curation, Project administration, Writing – review & editing. SG: Data curation, Formal analysis, Writing – review & editing. SN: Supervision, Writing – review & editing.

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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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University of South Florida, United States

*CORRESPONDENCE
Adrian Soto-Mota
adrian.sotom@incmnsz.mx

†These authors have contributed equally to this work

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Thyroid markers and body composition predict LDL-cholesterol change in lean healthy women on a ketogenic diet: experimental support for the lipid energy model

Isabella D. Cooper[1†], Claudio Sanchez-Pizarro[2,3†], Nicholas G. Norwitz[4], David Feldman[5], Yvoni Kyriakidou[1], Kurtis Edwards[1], Lucy Petagine[1], Bradley T. Elliot[1] and Adrian Soto-Mota[2,3*]

[1]Ageing Biology and Age-Related Diseases, School of Life Sciences, University of Westminster, London, United Kingdom, [2]Metabolic Diseases Research Unit, National Institute of Medical Science and Nutrition Salvador Zubiran, Mexico City, Mexico, [3]Tecnologico de Monterrey, Escuela de Medicina y Ciencias de la Salud, Mexico City, Mexico, [4]Harvard Medical School, Boston, MA, United States, [5]Citizen Science Foundation, Las Vegas, NV, United States

Introduction: There is a large heterogeneity in LDL-cholesterol change among individuals adopting ketogenic diets. Interestingly, lean metabolically healthy individuals seem to be particularly susceptible, with an inverse association between body mass index and LDL-cholesterol change. The lipid energy model proposes that, in lean healthy individuals, carbohydrate restriction upregulates systemic lipid trafficking to meet energy demands. To test if anthropometric and energy metabolism markers predict LDL-cholesterol change during carbohydrate restriction.

Methods: Ten lean, healthy, premenopausal women who habitually consumed a ketogenic diet for ≥6 months were engaged in a three-phase crossover study consisting of continued nutritional ketosis, suppression of ketosis with carbohydrate reintroduction, and return to nutritional ketosis. Each phase lasted 21 days. The predictive performance of all available relevant variables was evaluated with the linear mixed-effects models.

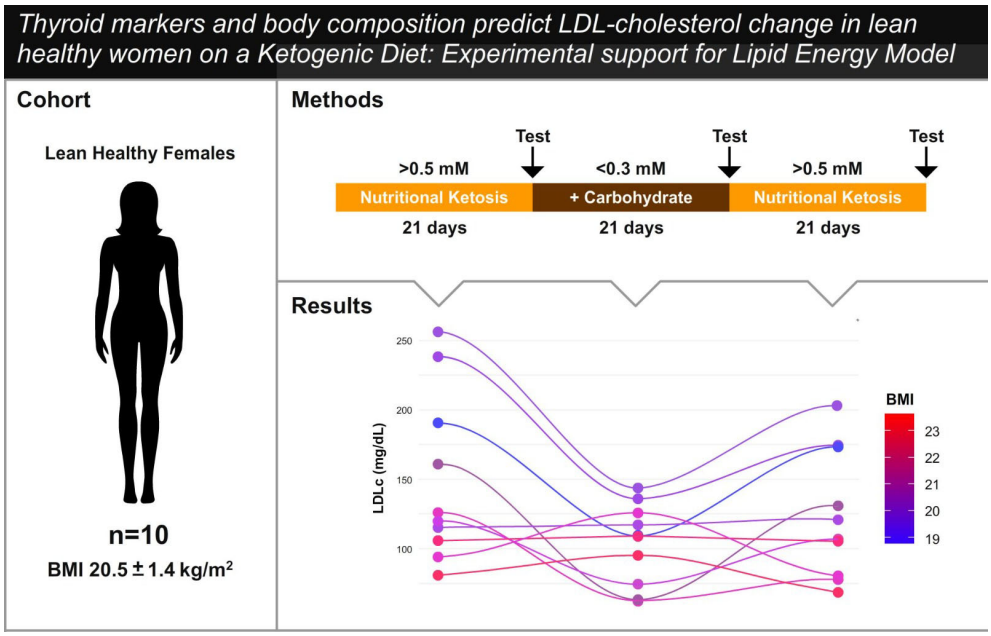
Results: All body composition metrics, free T_3 and total T_4, were significantly associated with LDL-cholesterol change. In an interaction model with BMI and free T_3, both markers were significant independent and interacting predictors of LDL-cholesterol change. Neither saturated fat, HOMA-IR, leptin, adiponectin, TSH, nor rT_3 was associated with LDL-cholesterol changes.

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Discussion: Among lean, healthy women undergoing carbohydrate restriction, body composition and energy metabolism markers are major drivers of LDL-cholesterol change, not saturated fat, consistent with the lipid energy model.

KEYWORDS

cholesterol, ketogenic diet, lean mass hyper-responder, lipid energy model, cardiovascular risk



GRAPHICAL ABSTRACT

1 Introduction

Ketogenic diets (KDs) are becoming increasingly popular for the treatment of a wide range of chronic medical conditions, including epilepsy (1), neurodegenerative diseases (2), mental health disorders (3), diabetes (4), and many other chronic conditions not necessarily associated with an elevated body mass index (BMI). However, there is a large degree of heterogeneity in LDL-cholesterol (LDLc) changes to KD, with some individuals exhibiting decreases in LDLc, many exhibiting minimal change, and yet some exhibiting large increases on the order of 100s of mg/dL (5, 6).

At present, the sources of this heterogeneity are poorly understood and/or misunderstood, deterring the clinical implementation of this nutritional therapy. Interestingly (and counterintuitively), a major source of heterogeneity in the LDLc response may be BMI, with a normal BMI (≤~25 kg/m^2) associated with larger increases in LDLc when compared with higher BMIs (6).

One hypothesis to explain this phenomenon is the lipid energy model (LEM) (7), which posits that the increase in LDLc exhibited by lean individuals on KD is a result of upregulation in systemic lipid trafficking to meet energy demands. Briefly, in lean individuals, a KD induces larger increases in circulating free fatty acids that are subsequently taken up by hepatocytes, resynthesized into triglycerides (TGs), and exported on very low-density lipoprotein

Abbreviations: βHB, D-β-hydroxybutyrate; BMI, body mass index; LDLc, low-density lipoprotein cholesterol; HDLc, high-density lipoprotein cholesterol; LMHR, lean mass hyperresponders; FM, fat mass; FMI, fat mass index; FatOx, fat oxidation; fT_3, free triiodothyronine; FFMI, fat-free mass index; LPL, lipoprotein lipase; NK, nutritional ketosis; RQ, respiratory quotient; rT_3, reverse triiodothyronine; SuK, suppressed ketosis; SUK, Standard United Kingdom Diet; TG, triglycerides; T_4, thyroxine; TSH, thyroid-stimulating hormone; VLDL, very low-density lipoprotein.

(VLDL) particles. These VLDLs are depleted of their TG cargo by lipoprotein lipase (LPL) at peripheral adipocytes and oxidative tissues, resulting in TG-depleted LDL particles and an increase in HDL-cholesterol (HDLc) as HDL particles accept cholesterol from VLDL during LPL-mediated turnover.

The LEM also predicts that energy demand can influence LDLc changes too, as VLDL export and peripheral turnover should increase to meet the needs of increased peripheral tissue demands, all else being equal. In this three-phase crossover study, involving 10 lean, healthy women on KDs, we aim to confirm prior reports of an inverse association between BMI and LDLc on KD and test if thyroid hormones—well-documented regulators of energy metabolism—also predict LDLc change.

2 Methods

2.1 Ethical approval

Ethical approval was obtained by the College of Liberal Arts and Sciences Research Ethics Committee, University of Westminster, United Kingdom (ETH2122-0634). All procedures were conducted in accordance with the Declaration of Helsinki and UK legislation.

2.2 Study design

This study was an open-labeled, non-randomized crossover trial with three phases, each 21 days in duration: nutritional ketosis (NK; P1); suppressed ketosis (SuK) on higher carbohydrate UK Eatwell Guidelines, Standard United Kingdom (SUK; P2) guidelines; and returned to NK (P3). Participants were n = 10 healthy women. Recruitment criteria included healthy premenopausal lean (BMI < 25 kg/m^2) women, who were not taking hormonal contraception, were non-smokers, and had been adherent to a KD for ≥6 months (mean 3.9 ± 2.3 years) to ensure sufficient time for metabolic adaptation. To confirm a state of NK, a daily capillary D-β-hydroxybutyrate (βHB) measurement was done between 16:00 and 18:00 before the evening meal during the 6 months prior to commencing the study. Standardized evening measurements were chosen as a more rigorous threshold (as compared with morning measurements after overnight fasting) to increase the probability that participants were in NK most of the time.

These analyses emerged from a larger recently published study. Further methodological details can be found here (8).

During the first 21-day phase, participants maintained NK, defined as βHB ≥0.5 mmol/L, and similar to those exhibited during their routine KD. On day 22, participants attended the Human Physiology Laboratory at the University of Westminster at 08:00 after an overnight fast (>12 h) for baseline anthropometric and biochemical testing.

Days 23–43 (21 days inclusive) marked phase 2 (SuK), in which participants aimed to suppress βHB to <0.3 mmol/L. To accomplish this, participants were instructed to adhere to the UK Eatwell Guideline (9), which recommends consuming a predominance of calories from carbohydrates (e.g., 55% kcal from carbohydrates on a 2,000 kcal diet is 275 g/day net carbohydrate), similar to most standard healthy eating guidelines worldwide. After the SuK phase on day 44, participants reported to the laboratory at 08:00 after an overnight fast for measurements, as previously. Days 45–65 marked phase 3, a return to NK.

All anthropometric measurements used Seca® (mBCA 514 Medical Body Composition Analyzer, Gmbh&Co. KG, 996 Hamburg, Germany). Fat oxidation (FatOx) was extrapolated from the respiratory quotient (RQ) and measured with Quark RMR 1004 (COSMED srl, Rome, Italy). All participants lay supine for 15 min before measurements were taken.

Since thyroid hormones are well-documented regulators of energy expenditure (10, 11), we also sought to analyze if they are independent predictors of LDLc throughout the study. Dietary composition was assessed from daily dietary records on days 1, 14, and 21 of each phase using Food Processor® (ESHA Research, Salem, Oregon, United States).

2.3 Statistical analysis

Data management and statistical analyses were performed using R version 2023.09.0 + 463, and all data manipulation was performed using *dplyr::*. To account for a repeated measures design, we used linear mixed-effects models with a random slope for each participant to compare the influence of different physiological variables on LDLc across the study. All models used the R function *lmerTest::lmer*.

Multidimensional predictive performance comparisons were made with *performance::compare_performance* and considered the Akaike information criterion (AIC), Bayes information criterion (BIC), root mean square error (RMSE), intraclass correlation coefficients (ICCs), and marginal R^2.

The sample size of the study was calculated based on pilot feasibility data with five participants who underwent all three phases with changes in insulin and insulin-like growth factor 1 (IGF-1) as the primary outcome. A sample size of n = 9 (n = 3 for IGF-1), was obtained using G*Power (v3.1) with an ICC = 0.5, an alpha level of 0.05, a target statistical power of 0.80, and a medium effect size of f = 0.5.

Our sensitivity analyses tested if other physiological variables, also known to influence lipid and lipoprotein metabolism [leptin, adiponectin, fasting insulin, homeostatic model for insulin resistance (HOMA-IR), fat oxidation, and saturated fat], took over the significance of BMI as an LDLc change predictor (suggestive of full mediation).

Additionally, we simulated data for every participant to test if a larger consumption of saturated fat (≥90% of total energy intake) would have changed our conclusions with the MS Excel function *randbetween* with lower and upper bounds (of 180 to 200 g/day for phases 1 and 3 and of 15 to 25 g/day for phase 2).

Finally, we tested if BMI and thyroid hormones were associated to rule out that their association with LDLc was simply due to potential collinearity.

The analysis code is available at https://github.com/AdrianSotoM/LMHRW.

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3 Results

Participants had a mean BMI of 20.5 ± 1.4 kg/m^2, were 32.3 ± 8.9 years of age, and had been on a KD for 3.9 ± 2.3 years. Adherence to each phase was confirmed with daily capillary βHB tests (NK, phase 1 = 1.9 ± 0.7; SuK, phase 2 = 0.1 ± 0.1; NK, phase 3 = 1.9 ± 0.6 mmol/L), and all 10 consistently achieved NK (βHB ≥ 0.5 mmol/L) and SuK (βHB < 0.03 mmol/L) as required by the study design. Table 1 summarizes the changes in variables of interest throughout the study.

As represented in Table 2, free triiodothyronine (fT_3) and thyroxine (T_4), but neither TSH nor reverse T_3 (rT_3), were associated with LDLc. All body composition markers—BMI, fat mass index (FMI), and fat-free mass index (FFMI), were associated with LDLc.

To build an interacting model between body composition and energy metabolism, fT_3 and BMI were chosen due to their overall superior predictive performance. Both were independently and significantly interacting predictors of LDLc. Figure 1 illustrates the multidimensional predictive performance comparisons in which the BMI:fT_3 interaction model was the best predictor of all models (patent in the largest area of its polygon).

As depicted in Figure 2, BMI remained stable throughout the study in all participants, and those with lower BMIs had higher LDLc during the first (NK) phase and larger LDLc changes across phases.

As shown in the sensitivity analyses in Table 3, BMI remained a robust and significant predictor of LDLc change despite the addition of any covariate, and no covariate remained significant after accounting for the effect of BMI. Even when tested against a saturated fat intake as high as 90% of total energy (1,800 kcal from pure saturated fat on a 2,000 kcal diet), BMI remained a significant, and virtually unchanged, predictor of LDLc change.

Finally, BMI was not significantly associated with any of the measured thyroid markers.

4 Discussion

These experimental results are consistent with prior observational data in showing that BMI is inversely related to LDLc change on a KD

TABLE 1 Anthropometric and lipid metabolism measurements throughout the study.

	Phase 1		Phase 2		Phase 3		*p*-value
	Mean	(± SD)	Mean	(± SD)	Mean	(± SD)	
BMI	20.5	1.4	21.5	1.3	20.8	1.5	0.28
Fat mass index	5.5	1.0	6.1	1.0	5.7	1.0	0.37
Fat-free mass index	15.0	1.1	15.3	1.0	15.1	1.1	0.76
Fat oxidation (%)	97.8	4.2	87.8	13.6	98.7	1.9	0.01
CHO oxidation (%)	2.2	4.2	12.2	13.6	1.3	1.9	0.01
Insulin (pmol/L)	33.6	8.6	59.8	14.7	31.6	9.4	<0.001
Glucose (mmol/L)	4.4	0.5	5.1	0.6	4.4	0.3	0.003
HOMA-IR	1.1	0.3	2.3	0.7	1.0	0.4	<0.001
Adiponectin (μg/mL)	9.1	4.2	10.8	6.8	8.7	3.3	0.62
LDLc (mg/dL)	148.0	61.2	102.5	29.4	124.1	45.8	0.12
HDLc (mg/dL)	70.1	10.4	72.7	13.6	69.8	11.8	0.83
Triglycerides (mg/dL)	66.8	28.0	66.1	21.1	79.3	45.9	0.61
fT_3 (pmol/L)	3.85	0.3	5.5	0.7	3.9	0.4	<0.001
T_4 (pmol/L)	13.5	1.6	13.2	1.5	12.7	0.7	0.34
TSH (mIU/L)	1.4	0.7	1.6	0.8	1.3	0.8	0.67
rT_3 (nmol/L)	0.3	0.1	0.3	0.1	0.3	0.1	0.64
Resting metabolic rate (kcal/day)	1,534	177	1,568	311	1,479	268	0.74
Energy intake (kcal/day)	1,834	236	1,830	232	1,757	176	0.66
CHO intake (g/day)	58.5	21.1	198.6	71.1	57.0	23.6	<0.001
Protein (g/day)	83.9	27.6	63.3	14.9	76.7	28.5	0.18
Sat fat intake (g/day)	33.7	12.4	20.9	7.1	28.9	15.1	0.07
Simulated sat fat (g/day)	196.3	3.3	19.9	3.3	194.6	3.3	<0.001

CHO, carbohydrate; sat fat, saturated fat.

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TABLE 2 Predictive performance of the models.

Model	Estimate	AIC	Marginal R^2	RMSE	*p*-value
LDLc ~ fT_3	−20.4	312.2	11%	22.9	0.003
LDLc ~ T_4	14.2	314.8	15%	29.6	0.03
LDLc ~ TSH	−5.6	320.1	0.07%	30.1	0.68
LDLc ~ rT_3	−46.7	325.5	0.07%	30.1	0.59
LDLc ~ BMI	−25.1	307.8	42%	25.0	0.001
LDLc ~ FMI	−32.5	315.2	26%	24.3	0.01
LDLc ~ FFMI	−30.1	311.3	37%	29.0	0.01
LDL ~ BMI: fT_3	BMI = −56.9 fT_3 = −191.5 BMI:fT_3 = 8.5	305.6	38%	20.2	0.005 0.03 0.04

AIC, Akaike information criterion; marginal R^2, variability explained by the fixed effects; RMSE, root mean square error.

(6). While prior data primarily report that lower BMI is associated with larger increases in LDLc upon adoption of KD, these data show the inverse is also true: those with lower BMI also exhibit larger decreases in LDLc upon carbohydrate reintroduction.

It is not surprising that all anthropometric measurements (BMI, FMI, FFMI) were significant predictors of LDLc given their expected collinearity among lean women. While BMI is an imperfect indicator of body composition, we focused on it as it is more widely available, and it yields the most conservative estimate size (β = −25) for our primary outcome and explains better the observed variability than the other anthropometric biomarkers (R^2 = 0.42 vs. 0.26 and 0.37 for FMI and FFMI, respectively).

How do anthropomorphic measurements compare in predictive power to a more widely recognized influencer of LDLc levels: saturated fat intake? As shown in our sensitivity analysis, BMI dominates over saturated for determining LDLc change, even when ≥90% of total calories were derived from saturated fat.

Consistent with what has been documented in randomized crossover trials (12), fT_3 was lower in the NK phases when compared with the SuK (higher carbohydrate) phase. Additionally, we found that fT_3 and T_4 (but not TSH or rT_3) were predictive of LDLc change, which is consistent with the role of thyroid hormones in regulating systemic lipid energy trafficking to meet energy demands during carbohydrate restriction.

While this thyroid marker pattern may appear reminiscent of a "sick euthyroid syndrome," a condition in which patients with critical illness demonstrate changes in peripheral thyroid hormone levels (13), we caution against this interpretation for the following reasons. i) We studied healthy participants. ii) As shown in Table 1, energy intake, weight, resting metabolic rate, TSH, and rT_3 remained unchanged across throughout the study. iii) It is not yet clear how macronutrient shifts affect thyroid hormone sensitivity, and akin to what is observed in insulin or leptin resistance, the levels of a hormone may be inversely associated with its sensitivity.

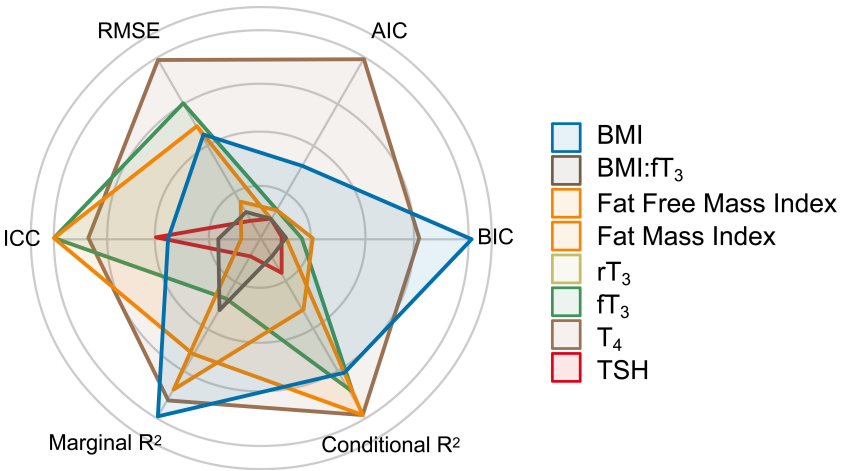


FIGURE 1
Multidimensional predictive performance comparison. AIC, Akaike information criterion; BIC, Bayes information criterion; conditional R^2, explained variability by both fixed and random effects; marginal R^2, explained variability by fixed effects; ICC, intraclass correlation coefficient; RMSE, root mean square error.

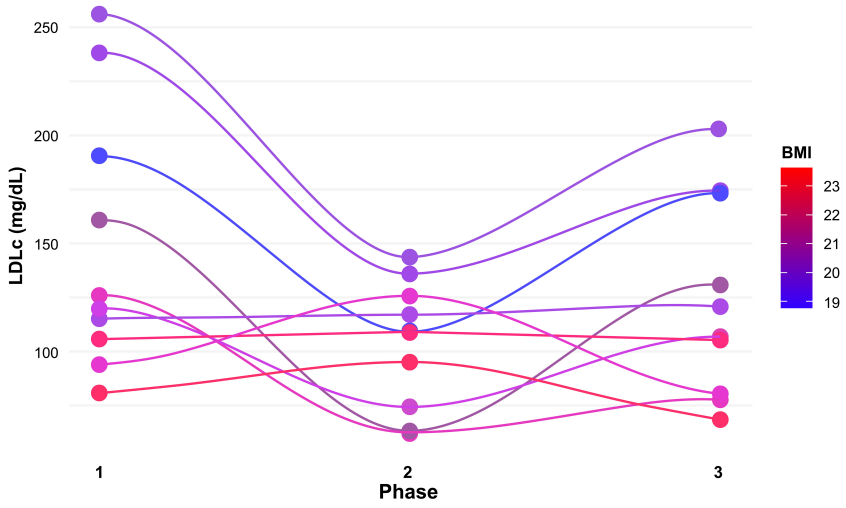


FIGURE 2
LDL-cholesterol change by phase and BMI.

A chief strength of this study included the rigor with which participants complied with the dietary regimen, as evidenced by all participants achieving consistent NK during the NK phases and consistent suppression of ketosis during the SuK phase, determined by blood capillary testing combined with dietary records. Additionally, all participants had a BMI ≤24 kg/m^2 and, therefore, were "lean" and remained weight-stable, increasing the internal validity of this study.

Limitations include that our sample size was not calculated with LDLc changes as the primary outcome. However, it is unlikely that the negative results in our sensitivity analyses are due to low statistical power, as none had borderline statistical significance. Another limitation is the relatively short duration of each phase, as noticeable from the fact that 21 days was not sufficient for participants to return to baseline (elevated) LDLc on a KD. Of note, it has been reported that some physiological adaptations to macronutrient shifting may persist after 3 months (14). Nonetheless, since all participants followed a KD for ≥6 months before enrolment, it is likely that a longer follow-up would only have caused phase 3 to mirror phase 1 more closely and have strengthened the main findings in this work. Future studies will investigate the potential role of other potentially relevant hormonal modulators in the LEM.

TABLE 3 Sensitivity analyses.

Model	Estimate	*p*-value
LDLc ~ BMI + adiponectin	BMI: −26.0 Adiponectin -1.9	0.001 0.26
LDLc ~ BMI + insulin	BMI: −22.1 Insulin: 0.4	0.02 0.40
LDLc ~ BMI + leptin	BMI: −33.7 Leptin: −0.2	0.01 0.84
LDLc ~ BMI + HOMA	BMI: −21.0 HOMA: −11.5	0.01 0.21
LDLc ~ BMI + ≥90% energy from saturated fat	BMI: −20.5 Sat fat: 0.09	0.02 0.23
fT_3 ~ BMI	0.15	0.21
FT_4 ~ BMI	−0.06	0.76
TSH ~ BMI	0.19	0.12
rT_3 ~ BMI	0.003	0.84

4.1 Conclusions

The amplitude of LDLc changes (both increases with KD adoption and decreases with carbohydrate reintroduction) is larger when BMI is lower. Additionally, fT_3 is an independent predictor of LDLc changes, separate from BMI. These findings are consistent with and build upon the lipid energy model and provide useful insight for lean people considering adopting carbohydrate-restricted ketogenic diets.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the College of Liberal Arts and Sciences Research Ethics Committee, University of Westminster, United Kingdom (ETH2122-0634). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

IC: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. CS-P: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. NN: Conceptualization, Formal analysis, Investigation, Methodology, Supervision, Visualization, Writing – original draft, Writing – review & editing. DF: Conceptualization, Investigation, Methodology, Supervision, Visualization, Writing – review & editing. YK: Investigation, Methodology, Project administration, Writing – review & editing. KE: Investigation, Methodology, Project administration, Writing – review & editing. LP: Investigation, Methodology, Project administration, Writing – review & editing. BE: Conceptualization, Project administration, Supervision, Writing – review & editing. AS-M: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

NN is co-author of a low-carbohydrate-diet cookbook; he donates all royalty payments to nutrition research and education. DF receives financial contributions from membership e.g., through Patreon for continued research and is a partner in Own Your Labs LLC, with all proceeds contributed to the Citizen Science Foundation.

The remaining 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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EDITED BY
Eduardo Hertel Ribeiro,
Escola Superior de Ciências da Santa Casa de Misericórdia de Vitória (EMESCAM), Brazil

REVIEWED BY
Zoran Gluvic,
University of Belgrade, Serbia
Chunheng Mo,
Sichuan University, China

*CORRESPONDENCE
Youdong Yang
708924865@qq.com

†These authors have contributed equally to this work and share first authorship

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A combined analysis of TyG index, SII index, and SIRI index: positive association with CHD risk and coronary atherosclerosis severity in patients with NAFLD

Wenyuan Dong[1,2†], Yuxin Gong[2,3†], Jianqi Zhao[4†], Yanan Wang[1], Bao Li[2] and Youdong Yang[1*]

[1]Vasculocardiology Department, The Third People's Hospital of Datong, Datong, Shanxi, China, [2]Key Laboratory of Cardiovascular Diseases, Second Hospital of Shanxi Medical University, Taiyuan, Shanxi, China, [3]Department of Pathology, Tongji Hospital Affiliated to Tongji Medical College Hust, Wuhan, Hubei, China, [4]Vasculocardiology Department, The First People's Hospital of Jinzhong, Jinzhong, Shanxi, China

Background: Insulin resistance(IR) and inflammation have been regarded as common potential mechanisms in coronary heart disease (CHD) and non-alcoholic fatty liver disease (NAFLD). Triglyceride-glucose (TyG) index is a novel biomarker of insulin resistance, System immune-inflammation index(SII) and Systemic inflammation response index(SIRI) are novel biomarkers of inflammation, these biomarkers have not been studied in CHD with NAFLD patients. This study investigated the correlation between the TyG index, SII index, and SIRI index and CHD risk among NAFLD patients.

Methods: This cross-sectional study included 407 patients with NAFLD in the Department of Cardiology, The Second Hospital of Shanxi Medical University. Of these, 250 patients with CHD were enrolled in the NAFLD+CHD group and 157 patients without CHD were enrolled as NAFLD control. To balance covariates between groups, 144 patients were selected from each group in a 1:1 ratio based on propensity score matching (PSM). Potential influences were screened using Lasso regression analysis. Univariate and multivariate logistic regression analyses and the Least Absolute Shrinkage and Selection Operator (LASSO) regression were used to assess independent risk and protective factors for CHD. Construction of nomogram using independent risk factors screened by machine learning. The receiver operating characteristic(ROC) curve was used to assess the ability of these independent risk factors to predict coronary heart disease. The relationship between the Gensini score and independent risk factors was reflected using the Sankey diagram.

Results: The LASSO logistic regression analysis and Logistic regression analyses suggest that TyG index (OR, 2.193; 95% CI, 1.242-3.873; P = 0.007), SII index (OR, 1.002; 95% CI, 1.001-29 1.003; P <0.001), and SIRI index (OR,1.483;95%CI,1.058-2.079,P=0.022) are independent risk factors for CHD. At the same time, Neutrophils, TG, and LDL-C were also found to be independent risk factors in patients, HDL-C was a protective factor for CHD in patients with NAFLD. Further analysis using three machine learning algorithms found these independent risk factors to have good predictive value for disease diagnosis, SII index shows the

highest predictive value. ROC curve analysis demonstrated that combining the SII index, SIRI index, and TyG index can improve the diagnostic ability of non-alcoholic liver cirrhosis patients with CHD.ROC curve analysis showed that the combined analysis of these independent risk factors improved the predictive value of CHD(AUC: 0.751; 95% CI: 0.704-0.798; P <0.001).

Conclusion: TyG index, SII index, and SIRI index are all independent risk factors for CHD in patients with NAFLD and are strongly associated with prediction and the severity of CHD.

KEYWORDS

triglyceride-glucose index, systemic immune-inflammation index, systemic inflammation response index, non-alcoholic fatty liver diseases, coronary heart disease

1 Introduction

Coronary heart disease (CHD) is one of the leading causes of death and disability worldwide, with a common cause being atherosclerosis (1). The formation and development of atherosclerotic plaques is the most important cause of CHD. Traditional risk factors include hypertension, dyslipidemia, smoking, diabetes, genetic predisposition and obesity (2). Recent studies have shown that Insulin resistance and inflammatory responses are also is involved in atherosclerotic plaque formation and remodeling independent of traditional risk factors (3). Despite significant progress in the treatment of CHD, further research is needed on early diagnosis and prevention. As an emerging field, increasing attention is being focused on the connection between IR, systemic inflammation, and the occurrence and development of CHD (4).

Non-alcoholic fatty liver disease (NAFLD) is one of the most common chronic diseases in the world (5). NAFLD has the potential to progress to more serious liver damage such as non-alcoholic steatohepatitis (NASH) (6).NASH is characterized by inflammatory changes that can lead to progressive liver damage, cirrhosis, and hepatocellular carcinoma (HCC) (7). Meanwhile, NAFLD is also a major risk factor for cardiovascular disease. An increasing number of studies have shown that NAFLD and CHD share common risk factors. Recent studies have shown that in addition to obesity, hypertension, dyslipidemia, diabetes, and genetic predisposition, Insulin resistance and inflammatory responses also play an important role in cardiovascular events in patients with NAFLD (8). Triglyceride-glucose index (TyG index) is a reliable indicator for the diagnosis of IR because of it performs better than the high insulin-glucose clamp test (9). SII index and SIRI index are two novel indicators of inflammation and immunity, that have been proven to be associated with the risks for CVDs and all-cause mortality (10), SII index was non-linear associated with all-cause mortality in individuals with NAFLD (11). A population-based study imply that the SII index and TyG index are related to NASH (12). however, the relationship between the SIRI index and CHD in NAFLD patients remains unclear. This study aims to analyze the potential associations between the TyG index, SII index, and SIRI index, and the occurrence as well as the severity of CHD in NAFLD patients.

Abbreviations: NAFLD, non-alcoholic fatty liver disease; CHD, coronary heart disease; SII index, system immune-inflammation index; SIRI index, systemic inflammation response index; TyG index, Triglyceride-glucose index; HT, hypertension; DM, Diabetes mellitus; HDL-C, High density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol; L, lymphocytes; N, Neutrophils; M, Monocytes; Plt, Platelet; Cr, Creatinine; TC, Total cholesterol; TG, Triglyceride; Glu, Bloodglucose.

2 Materials and methods

2.1 Experimental design and participant inclusion

This study is a retrospective study that included a total of 407 patients with NAFLD. These patients were divided into two groups, the non-alcoholic fatty liver disease group (NAFLD group) and the non-alcoholic fatty liver disease combined with coronary heart disease group (NAFLD+CHD group), coronary angiography was done at the Second Hospital of Shanxi Medical University. For patients with CHD, the Gensini score was further used to evaluate the severity of coronary stenosis (13). The diagnosis of NAFLD is based on the Asia-Pacific Working Party recommendations (14). Firstly, other factors that can cause fatty liver, such as excessive alcohol consumption, a history of viral hepatitis, and the use of hepatotoxic drugs, are excluded. Ultrasound technicians evaluate using standard methods (15).However, we cannot yet further confirm the presence of NASH patients among NAFLD patients because of the lack of liver biopsy results in the study. Additionally, this study excluded other potential confounding factors including

congenital heart disease, a history of coronary heart disease, oral lipid-lowering medications, autoimmune diseases, malignancies, the use of anti-platelet drugs, quality of diabetes regulation, and patients with cerebrovascular accidents. This study has been approved by the Ethics Committee of Shanxi Medical University Second Hospital and all participating patients have signed informed consent forms.

2.2 Data collection and computation

This study retrospectively collected the basic information and laboratory indicators of enrolled patients from the electronic medical record system of the Second Hospital of Shanxi Medical University. The basic information includes gender, age, height, weight, history of hypertension (systolic and diastolic blood pressure), history of diabetes, coronary angiography report, smoking history, alcohol consumption history, and medication use. The body mass index (BMI), Triglyceride glucose product index (TyG), Systemic immune-inflammation index (SII), and systemic inflammation response index (SIRI) are calculated as follows: BMI = W(kg)/H(m)2, TyG = Ln[TG(mg/dL)×FBG(mg/dL)/2] (16); SII = (Plt×N/L) (17); SIRI = (N×M/L) (18).

2.3 Machine learning

This study used four machine learning methods to select variables and assess their importance in diagnosis and prognosis. Lasso regression analysis was used to address confounding factors that could potentially affect the results. Based on the potential influencing factors, the random forest model (RF), support vector machine model (SVM), and generalized linear model (GLM) were constructed using the ‘ caret ‘ R software package (version 6.0-86). Seven potential influences were used as explanatory variables and CHD as a response variable (19). The three models obtained were analyzed using the “explain” function in the’DALEX’ R package, and the cumulative residual distributions were plotted. The cumulative residual distribution was plotted to determine the best predictive model.RF, GLM, and SVM were further utilized to analyze the significance of various risk factors in disease occurrence. All these procedures were conducted using R (version 4.3.1).

2.4 Propensity score matching analysis

Propensity score matching analysis (PSM) is a recognized method for eliminating the influence of potential confounding factors, and after PSM adjustment, data can be considered free from interference by these confounding factors. In non-alcoholic fatty liver disease patients, this study used Lasso regression to obtain confounding factors (including BMI, lymphocytes, monocytes, serum creatinine, and total cholesterol) as independent variables to establish a model with the occurrence of CHD as the dependent variable. The caliper value of the model was set at 0.02. After adjusting for these confounding factors, the validity of PSM was assessed using quartiles and absolute standard deviations. The results showed a good balance between the CHD+NAFLD and NAFLD groups after performing 1:1 PSM matching.

2.5 Statistical analysis

Statistical analysis software IBM SPSS (version 23.0) and GraphPad Prism (version 8.0) were used to analyze the data in this study. Continuous variables were described using mean ± standard deviation (SD) or median (IQR), while categorical variables were expressed as percentages. The Shapiro-Wilk test was employed to assess the normality of continuous variables, and the independent sample t-test or Mann-Whitney test was used for between-group comparisons of continuous variables. Between-group comparisons of categorical variables were conducted using the chi-square test or Fisher’s exact test.

2.6 Receiver operating characteristic

First, Lasso regression analysis was used to screen confounding factors, followed by Propensity Score Matching (PSM) to correct for these confounders. Logistic regression analysis was then performed using the corrected data to further identify potential risk factors for CHD in NAFLD patients. Multivariate analysis was conducted to determine independent factors that can influence the occurrence of the disease. A nomogram was constructed using the selected risk factors, and receiver operating characteristic (ROC) curve analysis and area under the curve (AUC) values were used to evaluate their predictive ability for the disease (20). Additionally, Spearman correlation analysis was employed to assess the relationship between Gensini scores and several independent risk factors.

3 Results

3.1 Baseline characteristics of the NAFLD and NAFLD+CHD groups

This study collected a total of 407 patients with NAFLD, among whom 250 patients had CHD. Among these patients, the average age was 59 years for those with NAFLD-CHD and 55 years for those with NAFLD alone. As shown in Table 1, there were no significant differences in BMI, platelet count(Plt), lymphocyte count(L), and total cholesterol levels(TC) between the two groups. However, the proportion of male patients, with hypertension, diabetes mellitus, and smokers was higher in the NAFLD+CHD group ($p<0.05$). Additionally, neutrophil count(N), triglyceride level(TG), low-density lipoprotein level(LDL-C) and fasting blood glucose(Glu) level were higher in the NAFLD+CHD group compared to the NAFLD group ($p<0.05$), while lymphocyte count and high-density lipoprotein level were lower in the NAFLD+CHD group compared to the NAFLD group ($p<0.05$).

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TABLE 1 Demographic and clinical characteristics of participants by the presence of CHD.

Characteristics	CHD +NAFLD (n=250)	NAFLD (n=157)	P
Age (years)	59 (51, 67)	55 (48, 62)	< 0.001
Sex (male)	176 (43.2%)	81 (19.9%)	< 0.001
BMI (kg/m^2)	25.96 (24.77, 27.775)	25.82 (24.16, 27.72)	0.429
HT [n (%)]	169 (41.5%)	73 (17.9%)	< 0.001
DM [n (%)]	96 (23.6%)	19 (4.7%)	< 0.001
Smoke [n (%)]	134 (32.9%)	49 (12%)	< 0.001
Plt (10^9/L)	216.5 (177.25, 258.25)	225 (195, 258)	0.117
N (10^9/L)	4.335 (3.4325, 5.9375)	3.48 (2.73, 4.61)	< 0.001
L (10^9/L)	1.84 (1.4525, 2.33)	1.94 (1.58, 2.37)	0.105
M (10^9/L)	0.475 (0.37, 0.6175)	0.47 (0.34, 0.54)	0.048
Cr (mmol/L)	67 (58.25, 76)	65 (56, 74)	0.027
TC (mmol/L)	4.4689 ± 1.1113	4.3691 ± 0.98557	0.358
TG (mmol/L)	1.805 (1.335, 2.6)	1.61 (1.19, 2.2)	0.001
HDL-C (mmol/L)	1.05 (0.91, 1.23)	1.17 (1, 1.34)	< 0.001
LDL-C (mmol/L)	2.485 (2.075, 2.975)	2.27 (1.78, 2.71)	< 0.001
Glu (mmol/L)	5.86 (4.9925, 7.2375)	5.3 (4.94, 5.77)	< 0.001
TyG	9.1 (8.76, 9.5275)	8.84 (8.53, 9.16)	< 0.001
SII	519.44 (356.27, 770.47)	410.04 (304.5, 558.74)	< 0.001
SIRI	1.1191 (0.71674, 1.7797)	0.81013 (0.51493, 1.1947)	< 0.001

All the above data are expressed as mean ± standard deviation or median (quartiles). The Chi-square test was used for categorical variables.Paired and unpaired t-tests were used for comparison of normal values, and Wilcoxon rank sum test or Mann-Whitney test was used for comparison of non-normally distributed values. p<0.05 was considered statistically significant.

BMI, body mass index; HT, hypertension; DM, diabetes mellitus; Plt, platelet; N, neutrophil; L, lymphocyte; M, monocyte; Cr serum, creatinine; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Glu, fasting plasma glucose; TyG, triglyceride-glucose index; SII, immune-Inflammation Index; SIRI, systemic inflammation response index.

3.2 Lasso regression screening for risk factors

Lasso regression analysis was used to identify potential risk factors for CHD from the aforementioned 16 variables. The results, as shown in Figures 1, 2, revealed that BMI, platelet count, lymphocytes, monocytes, creatinine, total cholesterol, and fasting random blood glucose were excluded from consideration as these seven indicators did not significantly impact CHD. The remaining nine indicators were considered potential risk factors for CHD (Supplementary Table 1).

3.3 Propensity score matching analysis

Propensity score matching analyses were then performed on the uncorrelated factors identified by the Lasso regression analyses to eliminate any potential effect of these confounding variables on the results. The results, as shown in Table 2, revealed that after propensity score matching, there were no statistically significant differences (p>0.05) between groups in terms of BMI, platelet count, lymphocyte count, monocyte count, creatinine level, total cholesterol level, and fasting random blood glucose. Additionally, after propensity score matching, there were also no statistically significant differences (p>0.05) between groups in terms of gender and smoking history.

3.4 Univariate and multivariate analysis of CHD-related factors in NAFLD

Univariate logistic regression analysis showed significant correlations between neutrophils, TG, HDL-C, LDL-C, TyG index, SII index, and SIRI index with the occurrence of CHD in NAFLD patients. Furthermore, multivariate logistic regression analysis revealed that neutrophils (OR 1.363; 95% CI 1.152 - 1.611; *P* <0.001), TG (OR 1.512; 95% CI 1.142 - 2.003; *P* =0.004), LDL-C (OR 1.601; 95% CI 1.114 -2.302; *P* =0.011), TyG index (OR,2.193;95% CI,1242-3.873;*P*=0.007),SII index(OR,1002;95%CI,10011-1003; *P*<0.001)and SIRI index(OR,1483;95%CI,1058-2079;*P*=002) remained are independent risk factors for NAFLD patients developing CHD while HDL-C (OR,0260;95%CI,0089-0762;p=014) was still an independent protective factor where elevated levels of HDL-C reduce risk of CHD in NAFLD patients (Table 3; Figure 3).

3.5 Machine learning models evaluate biomarkers

To further validate the accuracy of these risk factors in predicting the occurrence of CHD in NAFLD patients, this study used three machine learning algorithms to evaluate the importance of seven predictive factors in disease diagnosis. We chose Random Forest (RF), Support Vector Machine (SVM), and Generalize linear model(GLM) as our test models (21). We used the DALEX package to explore, interpret, and evaluate the models. The optimal model is selected for the next analysis based on the residual values. As shown in Figure 4A, the boxplot represents the accuracy of CHD prediction by the three machine.

learning models, with the GLM model having the smallest residual and thus indicating the highest accuracy. Figure 4B illustrates the importance of CHD prediction by the three machine learning models, while Figure 4C displays the importance of each factor in CHD prediction within the GLM model. The results demonstrate that all seven influencing factors have significant value in predicting CHD, with SII index, LDL-C, neutrophils(N), HDL-C, SIRI index, TyG index, and TG being sequentially ranked according to their predictive importance.

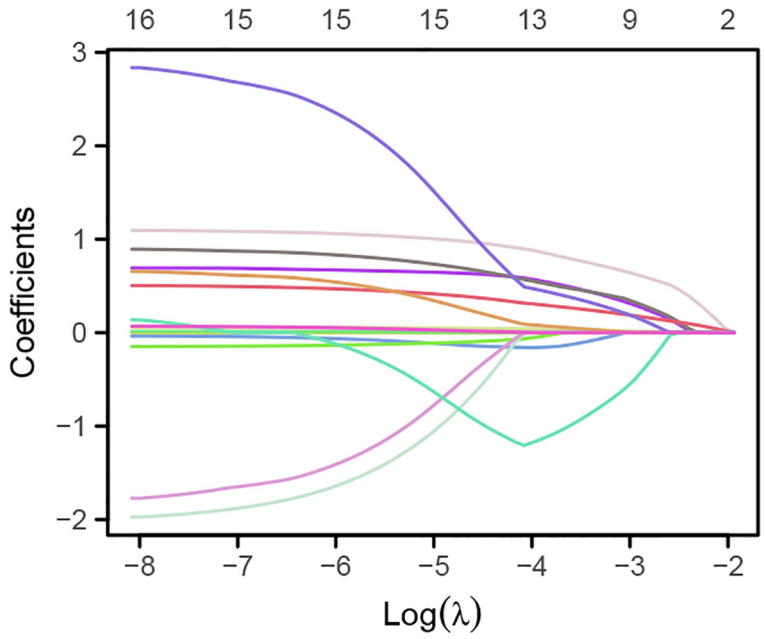


FIGURE 1
Variation of the 16 variables with the regularization parameter λ.

3.6 Establishing a diagnostic nomogram for CHD in patients with NAFLD

Through univariate and multivariate logistic regression analysis, as well as validation using three machine learning models, this study discovered that seven factors have significant potential for diagnosing and predicting CHD. Therefore, this study utilized these seven factors to construct a clinical nomogram model.

(Figure 5A). The calibration curve in Figure 5B demonstrates that the actual risk aligns closely with the predicted risk after bias correction, indicating a high level of consistency between them. Additionally, in the diagnostic PR curve, all factors except LDL-C

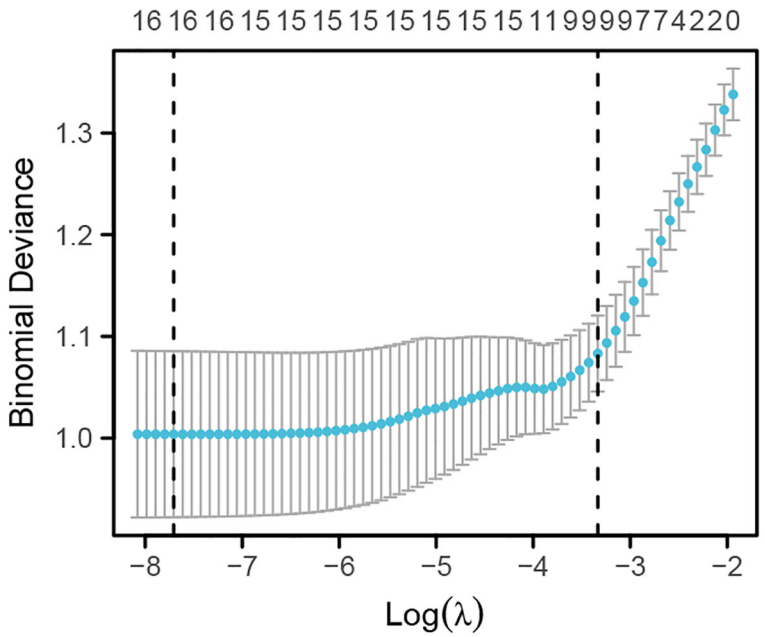


FIGURE 2
Lasso regression analysis of the optimal regularization parameter λ.

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TABLE 2 Demographic and clinical characteristics of participants after PSM analysis.

Characteristics	CHD +NAFLD (n=144)	NAFLD (n=144)	P
BMI (kg/m^2)	26 (24.22, 27.76)	25.995 (24.385, 28.04)	0.850
Plt (10^9/L)	223.5 (183.75, 268)	224 (188.75, 258)	0.887
L (10^9/L)	1.885 (1.4775, 2.33)	1.935 (1.595, 2.3625)	0.355
M (10^9/L)	0.43 (0.35, 0.5325)	0.475 (0.3675, 0.5525)	0.286
Cr (mmol/L)	64 (54, 72)	66 (56, 75)	0.449
TC (mmol/L)	4.3429 ± 1.0494	4.369 ± 0.98124	0.828
Glu (mmol/L)	5.345 (4.86, 6.2825)	5.365 (4.95, 5.8325)	0.626
Sex (male)	90 (31.2%)	79 (27.4%)	0.188
Age (years)	59 (51, 66)	54.5 (48, 62)	0.006
HT [n (%)]	93 (32.3%)	66 (22.9%)	0.001
DM [n (%)]	33 (11.5%)	17 (5.9%)	0.013
Smoke, n (%)	64 (22.2%)	48 (16.7%)	0.053
N (10^9/L)	4.085 (3.2675, 5.1725)	3.54 (2.7275, 4.78)	< 0.001
TG (mmol/L)	1.74 (1.3175, 2.3825)	1.595 (1.17, 2.2)	0.015
HDL-C (mmol/L)	1.1072 ± 0.23081	1.1859 ± 0.26976	0.008
LDL-C (mmol/L)	2.41 (2.0075, 2.8925)	2.265 (1.78, 2.715)	0.030
TyG	8.95 (8.67, 9.27)	8.835 (8.53, 9.155)	0.012
SII	502.45 (326.84, 680.06)	417.51 (316.18, 560.56)	0.006
SIRI	0.93323 (0.63843, 1.4049)	0.8698 (0.56531, 1.2157)	0.045

All the above data are expressed as mean ± standard deviation or median (quartiles). The Chi-square test was used for categorical variables.Paired and unpaired t-tests were used for comparison of normal values, and Wilcoxon rank sum test or Mann-Whitney test was used for comparison of non-normally distributed values. p<0.05 was considered statistically significant.

BMI, body mass index; HT, hypertension; DM, diabetes mellitus; Plt, platelet; N, neutrophil; L, lymphocyte; M, monocyte; Cr serum, creatinine; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Glu, fasting plasma glucose; TyG, triglyceride-glucose index; SII, immune-Inflammation Index; SIRI, systemic inflammation response index.

exhibit higher predictive ability than the baseline prediction line, indicating a high level of accuracy in these predictions (Figure 5C). As shown in Figure 5D, the predictive curves for all factors are mostly above both the ALL curve and None curve, suggesting a substantial net benefit for patients.

3.7 ROC analysis predicts factors

This study evaluated the accuracy of various predictive factors in predicting the occurrence of CHD in NAFLD patients using ROC curves (Figure 6A). The results showed that N (AUC = 0.679), TG (AUC = 0.595), HDL-C (AUC = 0.616), LDL-C (AUC = 0.598), TyG (AUC = 0.652), SII (AUC = 0.631), and SIRI (AUC = 0.656) had different levels of accuracy in prediction. Furthermore, this study assessed the diagnostic accuracy of all factors combined (Figure 6B) and found a significant improvement in predictive accuracy when using a combination of these seven predictive factors, with an AUC: 0.751 (CI: 0.704-0.798; P<0.001).

3.8 Association between predictors and coronary Gensini score

This study used Spearman's correlation analysis to examine the relationship between Gensini score and neutrophils, TG, HDL-C, LDL-C, TyG, SII, and SIRI. The results showed no significant correlation between the Gensini score and LDL-C (r=0.082, P=0.121).The Gensini score was weakly correlated with neutrophils (r=0.19, P<0.001), TG (r=0.329, P<0.001), HDL-C (r=-0.203, P<0.001), SII (r=0.132, P=0.004), and SIRI (r=0.184, P= 0.025) but strongly correlated with TyG (r= 0.435, P <0001) (Figure 7).

4 Discussion

NAFLD is an inflammation-associated metabolic disease, with steatosis as its most prominent feature. NAFLD is a continuum of disease with or without mild inflammation (non-alcoholic fatty liver) to non-alcoholic steatohepatitis (NASH) (5). NASH is characterised by a more rapid progression of necroinflammation and fibrosis than non-alcoholic fatty liver. NAFLD patients included in this study include non-alcoholic fatty liver and non-alcoholic steatohepatitis. Cardiovascular disease is one of the leading causes of death in NAFLD patients, and NAFLD patients are more likely to develop atherosclerosis, cardiovascular disease, and arrhythmias (22, 23). CHD is prevalent in patients with NAFLD, NAFLD is considered a high-risk group for CHD (8). NAFLD and CHD share many common pathogenic mechanisms, Many studies have suggested that IR and inflammation are underlying mechanisms common to both NAFLD and CHD.

IR is a systemic disease affecting multiple organs throughout the body, which makes it play an important role in the onset and progression of NAFLD and CHD (24). The cardiometabolic syndrome(CMS) is typically characterised by IR, IR may have an effect on cardiovascular disease through decreases in nitric oxide (NO) and increases in vascular stiffness (25). At the same time, IR is closely related to NAFLD, and IR is involved in various liver diseases such as steatosis, NASH and liver fibrosis (26). Patients with NAFLD have impaired liver function, resulting in an inability to suppress the production of glucose and fatty acids, Localised lipid and glucose accumulation leads to the onset and development of IR, IR leads to systemic disorders of glucose metabolism and lipid metabolism, ultimately leading to damage to the vascular endothelium (27), This explains why NAFLD is a high risk group for CHD. The TyG index is a reliable indicator of IR,A clinical study

TABLE 3 Univariate and multivariate analyses of factors associated with CHD and NAFLD.

Characteristics	Total (n)	Univariate analysis		Multivariate analysis (Model I)		Multivariate analysis (Model II)	
		Odds Ratio (95% CI)	P value	Odds Ratio (95% CI)	P value	Odds Ratio (95% CI)	P value
N	288	1.339 (1.139 - 1.575)	< 0.001	1.413 (1.196 - 1.670)	< 0.001	1.363 (1.152 - 1.611)	< 0.001
TG (mmol/L)	288	1.414 (1.091 - 1.832)	0.009	1.518 (1.156 - 1.994)	0.003	1.512 (1.142 - 2.003)	0.004
HDL-C (mmol/L)	288	0.283 (0.109 - 0.733)	0.009	0.185 (0.067 - 0.510)	0.001	0.260 (0.089 - 0.762)	0.014
LDL-C (mmol/L)	288	1.431 (1.013 - 2.023)	0.042	1.460 (1.029 - 2.070)	0.034	1.601 (1.114 - 2.302)	0.011
TyG	288	2.111 (1.257 - 3.546)	0.005	2.226 (1.313 - 3.775)	0.003	2.193 (1.242 - 3.873)	0.007
SII	288	1.002 (1.001 - 1.003)	< 0.001	1.002 (1.001 - 1.003)	< 0.001	1.002 (1.001 - 1.003)	< 0.001
SIRI	288	1.527 (1.092 - 2.137)	0.013	1.602 (1.137 - 2.257)	0.007	1.483 (1.058 - 2.079)	0.022

Model I was adjusted for age and sex. Model II was adjusted for age, sex,hypertension, diabetes mellitus and smoking history.

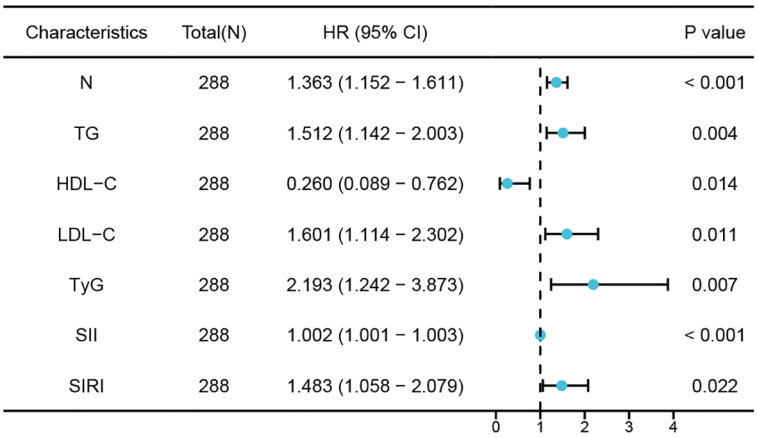


FIGURE 3
Forest plots of independent factors associated with CHD in NAFLD.

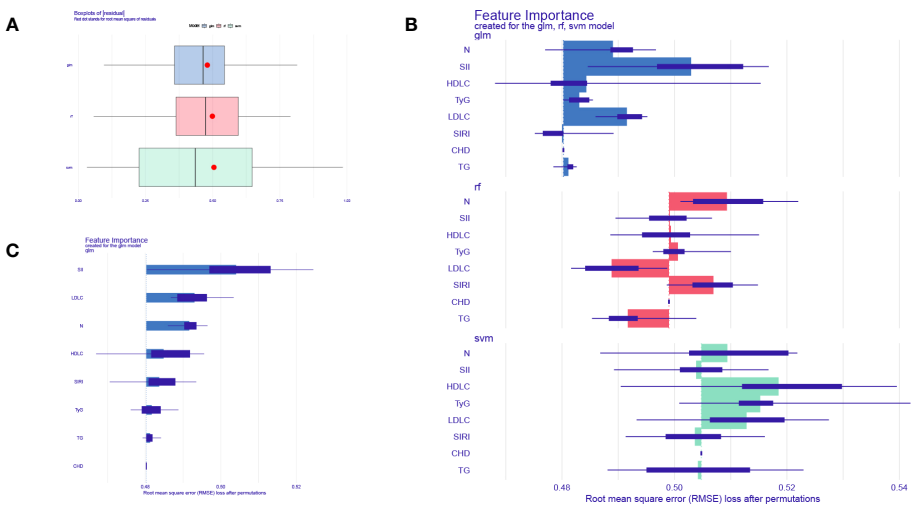


FIGURE 4
Machine learning models evaluate biomarkers. **(A)** Boxplots of the three machine learning models. **(B)** Three machine learning models to assess the importance of seven factors for CHD. **(C)** GLM model showing the importance of 7 factors for CHD prediction.

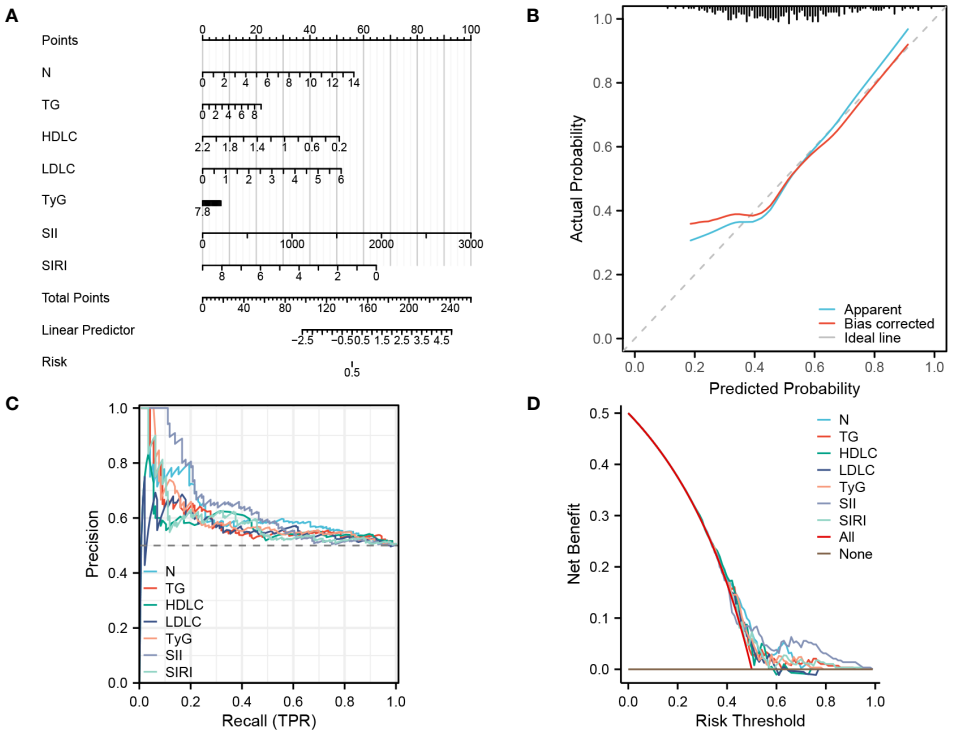


FIGURE 5
Establishing a diagnostic nomogram for CHD in patients with NAFLD **(A)** nomogram for the diagnosis of CHD. **(B)** Calibration curve for prediction accuracy. **(C)**, PR curve for diagnosis of CHD. **(D)**, DCA curve for the diagnosis of CHD.

enrolling 62,443 Chinese individuals suggests that the TyG index is an independent risk factor for cardiovascular disease and may help identify individuals potentially at high cardiovascular risk (28). Another clinical study, which lasted follow-up 10 years and included 6,095 CVD patients with undiagnosed diabetes mellitus, demonstrated that the TyG index can be used as a clinical.

predictor of CVD and CHD in the non-diabetic population (29). This suggests that TyG index can still be an independent risk factor for CHD in low-risk populations. However, there are still fewer studies on the TyG index in populations at high risk of CHD. TyG index also has good diagnostic value in NAFL,A cross-sectional study including 10,761 patients suggests that the TyG index is effective in identifying people at risk of NAFLD (30). Wang et al. showed that TyG index was independently and positively associated with NAFLD in the general population (31). And another cohort study showed that TyG index showed good diagnostic performance for NASH in obese patients. Thus, TyG index is an independent risk factor for both NAFLD and CHD.

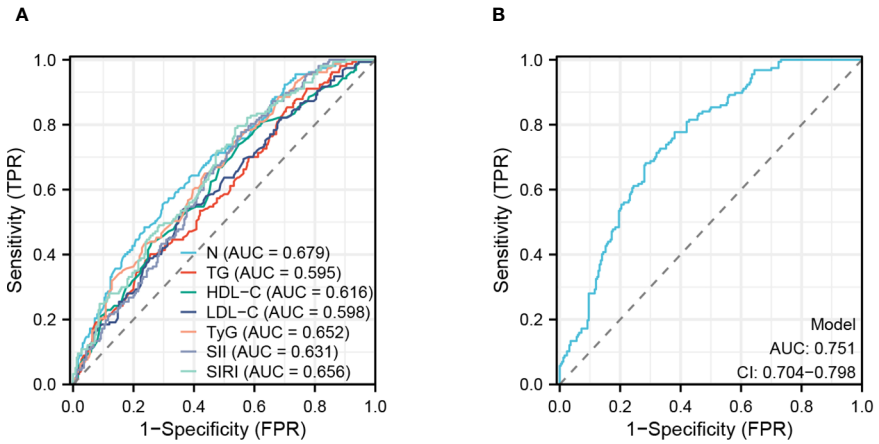


FIGURE 6
ROC analyses of risk factor. **(A)** Analysis of subject work characteristics (ROC) curves for the predictive ability of each risk factor for CHD. **(B)** Overall prediction curves for the combined individual factors.

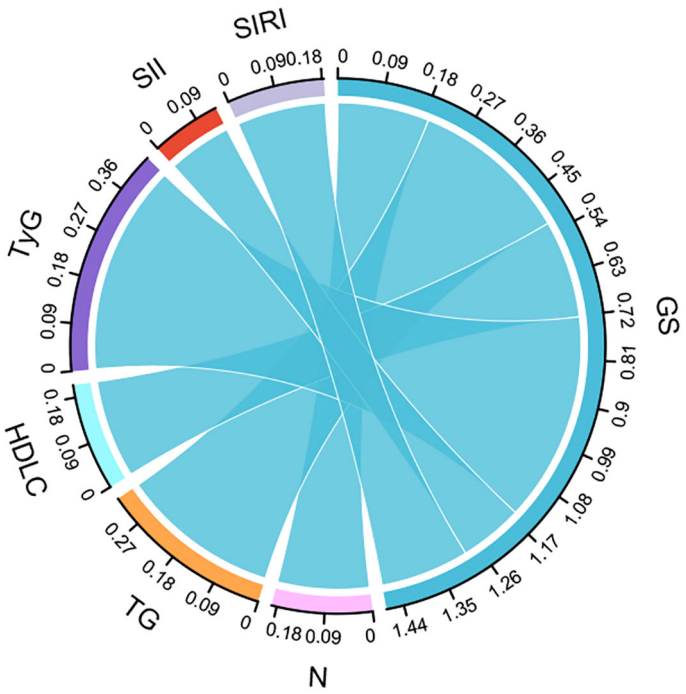


FIGURE 7
Sankey diagram reflecting the correlation between factors and CHD severity.

Systemic inflammation plays an important role in CHD and NAFLD development. Systemic inflammation is directly related to the development of atherosclerosis, it is well known that coronary atherosclerosis is the main cause of CHD, and immune cells and systemic inflammation play an important role in the accumulation of lipids in the matrix of intima of coronary arteries (2). Some studies have shown that patients with systemic inflammatory diseases have a much higher risk of CVD than the general population (32). Similarly, systemic inflammation can lead to IR, and promotes the development of NAFLD. By analysing 47 high-quality serum samples from patients with NAFLD, Haukeland et al. found that mild systemic inflammation was prevalent in patients with NAFLD and that CCL2 (chemokine (C-C motif) ligand 2) and MCP1 (monocyte chemoattractant protein) may play a key role in the disease (33). Lipids accumulation in hepatocytes leads to local inflammation and immune cell activation. On the one hand, activated immune cells cause systemic inflammation by releasing pro-inflammatory cytokines and chemokines (34). On the other hand, activation and recruitment of hepatic immune cells via signals from adipose tissue may promote inflammatory responses leading to cell injury and death, thereby promoting NAFLD disease progression (35). In summary, NAFLD can promote CHD through IR and systemic inflammation. NAFLD is not only associated with morbidity and mortality from liver disease, but is also an important risk factor for the development and progression of CHD. SII index and SIRI index are two novel systemic inflammatory biomarkers, A 20-Year Follow-Up Cohort Study of 42,875 US Adults found that high SII or SIRI increased adverse cardiovascular outcomes in the general population (36). Another cohort study has also shown that the SII index can be a useful marker for predicting the development of cardiovascular disease in middle-aged and older adults (37). Clinical studies have shown that inflammation is associated with the progression of NAFLD, A cross-sectional study of 6,792 adult Chinese on fatty liver showed a significant positive correlation between SII index and hepatic steatosis (17), NAFLD is an inflammation-related metabolic disease, and hepatic steatosis is the most prominent manifestation of the disease. Given that both IR and systemic inflammation lead to an increased risk of NAFLD with CVD and that NAFLD is a high-risk factor for CHD, both the TyG index, SII index, and SIRI index can predict NAFLD and CVD. There are no studies that have simultaneously explored the effects of IR and systemic inflammation on CHD in patients with NAFLD. Therefore, this work aims to explore this association.

Our findings suggest that TyG index, SII index, and SIRI index are all independent risk factors for CHD in patients with NAFLD. Neutrophils, TG, and LDL-C were also screened as independent risk factors for CHD by using three machine learning algorithms, while HDL-C was an independent protective factor for CHD, Of these, TG, LDL-C and HDL-C are all traditional independent risk factors for CHD (38), Interestingly, Neutrophil thought to cause CHD in NAFLD patients, There is still a lack of clarity regarding the role of neutrophil in NAFLD and CHD, Emerging evidence suggests that neutrophils are important players in several chronic diseases, such as atherosclerosis, nonalcoholic fatty liver disease and autoimmune disorders (39, 40). Neutrophils are normally the first responders to acute inflammation and contribute to the resolution of inflammation, However, in chronic inflammation neutrophils may promote

inflammation through different mechanisms such as neutrophil accumulation within the tissue, protease release and NET formation (41). In addition, ROC analyses showed that TyG index (AUC = 0.652),SII index (AUC = 0.631),and SIRI index (AUC = 0.656) had average predictive power for CHD occurrence, this may be related to the sample size included in the study. By constructing a nomogram, this study designed a clinical diagnostic model for predicting CHD in patients with NAFLD, ROC analysis shows good diagnostic power for CHD on combined analysis of 7 independent risk factors (AUC: 0.751;CI: 0.704-0.798; P <0.001).

This study also has some limitations. Firstly, this is a retrospective study with a limited sample size included. Second, because of the lack of liver biopsy results, no distinction was made between NAFLD and NASH at the time of patient inclusion. Finally, because this was a cross-sectional study, the results showed that the TyG index, SII index, and SIRI index was positively associated with the occurrence of CHD in patients with NAFLD, but it was not able to determine whether it had predictive value. Although this study attempted to construct a diagnostic model to predict CHD, a large number of large-scale, multicentre prospective studies will be needed in the future to illustrate further the predictive value of these independent risk factors for the occurrence of CHD in patients with NAFLD.

5 Conclusion

This study found that in the NAFLD patient population, patients with concurrent CHD had significantly elevated levels of TyG index, SII index, and SIRI index in their serum. Furthermore, the TyG index, SII index, and SIRI index were positively correlated with the severity of CHD. This study also constructed a nomogram to help predict the occurrence of CHD by incorporating abnormal lipid metabolism and inflammation levels into the diagnostic system. Due to their simplicity, convenience, and non-invasive nature, these indicators have great value in routine clinical diagnosis as they can help identify high-risk cardiovascular patients within the NAFLD patient population. However, further research is needed to determine their clinical diagnostic value.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by Ethics Committee of the Second Hospital of Shanxi Medical University; Second hosptial of Shanxi medical university. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

WD: Writing – original draft. YG: Formal Analysis, Writing – original draft. JZ: Data curation, Investigation, Writing – original draft. YW: Software, Writing – review & editing. BL: Writing – review & editing. YY: Writing – review & editing.

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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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Supplementary material

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EDITED BY
Eduardo Hertel Ribeiro,
Escola Superior de Ciências da Santa Casa de Misericórdia de Vitória (EMESCAM), Brazil

REVIEWED BY
Georgios A. Christou,
University of Ioannina, Greece
Soonil Kwon,
Seoul National University Hospital, Republic of Korea

*CORRESPONDENCE
Zhenhua Xing
xing2012x@csu.edu.cn

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Exploring the association between regional fat distribution and atrial fibrillation risks: a comprehensive cohort study

Chenkai Wu[1], Yuefei Xu[1] and Zhenhua Xing[2,3]*

[1]Department of Cardiology, The Second Xiangya Hospital, Central South University, Changsha, Hunan, China, [2]Department of Emergency Medicine, Second Xiangya Hospital, Central South University, Changsha, Hunan, China, [3]Emergency Medicine and Difficult Diseases Institute, Central South University, Changsha, Hunan, China

Background: The contribution of total fat mass and regional fat distribution to the risk of AF has rarely been studied.

Methods: This prospective cohort study(N=494,063) evaluated the association of total fat mass measured by fat percentage (FP) and regional fat measured by arm fat percentage (AFP), trunk fat percentage (TFP), and leg fat percentage (LFP) with incident AF. A subgroup (N = 25,581) underwent MRI, which allowed us to further assess whether visceral adipose tissue (VAT) and abdominal subcutaneous adipose tissue (ASAT) of the trunk fat exert different effects on AF incidence.

Results: Over, a median 12.9 ± 1.86 years of follow-up, 29,658 participants (cumulative rate: 6.0%) developed AF. Each 1-standard deviation (SD) increase in LFP was associated with a 16% lower risk of AF (HR: 0.84, 95% CI: 0.82, 0.85). The association between FP and AF was weaker than that between LFP and AF (HR: 0.90, 95% CI: 0.89, 0.92). AFP and TFP only had a marginal association with a lower incidence of AF. Both the VAT and ASAT showed a U-shaped relationship with incident AF.

Conclusions: Fat mass, mainly leg fat mass, was associated with a lower risk of AF. ASAT did not exert protective effects.

KEYWORDS

fat mass distribution, total fat mass, atrial fibrillation, visceral adipose tissue, abdominal subcutaneous adipose tissue

Introduction

The incidence of atrial fibrillation (AF) has surged globally and poses significant risks, including dementia, stroke, and heart failure (HF), impacting health-related quality of life (1–3). Therefore, identifying modifiable risk factors for AF is extremely important for its prevention. Among these, obesity is recognized as a major risk factor. Common obesity-related anthropometric measures, such as body mass index (BMI), waist circumference (WC), and waist-to-hip ratio, are associated with AF, likely due to inflammation and metabolic disorders induced by obesity (4). Obesity can further lead to increased LV filling pressures and LA pressure due to the increased volume of adipose tissue, which is a well-established risk factor for AF (4).

However, recent studies have challenged this hypothesis (5, 6). Lean body mass (LBM) emerges as a primary anthropometric risk factor for AF, challenging the clarity of the association between obesity and AF (6). Frost et al. observed that both LBM and fat mass correlate with a higher AF risk, with fat mass showing a correlation to LBM (4). Furthermore, recent studies have demonstrated that obesity is a heterogeneous and complex condition and that regional fat mass may contribute differently to the development of obesity-related diseases such as AF (7). For instance, trunk fat mass links significantly to metabolic complications and inflammation like cardiovascular diseases (CVD) and type 2 diabetes, whereas leg fat mass (LFM) exhibits a protective effect (8, 9).

Despite these insights, previous studies on regional fat mass effects on AF primarily focused on inflammation and complications induced by excess trunk mass (measured by WC), overlooking the potential protective role of LFM (4, 10). While dual-energy X-ray absorptiometry (DEXA), magnetic resonance imaging (MRI), and computed tomography (CT) are preferred methods for accurate fat mass and LBM measurement, their practicality is limited in large cohort studies and routine clinical practice due to accessibility, radiation, and cost concerns. Bioimpedance analysis (BIA) emerges as a simple and effective alternative for measuring fat mass, offering accuracy comparable to DEXA (11). In this context, we aimed to evaluate the association between total and regional fat mass (arm fat, trunk fat, and leg fat) measured by BIA and the risk of incident AF, aiming to provide a comprehensive understanding of these relationships.

Methods

Study design and population

The Biobank study, a population-based prospective cohort study conducted in the UK between 2006 and 2010, encompassed over 500,000 residents aged 40–69 years. The study's design has been previously outlined (12). BIA measurements did not distinguish between visceral adipose tissue (VAT) and abdominal subcutaneous adipose tissue (ASAT). However, VAT and ASAT may have different roles in metabolism and inflammation (7). A subgroup of the Biobank study underwent MRI scanning (N = 25,581), which provided us the opportunity to assess the different roles of VAT and ASAT in AF. Ethical approval for the Biobank study was granted by the Northwest Multi-Center Research Ethics Committee(11/NW/0382), and all participants provided written informed consent. Comprehensive details about this study are available at www.ukbiobank.ac.uk. Participants with AF at baseline were excluded from this study.

Exposure and covariates

Body weight was measured and segmental single-frequency BIA was performed using a Tanita BC-418 MA (Tanita Corporation, Arlington Heights, IL, USA). Through BIA, measurements of total and regional fat mass (leg, arm, and trunk) were obtained. Height, measured using the Seca 202 scale (Seca, Hamburg, Germany) without shoes, was utilized to calculate Body Mass Index (BMI), an indicator of general obesity (weight in kilograms divided by the square of height in meters).Regional fat percentage, specifically arm fat percentage (AFP), trunk fat percentage (TFP), and leg fat percentage (LFP), served as indicators of regional fat. These percentages were calculated as fat mass divided by total mass for the corresponding body parts (arm, trunk, and leg). Fat percentage (FP) was used as an indicator of total fat mass, calculated as total fat mass divided by total weight (11). The volumes of the VAT, ASAT, and total trunk fat (TTF) were calculated based on previous studies (13).

Baseline data on participants' demographic and clinical characteristics, encompassing lifestyle and health information, medical history, and biological samples, were acquired through physical examinations, interviews, and laboratory tests. These included age, sex, race, BMI, history of myocardial infarction, HF, and diabetes, smoking and alcohol consumption, diet, physical activity, systolic blood pressure (SBP), diastolic blood pressure (DBP), and medication for hypertension. History of myocardial infarction, diabetes, HF, and hypertension medication use was identified using questionnaires, interviews, and medical records. Physical activity was measured using the metabolic equivalent task (MET) based on the International Physical Activity Questionnaire (IPAQ). Diet score (ranging from 0 to 4) was calculated based on the following diet factors: cooked or raw vegetable intake ≥ four tablespoons/day; fresh fruit intake ≥ three pieces/day; fish intake ≥ twice/week, and processed meat intake ≤ twice/week. SBP and DBP were measured at the assessment center by registered nurses.

Definition of AF

In the current analysis, atrial flutter was considered equivalent to atrial fibrillation (AF). The diagnosis of AF (ICD10 code I48) was established through diagnostic codes linked to hospital encounters and death records. Baseline and incident AF cases were identified based on the "first occurrence of health outcomes defined by a three-character International Statistical Classification of Diseases and Related Health Problems 10th Revision code" (field ID 131351). The follow-up period for each participant was determined from the date of attending the assessment center (between 2006 and 2010) to the date of AF diagnosis, death, or censoring (May 31, 2022, field ID 131350), whichever occurred first.

Statistical analysis

The baseline characteristics of the included participants are presented as mean ± standard deviation (SD) with normal distribution or as median (25th and 75th percentiles) with skewed distribution. The continuous variables were compared using Student's t-test or Mann–Whitney U test according to the distribution type, and the categorical variables were compared using chi-square tests. Missing data were coded as missing indicator categories for categorical variables, such as race, and mean values for continuous variables. The correlations between total and regional fat mass percentage or total and regional fat percentage were assessed using Pearson's coefficient. The relationship between total and regional fat mass percentage/index, treated as both continuous and categorical variables (Q1, Q2, and Q3), and the risk of atrial fibrillation (AF) was evaluated using the Cox proportional-hazard model. Model 1 was adjusted for age, race, sex, and BMI. Model 2 was adjusted for age, race, sex, myocardial infarction (yes/no), diabetes (yes/no), HF (yes/no), hypertension medication use (yes/no), diet score (0–4), smoking and alcohol consumption, MET, SBP, DBP, and BMI. Both models were adjusted for BMI, which accounts for the fact that participants with the same fat mass who differ in BMI will have totally different fat distributions (4). Restricted cubic splines with four knots at the 5th, 35th, 65th, and 95th percentiles were used to explore the dose-response association between the total and regional fat mass percentage and incident AF. We found no evidence of violation of the proportional hazard assumption based on tests using Schoenfeld residuals. Discrimination in the models was assessed using Harrell's C-statistic.

Sensitivity analysis was performed to verify the robustness of our results. First, arm, trunk, and leg fat were normalized for height by dividing by the square of height in meters, similar to the calculation of BMI. This normalization was implemented to address the variability in body composition among participants with the same weight and regional fat mass but different heights (11).we used total and regional fat mass indicators (arm fat index [AFI], trunk fat index [TFI], and leg fat index [LFI]) instead of fat mass percentage to reevaluate the association between total and regional fat mass and incident AF. Additionally, to avoid bias in the Cox proportional-hazard model, we used competing risk models (modeling sub-distributional hazard ratios) for HF. Competing events of all-cause mortality, as a permanent condition, may prevent the occurrence of AF. Furthermore, we utilized propensity score matching to assess the connection between total and regional fat percentage and AF. This involved employing a 1:1 nearest-neighbor matching approach without replacement and setting a matching tolerance (caliper) of 0.05. The goal was to ensure balanced baseline characteristics between the groups. To generate the propensity score, we employed a logistic regression model, with AF as the outcome variable and all potential confounding factors listed in Model 2 and compared total and regional fat percentage using t test. Subgroup analyses were conducted according to age (<60 and ≥60 years), sex, race, smoking status, and history of myocardial infarction, and obesity categories (non-obese <30 kg/m² and obese ≥30 kg/m²) and HF.

All statistical analyses were two-sided, and a *P*-value <0.05 was considered statistically significant. All analyses were performed using the STATA 17 software (StataCorp) between April 1, 2022 and June 31, 2022.

Results

Baseline characteristics of included participants

Of the 502,461 participants in the Biobank study, 8,398 were excluded from the present study for the following reasons: baseline AF, lack of total or regional fat data, and loss of follow-up. A total of 494,063 participants (54.8% female, 88% white, 5.1% with a history of diabetes, and 2.1% with myocardial infarction) remained for further analysis. The included participants had an average age of 56.4 ± 8.1 years. Over a median follow-up period of 12.9 ± 1.86 years, 29,658 participants (4.64 per 1000 persons per year) developed AF. Table 1 presented the baseline characteristics of the participants based on the incidence of AF. Participants who developed incident AF tended to have higher BMI, SBP, and DBP and lower diet scores. They were also more likely to be older, male, non-white, current smokers, had a history of diabetes, HF, and myocardial infarction, and consumed more cigarettes and alcohol.

TABLE 1 Characteristics of included participants.

N	Incidence AF		P-value
	No (N=464,405)	Yes (N=29,658)	
FP (%)	31.5 ± 8.53	31.6 ± 8.54	<0.01
AFP (%)	30.1 ± 10.2	30.0 ± 29.8	0.15
TFP (%)	31.1± 8.00	32.8 ± 8.01	<0.01
LFP (%)	32.7 ± 10.6	30.7 ± 10.9	<0.01
BMI (Kg/m^2)	27.31 ± 4.73	28.99 ± 5.42	<0.01
Age (years)	56.09 ± 8.08	61.90 ± 6.08	<0.01
Sex, male (%)	205,044 (44.15%)	18,218 (61.43%)	<0.01
Race, White (%)	408,349 (87.93%)	27,049 (91.20%)	<0.01
Smoker (%)			<0.01
Never	256,472 (55.23%)	13,232 (44.62%)	
Current	156,239 (33.64%)	12,889 (43.46%)	
Quit	49,036 (10.56%)	3,310 (11.16%)	
Alcohol, times (%)			<0.01
Daily	92,594 (20.00%)	7,122 (24.10%)	
3-4/week	107,141 (23.14%)	6,490 (21.96%)	
1-2/week	120,345 (25.99%)	7,029 (23.78%)	
1-3/month	52,258 (11.29%)	2,798 (9.47%)	

(Continued)

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TABLE 1 Continued

N	Incidence AF		P-value
	No (N=464,405)	Yes (N=29,658)	
Occasionally	53,645 (11.59%)	3,457 (11.70%)	
never	37,056 (8.00%)	2,657 (8.99%)	
Diet Score			0.04
0	6,143 (1.36%)	304 (1.14%)	
1	54,391 (12.09%)	3,278 (12.28%)	
2	138,650 (30.81%)	8,400 (31.46%)	
3	163,569 (36.34%)	9,585 (35.89%)	
4	87,311 (19.40%)	5,137 (19.24%)	
Diabetes (%)	22,276 (4.80%)	3,121 (10.52%)	<0.01
Heart failure (%)	1,344 (0.29%)	462 (1.56%)	<0.01
Myocardial infarction (%)	8,533 (1.84%)	2,002 (6.75%)	<0.01
Previous stroke (%)	3250 (0.70%)	201 (0.68%)	0.39
Previous cancer (%)	3954 (0.85%)	258 (0.86%)	0.10
MET (min/week)	965 ± 941	974 ± 952	0.57
C2HEST score (IQR)	1 (0-1)	1 (0-1)	0.75
Stroke (%)	5,451 (1.1%)	315 (1.1%)	0.09
Medication for hypertension (%)	415,710 (89.51%)	21,557 (72.69%)	<0.01
MET (min/week)	1,041.15 ± 974.25	1,030.27 ± 949.13	0.06
DBP (mmHg)	82.17 ± 10.28	82.91 ± 10.66	<0.01
SBP (mmHg)	139.37 ± 18.90	145.60 ± 19.59	<0.01
Creatinine (umol/L)	72.85 ± 13.2	73.25 ± 14.2	0.52

AF, atrial fibrillation; FP , fat percentage; AFP, arm fat percentage; TFP, trunk fat percentage; LFP, leg fat percentage; BMI, body mass index; MET, the metabolic equivalent task; DBP, diastolic blood pressure; SBP, systolic blood pressure; IQR, interquartile range.

Supplementary Figure 1 displays the correlation of FP, AFP, TFP, LFP, AFI, TFI, and LFI (all P < 0.01).

Associations between total and regional fat mass and incident AF

The associations between total and regional fat mass and AF risk are presented in Table 2. There was a robust and statistically significant association between LFP and FP with a reduced risk of AF across all models. Following multivariable adjustment, including BMI, each standard deviation (SD) increase in LFP was linked to a notable 16% lower risk of AF (hazard ratio [HR]: 0.84, 95% confidence interval [CI]: 0.82, 0.85, P < 0.01, Model 2). In comparison, the association between FP and AF was weaker. Each SD increase in FP was associated with a 10% lower risk of AF (HR: 0.90, 95% CI: 0.89, 0.92, P < 0.01, Model 2). However, AFP and trunk fat percentage TFP exhibited only marginal associations with a lower incidence of AF (AFP, HR: 0.98, 95% CI: 0.96, 1.00; TFP, HR: 0.96, 95% CI: 0.94, 0.97, Model 2).

TABLE 2 Association between total and regional fat percentage and AF incidence.

	Model 1	Model 2
	HR (95%CI)	HR (95%CI)
FP		
Q1	Ref	Ref
Q2	0.87 (0.84,0.90)	0.86 (0.83,0.89)
Q3	0.93 (0.89,0.98)	0.84 (0.81,0.88)
P for trend	<0.01	<0.01
Per SD increase	0.92 (0.90,0.94)	0.90 (0.89,0.92)
AFP		
Q1	Ref	Ref
Q2	0.90 (0.87,0.93)	0.89 (0.86,0.92)
Q3	0.96 (0.92,1.00)	0.93 (0.89,0.97)
P for trend	0.09	0.01
Per SD increase	1.00 (0.98,1.03)	0.98 (0.96,1.00)
TFP		
Q1	Ref	Ref
Q2	0.89 (0.86,0.92)	0.88 (0.86,0.91)
Q3	0.93 (0.90,0.97)	0.90 (0.87,0.94)
P for trend	<0.01	<0.01
Per SD increase	0.97 (0.96,0.99)	0.96 (0.94,0.97)
LFP		
Q1	Ref	Ref
Q2	0.82 (0.79,0.84)	0.81 (0.78,0.84)
Q3	0.75 (0.72,0.78)	0.73 (0.70,0.76)
P for trend	<0.01	<0.01
Per SD increase	0.84 (0.83,0.86)	0.84 (0.82,0.85)

Model 1: age, race, sex, BMI.
Model 2: adjusted for age, sex, race, myocardial infarction, diabetes, heart failure, diet score, smoking, and alcohol, MET, systolic blood pressure, diastolic blood pressure, medication for hypertension, BMI.
AF, atrial fibrillation; FP, fat percentage; AFP, arm fat percentage; TFP, trunk fat percentage; LFP, leg fat percentage; SD, standard deviation.

The categorical analysis and restricted cubic splines demonstrated a nonlinear relationship between total and regional fat and incidence of AF after adjusting for confounding factors (Table 2; Figure 1). Specifically, a U-shaped relationship emerged for AFP and TFP in relation to the incidence of AF (P for nonlinear trend < 0.01; Figures 1B, C). The plot illustrated a substantial risk reduction within the lower range of AFP or TFP, reaching the lowest risk of approximately 27% for AFP and 32% for TFP, followed by a slow increase for AFP and a sharp increase for TFP thereafter. Additionally, a negative association was observed between FP and LFP and AF risk. The risk of AF sharply decreased to 30% for FP and 32% for LFP, then decreased slowly for LFP, and did not decrease for FP (P for nonlinear trend < 0.01, Figures 1A, D).

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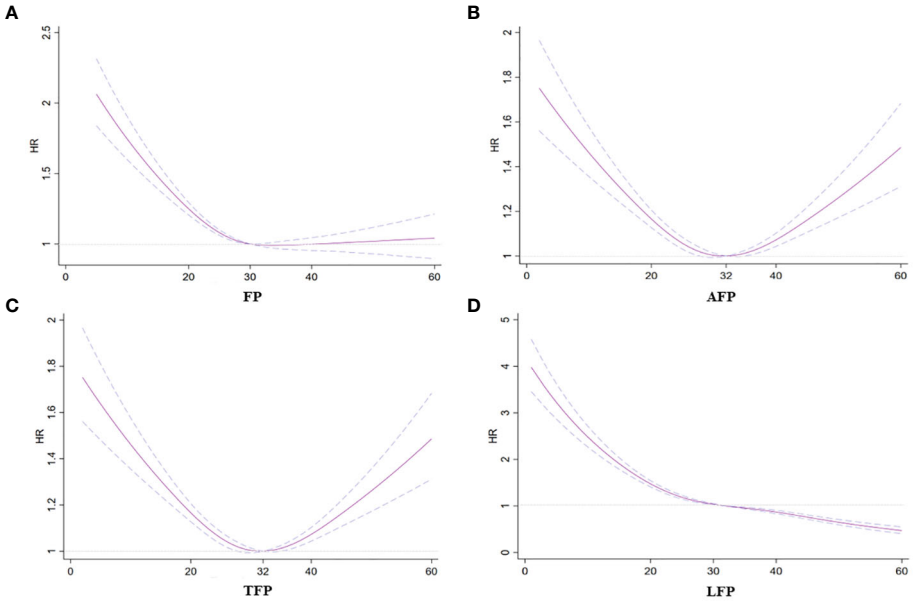


FIGURE 1
Association between fat mass and regional fat mass percentage with incident AF. **(A)** FP and AF. **(B)** AFP and AF. **(C)** TFP and AF. **(D)** LPF and AF. Hazard ratios are indicated by solid lines and 95% CIs are indicated by a dummy line.

Associations between VAT, ASAT, TTF, and AF

We did not observe statistical associations between VAT, ASAT, and TTF and AF incidence using ASAT, VAT, and TTF as continuous variables (Supplementary Table 1). Restricted cubic splines demonstrated a nonlinear relationship between VAT, ASAT, and TTF and incidence of AF after adjusting for confounding factors. A U-shaped relationship was found between VAT, ASAT, and TTF and AF incidence (P for nonlinear trend<0.01, Figure 2).

Sensitivity and subgroup analyses

Our findings remained robust in several sensitivity analyses. The association between regional fat mass and the incidence of atrial fibrillation AF did not change when using AFI, TFI, and LFI instead of AFP, TFP, and LFP (Supplementary Table 2). An increase in LFI was associated with a lower risk of AF, and AFP and TFP showed marginal associations with the incidence of AF. Furthermore, when applying the competing risk model instead of the Cox proportional hazard model, the results remained robust (Supplementary Table 3). We compared the total and regional fat percentages in participants with AF and matched non-AF participants. Participants with AF had lower LFP and FP percentages (Supplementary Table 4). Subgroup and interactive analyses revealed that sex moderated the association between total and regional fat mass and AF incidence (Supplementary Figure 2). The protective role of fat mass was weaker in female participants.

Discussion

In this large, population-based, prospective cohort study including more than 480,000 participants and 29,658 AF outcomes, fat mass, mainly leg fat mass, was associated with a lower risk of AF after adjusting for BMI and traditional risk factors. A U-shaped relationship was observed between arm fat, trunk fat (including VAT and ASAT), and the risk of atrial fibrillation (AF).

Consistent with our findings, Azarbal et al. found that fat mass is associated with a lower risk of AF in postmenopausal women (5). However, Frost et al. showed contrasting results, indicating that higher fat mass was linked to an increased risk of AF (HR: 1.29 per standard deviation increment in fat mass) (4). The controversy in these findings may be attributed to the confirmed correlations between LBM, fat mass, and other anthropometric parameters, as highlighted in our and previous studies (4). Recent investigations have emphasized LBM as the primary anthropometric risk factor for AF (5, 6), suggesting that individuals with higher fat mass may have higher proportions of LBM, contributing to an elevated AF risk. Therefore, the crucial aspect in this context involves the systematic mutual adjustment of relevant anthropometric measures associated with fat mass. Both our study and that by Azarbal et al. included BMI adjustment, assuming that all participants had similar body shapes, although this assumption may be more suited for elucidating methodological mechanisms than offering a clear biological interpretation.

This study represents the first investigation of arm and leg fat mass in relation to atrial fibrillation (AF) risk within a large prospective cohort. The unique findings suggest that the associations between fat mass and AF risk are region-specific, reflecting diverse fat masses in

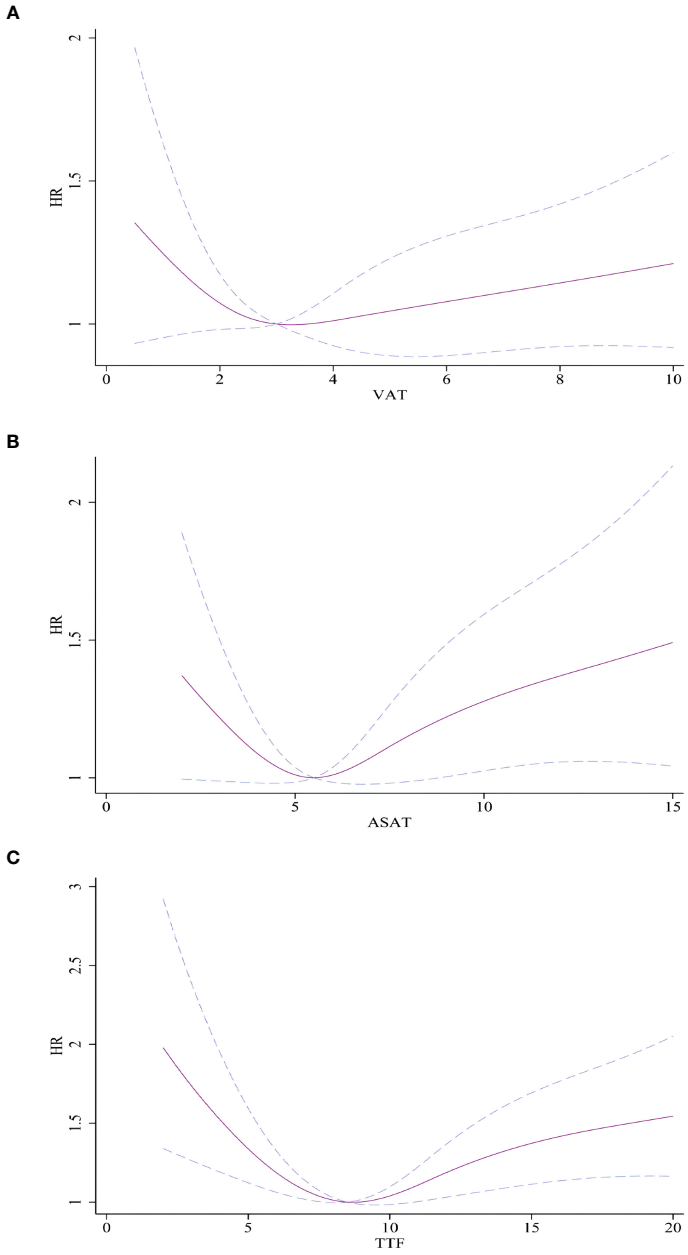


FIGURE 2
Association between VAT, ASAT, and TTF and with incident AF. **(A)** VAT and AF. **(B)** ASAT and AF. **(C)** TTF and AF. Hazard ratios are indicated by solid lines and 95% CIs are indicated by a dummy line. AF, atrial fibrillation; VAT, Visceral adipose tissue volume; ASAT, Abdominal subcutaneous adipose tissue volume; TTF, total trunk fat volume.

different regions with distinct inflammatory and metabolic functions (14). Our present study expand prior research that has demonstrated associations between fat mass and AF risk factors. Previous study have left gap in our understanding the association between regional fat mass and AF risk in prospective cohort studies, particularly in determining whether additional measurements of regional fat mass can further discriminate participants at high risk of AF in addition to BMI. The varying connections between body fat and cardiovascular risk factors or events across different regions make sense, considering the distinct fat depots in the upper and lower body, each with unique biological functions. Numerous potential mechanisms contribute to these region-specific associations, such as differences in adipose inflammation severity, lipid storage and turnover, release of adipokines, and endocrine effects (14–17). Inflammation and metabolic dysregulation are established mechanisms contributing to the development and progression of AF. Inflammation plays a pivotal role in the initiation and perpetuation of AF by promoting atrial structural remodeling, fibrosis, and electrical instability. Metabolic disturbances, such as insulin resistance and dyslipidemia, have been linked to atrial electrical and structural remodeling, creating a substrate conducive to AF (17, 18). The physiological functions of beige fat extend beyond metabolic regulation, encompassing its role in modulating systemic inflammation and metabolic homeostasis. By virtue of its ability to enhance energy expenditure and improve lipid and glucose

metabolism, beige fat activation may mitigate the inflammatory and metabolic perturbations that underlie AF pathogenesis (19). Moreover, recent evidence suggests that beige fat-derived factors, such as adipokines and thermogenic mediators, possess anti-inflammatory and cardioprotective properties (20). Lower limb fat typically contains more beige fat, which is associated with low systemic inflammation and healthy metabolism. These factors may directly influence atrial electrophysiology and remodeling processes, thereby attenuating the susceptibility to AF.

In contrast, a U-shaped relationship was identified between trunk fat mass (including VAT and ASAT) and AF risk in this study. Interestingly, ASAT did not exhibit a protective role, as both high volumes of ASAT and VAT were associated with a higher risk of AF. This aligns with findings from Fox et al., who also observed that elevated levels of both VAT and ASAT were linked to metabolic disorders (20). The present study did not find an exact association While the study did not find a clear association between arm fat mass and AF risk, AFP showed a marginal association with AF incidence, which was not supported by sensitivity analysis. As the arm is not a primary fat storage site and constitutes a small percentage of total fat mass, its impact on inflammation and metabolic factors may be insufficient. However, a U-shaped relationship was noted between arm fat and AF incidence, suggesting that arm fat mass may serve as an indicator of nutritional status (21). Both malnutrition and overnutrition have associations with metabolic disorders, potentially contributing to a higher risk of AF. Further research is warranted to establish the causal association.

Study strength and limitations

Our study has a multitude of strengths. The incidence of AF is very low in the general population (6.0% in our study). Previous studies with limited samples might have been underpowered to verify the association between total or regional fat distribution and incident AF. Our study included approximately 500,000 participants with a long-term follow-up period (12.1 years), and approximately 30,000 participants developed AF. The sample size was sufficiently large to evaluate the association and conclusively examine sex or race interactions. We used different indicators of regional fat distribution and statistical methods to verify the robustness of our findings. These findings have important clinical and public-health implications. As a simple, effective, and economic method, BIA can further improve risk stratification and reduce the incidence of AF. Furthermore, our findings prompt a reevaluation of obesity management strategies. Traditional measures of obesity, such as BMI, may not capture the nuances of fat distribution that influence AF susceptibility. Clinicians managing obesity patients should consider evaluating regional fat distribution alongside BMI to obtain a more comprehensive assessment of AF risk. Tailoring interventions based on regional fat distribution could lead to more effective strategies for reducing AF incidence among obese individuals.

On the other hand, our study also has several limitations. First, residual confounding is always an issue that cannot be averted in observational studies; however, we believe that residual confounding was a minor issue in the present study, as the relative confounding of AF incidence was fully adjusted. Furthermore, the adjustments had little effect on effect size. Second, the regional fat percentage or index was measured using BIA rather than DXEA/CT/MRI, although relevant studies have demonstrated that BIA has comparable accuracy to that of DEXA (11). Third, epicardial fat is of particular interest in AF due to its proximity to the myocardium and potential implications for cardiovascular health (22). It's noteworthy that bioimpedance analysis (BIA) cannot directly measure epicardial fat. Fourth, The Biobank study indeed did not measure adipokines secreted by adipose tissue, a factor of considerable relevance in understanding the association between regional fat distribution and AF risk (22).

Conclusion

In conclusion, fat mass, mainly leg fat mass, was associated with a lower risk of AF after adjusting for BMI and traditional CVD risk factors. ASAT did not exert protective effects.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical approval for the Biobank study was granted by the Northwest Multi-Center Research Ethics Committee(11/NW/0382). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

CW: Writing – original draft, Writing – review & editing. YX: Writing – original draft, Writing – review & editing. ZX: Conceptualization, Writing – review & editing.

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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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1367653/full#supplementary-material

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EDITED BY
Daigo Sawaki,
Jichi Medical University, Japan

REVIEWED BY
Shaoyi Zhang,
University of California, San Francisco, United States
Giuseppe Mascia,
University of Genoa, Italy

*CORRESPONDENCE
Liming Xia
xialiming2017@outlook.com

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Association of serum uric acid with right cardiac chamber remodeling assessed by cardiovascular magnetic resonance feature tracking in patients with connective tissue disease

Yuanyuan Tang, Zhaoxia Yang, Jinyang Wen, Dazhong Tang, Yi Luo, Chunlin Xiang, Lu Huang and Liming Xia*

Department of Radiology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China

Background: Right cardiac chamber remodeling is widespread in patients with connective tissue disease (CTD). Serum uric acid (SUA) is considered a potential independent risk factor for cardiovascular disease, and elevated SUA levels are often observed in patients with CTD. The correlation between SUA levels and right cardiac chamber remodeling remains unclear. This study investigated the association of SUA with right cardiac chamber remodeling as assessed by cardiac magnetic resonance feature-tracking (CMR-FT) in CTD patients.

Methods and results: In this cross-sectional study, a total of 104 CTD patients and 52 age- and sex-matched controls were consecutively recruited. All individuals underwent CMR imaging, and their SUA levels were recorded. The patients were divided into three subgroups based on the tertiles of SUA level in the present study. CMR-FT was used to evaluate the right atrial (RA) longitudinal strain and strain rate parameters as well as right ventricular (RV) global systolic peak strain and strain rate in longitudinal and circumferential directions for each subject. Univariable and multivariable linear regression analyses were used to explore the association of SUA with RV and RA strain parameters. Compared with the controls, the CTD patients showed significantly higher SUA levels but a lower RV global circumferential strain (GCS) and RA phasic strain parameters (all $p < 0.05$), except the RA booster strain rate. RV GCS remained impaired even in CTD patients with preserved RV ejection fraction. Among subgroups, the patients in the third tertile had significantly impaired RV longitudinal strain (GLS), RV GCS, and RA reservoir and conduit strain compared with those in the first tertile (all $p < 0.05$). The SUA levels were negatively correlated with RV GLS and RV GCS as well as with RA reservoir and conduit strain and strain rates (the absolute values of β were 0.250 to 0.293, all $P < 0.05$). In the multivariable linear regression analysis, the SUA level was still an independent determinant of RA conduit strain (β = -0.212, P = 0.035) and RV GCS (β = 0.207, P = 0.019).

Conclusion: SUA may be a potential risk factor of right cardiac chamber remodeling and is independently associated with impaired RA conduit strain and RV GCS in CTD patients.

KEYWORDS

connective tissue disease, serum uric acid, cardiovascular resonance magnetic imaging, feature tracking, right ventricular strain, right atrial strain

1 Introduction

Connective tissue disease (CTD) is a heterogeneous group of chronic inflammatory diseases due to abnormal auto-immunity regulation and characterized by multiorgan involvement, including systemic sclerosis (SSc), systemic lupus erythematosus (SLE), primary Sjögren's syndrome (pSS), idiopathic inflammatory myopathy (IIM), rheumatoid arthritis, and many other autoimmune diseases. Cardiovascular and pulmonary involvement is a common complication and one of the leading causes of death in patients with CTD. There are multiple clinical manifestations, presented as myocarditis, heart failure, valvular heart disease, coronary artery disease, pulmonary hypertension (PH) (1), and interstitial lung disease (ILD) (2). Right cardiac chamber remodeling, including chamber dilatation, wall hypertrophy, myocardial fibrosis, and cardiac insufficiency, plays an important role in the progression and prognosis of CTD. Both the primary involvement of the right ventricular (RV) myocardium and the secondary impact of ILD and PH on RV afterload can result in right cardiac chamber remodeling in patients with CTD. Echocardiography is limited by an acoustic window and poor reproducibility in assessing the right heart. Cardiac magnetic resonance (CMR) imaging is the current gold standard for quantitatively evaluating cardiac structure and function with high reproducibility. CMR feature tracking (CMR-FT) is a promising technique that evaluates myocardial deformation and provides additional information for subclinical cardiac dysfunction (3). Thus, RV and right atrial (RA) strain parameters may be more sensitive to assess the changes in right heart function and mechanics. Evidence supporting the early diagnostic and prognostic value of RV and RA strain parameters in CTD patients with cardiovascular involvement has accumulated over the years (4–6).

Serum uric acid (SUA) is the final metabolic product of purine degradation, which correlated with multiple cardiovascular risk factors and could be considered an independent predictor for several adverse cardiovascular outcomes (7). Previous studies demonstrated that an elevated SUA level was a potential risk factor of cardiovascular damage in patients with CTD (8, 9). Elevated SUA levels not only increase the risk of developing PH and serve as a marker for screening PH in CTD (10, 11) but may also be predictors of clinical prognosis in patients with CTD-associated pulmonary arterial hypertension (CTD-PAH) (12). Another study showed that SUA levels were correlated with RA pressures and RV function in patients with heart failure with preserved ejection fraction (13). However, the correlation between SUA levels and right cardiac chamber remodeling in patients with CTD remains unclear. Accordingly, the present study aimed to evaluate the right cardiac chamber remodeling in patients with CTD and to investigate the association of SUA with impaired RV and RA strain parameters as assessed by CMR-FT.

2 Materials and methods

2.1 Study design and population

This cross-sectional study enrolled 159 consecutive patients with CTD who underwent 3.0T CMR examination from January 2015 to July 2023 in our hospital. The diagnosis of CTD was according to the American College of Rheumatology diagnostic criteria or the European League Against Rheumatism for IIM (14), SLE (15), SSc (16), pSS (17), and rheumatoid arthritis (18) as well as sharp criteria for mixed connective tissue disease (MCTD) (19). Overlap syndrome was defined as patients with two or more clinical features of CTD at the same time. Undifferentiated connective tissue disease (UCTD) was defined as patients with clinical and laboratory features suggestive of systemic autoimmune diseases but cannot be classified into any of the defined CTD classification criteria. The exclusion criteria were as follows: (1) age <18 years; (2) a history of coronary artery disease; (3) severe heart valvular disease, cardiomyopathy, and congenital heart disease; (4) left ventricular ejection fraction (LVEF) <50%; (5) severe kidney dysfunction (estimated glomerular filtration rate <30 ml/min/1.73 m^2); (6) poor image quality; and (7) incomplete clinical data. Finally, a total of 104 CTD patients (comprising 49 patients with IIM, 29 with SLE, six with SSc, five with pSS, eight with overlap syndrome, two with rheumatoid arthritis, four with UCDT, and one with MCTD) were retrospectively included. The patients were divided into three subgroups based on the tertiles of SUA level in the present study. CTD patients with preserved RVEF (defined as RVEF ≥50%) were analyzed as a subgroup, too. Meanwhile, 52 age- and gender-matched individuals with no history of cardiovascular or systematic diseases were selected as the control group. This study protocol was approved by the Institutional Review Board of our

hospital (TJ-IRB20230914), and the requirement for written informed consent was waived due to the retrospective design. The patients' flowchart of this study is shown in Figure 1.

2.2 Clinical and laboratory data

Venous blood samples were obtained from a peripheral vein within 48 h of the CMR examination as well as without receiving any urate-lowering therapies. SUA, estimated glomerular filtration rate, total cholesterol, triglyceride, high-density lipoprotein-cholesterol, and low-density lipoprotein-cholesterol were measured in the laboratory department of our hospital. Pulmonary fibrosis was defined as reported on thoracic computed tomography imaging by specialist radiologists. PH was defined as systolic pulmonary arterial pressure >36 mmHg on echocardiography as measured by experienced doctors or mean pulmonary arterial pressure ≥20 mmHg on right-sided heart catheterization (20).

2.3 CMR scanning protocol

All patients and controls underwent a standard clinical protocol using 3.0T magnetic resonance imaging (MRI) scanners (MAGNETOM Skyra, Siemens Healthcare, Erlangen, Germany) with an 18-channel phased-array receive coil in the supine position. A steady-state free-precession sequence with electrocardiogram triggering and respiratory gating was used to assess cardiac structure and function. Short-axis cine images covering the right ventricle (RV) from apex to tricuspid annulus, as well as standard cine long-axis four-chamber view, were acquired. Typical imaging parameters were as follows: echo time/repetition time, 1.4/37.7 ms; flip angle, 55°; field of view, 360 × 360 mm^2; acquisition matrix size, 192 × 146; slice thickness, 8 mm; 25 phases per cardiac cycle.

2.4 Cardiac function and feature tracking analysis

Post-processing of all CMR images was analyzed using an offline customized software CVI42 (version 5.13.9, Circle Cardiovascular Imaging Inc., Calgary, Canada). Right and left ventricular endocardial and epicardial contours were drawn manually at the end of diastole and systole in all short-axis cine images excluding the papillary muscles and trabeculae. Biventricular end-diastolic volume (EDV), end-systolic volume (ESV), stroke volume, myocardial mass, and ejection fraction (EF) were calculated. Right atrial (RA) volumes were measured in CVI42 function LAX biplanar module using the biplane area–length method on the cine CMR 4-chambers views, excluding the confluence of the superior and inferior vena cava, as well as the atrial appendage. RA maximum (V_{max}) and minimum volumes (V_{min}) were assessed at the end of ventricular systole and diastole, respectively. Pre-atrial volumes ($V_{pre\text{-}A}$) were obtained before atrial contraction. The functions of RA were calculated by the following equations as previously described (21): (1) reservoir function: total emptying fraction (EF_t) = (V_{max} − V_{min})/V_{max} × 100%; (2) conduit function: passive emptying fraction (EF_p) = (V_{max} − $V_{pre\text{-}A}$)/V_{max} × 100%; (3) booster pump function: active emptying fraction (EF_a) = ($V_{pre\text{-}A}$−V_{min})/$V_{pre\text{-}A}$ × 100%. All volumes and RV myocardial masses were indexed to body surface area before analysis.

The myocardial deformation parameters of the RV free wall (RVFW) as well as the RA were evaluated in CVI42 feature-tracking module. The endocardial and epicardial contours of RV and RA were manually drawn using a point-and-click approach on the end-diastolic images, respectively. Subsequently, the contours were automatically tracked throughout the cardiac cycle while also checking the quality of the automatic tracking and adjusting the initial contour manually if necessary. RV global circumferential

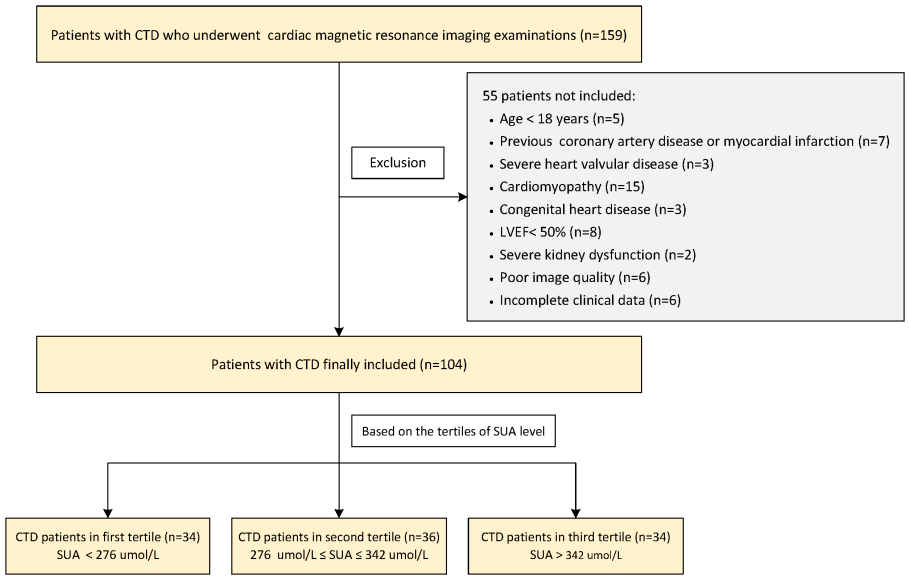


FIGURE 1
Flowchart of the CTD patients' enrollment. CTD, connective tissue disease; LVEF, left ventricular ejection fraction; SUA, serum uric acid.

strain (GCS) and global circumferential strain rate (GCSR) were derived from tracking the RVFW in the four-chamber view and short-axial views, while the global longitudinal RV and RA strain and strain rates were quantified based on the four-chamber view only (Supplementary Figures S1, S2). RV radial strain parameters were not evaluated due to the high variability as previously described (22). Three aspects of RA phasic function were quantified for each subject as follows: (1) RA reservoir function (total strain [ε_s] and strain rate [SRs]), (2) RA conduit function (passive strain [ε_e] and strain rate [SRe]), and (3) RA booster function (active strain [ε_a] and strain rate [SRa]). Above all, the results were based on the average of three independent repeated measurements.

2.5 Reproducibility of RV and RA strain analysis

The inter- and intra-observer variabilities for RV and RA strain and strain rate parameters were obtained from 40 randomly selected subjects (10 controls and 30 CTD patients). Inter-observer variability was assessed by two radiologists (YT and JW, with 3 and 5 years of experience in CMR imaging, respectively) blinded to all clinical information. Intra-observer variability was measured twice by radiologist YT, with at least 1 month between repeated analysis.

2.6 Statistical analysis

Statistical analysis was performed using SPSS version 26.0 (IBM Deutschland GmbH, Armonk, NY, USA) and GraphPad Prism version 10.0.2 (GraphPad Prism Software Inc., San Diego, CA, USA). Continuous variables were summarized as mean ± standard deviation (SD) or median with interquartile range (IQR) and were compared between groups using Student's *t*-test or Mann–Whitney *U*-test as appropriate based on their normality. Comparisons among three groups were carried out using analysis of variance (ANOVA) with Bonferroni or Games Howell *post hoc* test for normally distributed data or the Kruskal–Wallis test for non-normally distributed data. Categorical variables were presented as frequencies (percentages) and were compared using the Pearson χ^2 test. Univariable and multivariable linear regression analyses were used to investigate the association of SUA levels with CMR-derived RV and RA strain parameters. Multivariable analyses were adjusted to all variables without collinearity and a *P*-value <0.05 in univariable analysis as well as age, sex, hyperlipidemia, disease duration, pulmonary fibrosis, and PH. Intra-class correlation coefficient (ICC) was calculated to determine the inter- and intra-observer reproducibility of RV and RA strain parameters. A two-sided *P*-value <0.05 was considered statistically significant.

3 Results

3.1 Patients' characteristics

The clinical characteristics are summarized in Table 1. CTD patients had significantly higher SUA levels [308.5 (249.00, 357.800) vs. 265.30 (222.80, 297.35) umol/L, *P*=0.001] than controls. According to the tertiles of SUA level distribution in the present study, the CTD patients were divided into three subgroups: the first tertile (SUA level <276 umol/L), the second tertile (276–342 umol/L), and the third tertile (>342 umol/L). Approximately, a third of CTD patients had pulmonary involvement and presented in New York Heart Association (NYHA) class III–IV, which had a higher percentage in the third tertile. The median disease duration was 17.50 (5.00, 60.00) months. The CTD patients in the third tertile also had a significantly longer disease duration than those in the first tertile [21.00 (6.00, 66.00) vs. 7.00 (2.00, 52.75) months, *P* =

TABLE 1 Clinical characteristics of patients with connective tissue disease and controls.

	Controls (*n* = 52)	CTD (*n* = 104)	CTD			CTD with preserved RVEF (*n* = 70)
			First tertile (*n* = 34)	Second tertile (*n* = 36)	Third tertile (*n* = 34)	
Female, *n* (%)	40 (76.9%)	84 (80.8%)	32 (94.1%)	30 (83.3%)	22 (64.7%)[b]	60 (85.7%)
Age, years	46.17 ± 14.02	48.51 ± 12.49	47.47 ± 12.99	48.94 ± 13.25	49.09 ± 11.43	48.80 ± 12.35
BMI, kg/m^2	22.02 ± 3.43	22.40 ± 3.52	21.97 ± 3.80	22.48 ± 3.69	22.74 ± 3.08	22.52 ± 3.50
BSA, m^2	1.60 ± 0.15	1.62 ± 0.16	1.59 ± 0.13	1.61 ± 0.16	1.67 ± 0.17	1.61 ± 0.14
Heart rate, beats/min	74.40 ± 15.48	75.22 ± 13.79	73.35 ± 9.63	75.94 ± 16.22	76.32 ± 14.69	75.57 ± 13.41
Systolic blood pressure, mmHg	120.95 ± 16.20	117.96 ± 15.84	116.59 ± 15.44	117.36 ± 15.48	119.97 ± 16.86	119.39 ± 16.54
Diastolic blood pressure, mmHg	76.86 ± 12.69	77.99 ± 12.14	76.97 ± 11.25	79.08 ± 11.28	77.85 ± 14.01	78.60 ± 11.02
Underlying CTD, *n* (%)-						
IIM	–	49 (47.1%)	18 (52.9%)	18 (50.0%)	13 (38.2%)	39 (55.7%)
SLE	–	29 (27.9%)	7 (20.6%)	11 (30.6%)	11 (32.4%)	15 (21.4%)

(Continued)

TABLE 1 Continued

	Controls (*n* = 52)	CTD (*n* = 104)	CTD			CTD with preserved RVEF (*n* = 70)
			First tertile (*n* = 34)	Second tertile (*n* = 36)	Third tertile (*n* = 34)	
Underlying CTD, *n* (%)-						
SSc	–	6 (5.8%)	1 (2.9%)	1 (2.8%)	4 (11.8%)	2 (2.9%)
pSS	–	5 (4.8%)	1 (2.9%)	2 (5.6%)	2 (5.6%)	2 (2.9%)
RA	–	2 (1.9%)	1 (2.9%)	1 (2.9%)	–	1 (1.4%)
MCTD	–	1 (1.0%)	–	1 (2.8%)	–	–
UCTD	–	4 (3.8%)	4 (11.8%)	–	–	4 (5.7%)
Overlap syndrome	–	8 (7.7%)	2 (5.9%)	2 (5.6%)	4 (11.8%)	7 (10.0%)
Disease duration, months	–	17.50 [5.00, 60.00]	7.00 [2.00, 52.75]	24.00 [9.75, 81.00]	21.00 [6.00, 66.00] [b]	12.00 [3.75, 48.75]
NYHA class III–IV, *n* (%)	–	22 (21.2%)	2 (5.9%)	9 (25.0%)	11 (32.4%)	7 (10.0%)
Pulmonary fibrosis[*], *n* (%)	–	42 (40.4%)	9 (26.5%)	17 (47.2%)	16 (47.1%)	27 (38.6%)
PH, *n* (%)	–	32 (30.8%)	7 (20.6%)	10 (27.8%)	15 (44.1%)	14 (20.0%)
Pericardial effusion, *n* (%)	–	43 (41.3%)	13 (38.2%)	14 (38.9%)	16 (47.1%)	27 (38.6%)
Hypertension, *n* (%)	7 (13.5%)	21 (20.2%)	5 (14.7%)	11 (30.6%)	5 (14.7%)	17 (24.3%)
Hyperlipidemia[†], *n* (%)	5 (9.6%)	36 (34.6%)[a]	12 (35.3%)a	13 (36.1%)a	11 (32.4%)[a]	26 (37.1%)[a]
Diabetes mellitus, *n* (%)	5 (9.6%)	6 (5.8%)	3 (8.8%)	1 (2.8%)	2 (5.9%)	4 (5.7%)
SUA, umol/L	265.30 [222.80, 297.35]	308.50 [249.00, 357.80] [a]	208.00 [190.25, 249.00] [a]	308.50 [286.25, 326.00] [ab]	412.15 [357.80, 467.50] [abc]	282.00 [221.93, 343.50]
eGFR, mL/min/1.73 m^2	102.00 [90.75, 111.35]	105.45 [87.25, 122.90]	110.60 [90.00, 126.88]	106.45 [95.95, 121.58]	100.30 [79.48, 114.65]	106.60 [90.58, 123.08]
TC, mmol/L	3.99 [3.37, 4.63]	4.27 [3.59, 5.09]	4.42 [3.71, 5.09]	4.31 [3.82, 5.19] [a]	3.99 [3.41, 5.10]	4.35 [3.78, 5.09]
TG, mmol/L	1.13 [0.83, 1.47]	1.63 [1.03, 2.64] [a]	1.45 [0.89, 3.00]	1.72 [1.25, 2.36] [a]	1.79 [1.13, 2.58] [a]	1.66 [0.98, 2.74] [a]
HDL-C, mmol/L	1.25 [1.09, 1.43]	1.10 [0.85, 1.32] [a]	1.14 [0.93, 1.41]	1.12 [0.97, 1.33]	0.95 [0.74, 1.25] [a]	1.14 [0.94, 1.35]
LDL-C, mmol/L	2.42 [2.01, 3.14]	2.43 [2.01, 3.23] [a]	2.36 [1.85, 3.02]	2.57 [2.18, 3.31]	2.34 [2.01, 3.35]	2.45 [1.95, 3.34]

Values are presented as the mean ± SD, number (percentage), or median [interquartile range].
CTD, connective tissue disease; BMI, body mass index; BSA, body surface area; IIM, idiopathic inflammatory myopathy; SLE, systemic lupus erythematosus, SSc, systemic sclerosis; pSS, primary Sjogren Syndrome; RA, rheumatoid arthritis; MCTD, mixed connective tissue disease; UCTD, undifferentiated connective tissue disease; NYHA, New York Heart Association; PH, pulmonary hypertension; SUA, serum uric acid; eGFR, estimated glomerular filtration rate; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; a, $P < 0.05$ versus controls; b, $P < 0.05$ versus patients in the first tertile; c, $P < 0.05$ versus patients in the second tertile.
[*]As reported on CT thorax imaging by specialist radiologists.
[†]Total cholesterol ≥ 6.2 mmol/L, triglycerides ≥ 2.3 mmol/L, or low-density lipoprotein ≥ 4.1 mmol/L.

0.030]. No significant differences were found among age, sex, BMI, BSA, heart rate, systolic blood pressure, and diastolic blood pressure as well as the proportion of hypertension and diabetes mellitus between the CTD patients and the controls (all $P > 0.05$), but the percentage of hyperlipidemia was higher in CTD patients than in the controls (34.6 vs. 9.6%, $P = 0.001$).

3.2 CMR characteristics

The conventional CMR characteristics as well as RV and RA strain parameters of all participants are shown in Table 2. The CTD patients had significantly increased RVEDVi, RVESVi, and LV mass index, whereas they have decreased RVEF and RV GCS compared with the controls (all $p < 0.05$), and RV GCS remains impaired even in CTD patients with preserved RVEF (-17.81 ± 3.56 vs. -19.17 ± 2.84%, $P = 0.025$). Although no differences in RA morphological and functional parameters were observed among CTD patients and controls, the CTD patients showed significantly impaired RA strain parameters except SR_a [ε_s: 49.52 ± 16.90 vs. 58.04 ± 12.60 %, $P<0.001$; ε_e: 25.92 (17.13, 34.17) vs. 33.95 (25.66, 39.81) %, $P = 0.002$; ε_a: 22.20 (15.73, 27.68) vs. 25.31 (18.39, 30.24) %, $P = 0.025$; SRs: 2.62 ± 0.83 vs. 3.04 ± 0.95 1/s, $P = 0.005$; SR_e: -2.21 (-3.05, -1.46) vs. -2.62 (-3.62, -2.04) 1/s, $P = 0.004$]. In CTD patients with preserved RVEF, there are significantly increased LV mass index, RVEDVi, RVSVi, $RAEF_t$ and $RAEF_a$, whereas

decreased RAV_{min} index compared with controls (all $P < 0.05$). Both RV and RA strain parameters tended to worsen with increasing SUA levels among subgroups. Compared with patients in the first tertile, those in the third tertile had significantly impaired RV GLS and RV GCS as well as RA reservoir and conduit strain parameters. RV GLS was also significantly lower in the second tertile (all $P < 0.05$). The RV GLSR, RV GCSR, and RA booster strain parameters had no significant difference across tertiles of SUA in CTD patients (all $P > 0.05$). Figures 2–4 show the RV and RA strain parameters in CTD subgroups based on the tertiles of SUA and controls.

3.3 Association of SUA and CMR-derived RV and RA strain parameters in CTD patients

In univariable linear regression analyses, the elevated SUA levels were significantly associated with the impaired RV GLS and RV GCS as well as RA reservoir and conduit strain and strain rates (absolute value of β = 0.250 to 0.293, all $P < 0.05$). Multivariable linear regression analyses showed that the elevated SUA level was still independently correlated with impaired ε_e (β = -0.212, P = 0.035) and RV GCS (β = 0.207, P = 0.019) in CTD patients after adjustment for age, sex, hyperlipidemia, disease duration, pulmonary fibrosis, and PH as well as excluded the parameters with collinearity (including RVEDVi, RVESVi, RVSVi, $RAEF_t$, $RAEF_p$, $RAEF_a$, RAV_{max} index, and $RAV_{pre\text{-}A}$ index). There were no independent associations between SUA levels and right cardiac strain rates (all $P > 0.05$). Other details are presented in Table 3 and Supplementary Table S1.

3.4 Reproducibility of RV and RA strain analysis

The inter-observer and intra-observer variability values of the RV and RA strain parameters are presented in Table 4. There were excellent intra- and inter-observer agreements in the measurement of RV strain and strain rates (ICC = 0.795 to 0.959 and 0.764 to 0.936, respectively) as well as RA phasic strain and strain rate (ICC = 0.850 to 0.947 and 0.830 to 0.936, respectively).

TABLE 2 Cardiac magnetic resonance characteristics of patients with connective tissue disease and controls.

	Controls (n = 52)	CTD (n = 104)	CTD			CTD with preserved RVEF (n = 70)
			First tertile (n = 34)	Second tertile (n = 36)	Third tertile (n = 34)	
LV parameters						
LVEF, %	62.97 ± 5.94	62.55 ± 6.40	63.30 ± 5.98	61.29 ± 6.12	63.14 ± 7.06	63.75 ± 5.82
LVEDVi, mL/m^2	68.51 ± 9.76	71.44 ± 13.36	71.58 ± 11.71	71.64 ± 12.25	71.07 ± 16.15	71.58 ± 12.45
LVESVi, mL/m^2	25.02 [21.45, 29.55]	25.68 [21.66, 30.27]	24.95 [21.85, 30.11]	27.52 [22.00, 33.64]	24.32 [20.11, 30.15]	24.86 [21.75, 28.53]
LVSVi, mL/m^2	43.18 ± 7.40	44.18 ± 7.40	45.01 ± 7.54	43.49 ± 7.30	44.07 ± 7.50	45.29 ± 7.40
LV mass index, g/m^2	38.82 [35.30, 44.75]	44.55 [39.44, 51.05] [a]	42.57 [38.12, 48.32]	47.46 [39.25, 50.80] [a]	45.86 [42.92, 58.90] [a]	45.14 [39.44, 50.31][a]
RV parameters						
RVEF, %	57.28 ± 5.21	52.64 ± 10.23 a	56.51 ± 5.98	51.30 ± 9.59 ab	50.18 ± 13.02 [a]	57.95 ± 5.79
RVEDVi, mL/m^2	62.23 [55.35, 70.40]	70.38 [61.56, 82.89] [a]	67.47 [61.22, 81.17]	71.71 [62.58, 82.79]	71.78 [62.75, 91.56] [a]	67.47 [61.22, 76.30] [a]
RVESVi, mL/m^2	26.55 [23.87, 30.25]	30.77 [25.73, 40.13] [a]	28.61 [24.73, 36.30]	30.77 [27.04, 40.46] [a]	38.47 [24.05, 52.69] [a]	28.29 [23.88, 34.84]
RVSVi, mL/m^2	36.68 ± 7.88	37.72 ± 7.88	39.33 ± 7.07	36.56 ± 6.86	37.34 ± 9.48	39.48 ± 7.12 [a]
RV mass index, g/m^2	8.86 [8.04, 10.42]	9.87 [7.88, 11.95]	8.67 [7.57, 11.08]	9.37 [7.56, 11.38]	10.93 [8.98, 13.65] [ab]	8.85 [7.52, 10.73]
GLS, %	-25.67 ± 3.38	-24.42 ± 4.71	-26.49 ± 3.93	-23.12 ± 4.66 [b]	-23.12 ± 4.90 [b]	-25.26 ± 3.72
GCS, %	-19.17 ± 2.84	-16.17 ± 4.44 [a]	-17.91 ± 3.32	-15.79 ± 4.37 [a]	-14.83 ± 5.03 [ab]	-17.81 ± 3.56 [a]
GLSR, 1/s	-1.40 [-1.55, -1.22]	-1.36 [-1.60, -1.17]	-1.35 [-1.67, -1.19]	-1.36 [-1.55, -1.19]	-1.39 [-1.67, -1.10]	-1.37 [-1.53, -1.16]
GCSR, 1/s	-1.04 [-1.27, -0.76]	-0.90 [-1.18, -0.74]	-0.95 [-1.14, -0.80]	-0.86 [-1.27, -0.77]	-0.79 [-1.21, -0.65]	-0.95 [-1.17, -0.71]
RA parameters						
RAEFt, %	57.40 [53.14, 64.34]	60.12 [51.52, 67.18]	61.40 [51.94, 70.88]	61.12 [55.24, 69.32]	55.92 [49.29, 63.55]	61.94 [54.31, 69.37] [a]
RAEFp, %	26.62 ± 9.67	23.40 ± 11.22	27.31 ± 12.41	23.63 ± 10.49	19.24 ± 9.43 [ab]	25.53 ± 11.46
RAEFa, %	42.65 ± 10.14	46.33 ± 11.77	45.70 ± 10.89	47.35 ± 13.12	45.89 ± 11.37	47.53 ± 10.73 [a]
RAV_{max} index, mL/m^2	31.85 [25.28, 35.91]	30.28 [24.54, 36.99]	28.82 [23.45, 35.80]	32.26 [25.30, 36.24]	32.65 [23.22, 54.12]	28.10 [22.71, 35.59]
RAV_{min} index, mL/m^2	13.15 [10.01, 15.21]	12.29 [8.41, 16.87]	11.93 [7.86, 14.70]	11.56 [8.79, 14.95]	13.98 [9.03, 18.71]	11.51 [7.93, 13.92] [a]
$RAV_{pre\text{-}A}$ index, mL/m^2	22.08 [18.17, 27.29]	23.63 [18.09, 29.09]	23.31 [14.61, 27.40]	22.82 [18.31, 28.14]	26.51 [18.72, 31.69]	22.59 [16.39, 25.85]

(Continued)

TABLE 2 Continued

	Controls (*n* = 52)	CTD (*n* = 104)	CTD			CTD with preserved RVEF (*n* = 70)
			First tertile (*n* = 34)	Second tertile (*n* = 36)	Third tertile (*n* = 34)	
RA parameters						
ε_s, %	58.04 ± 12.60	49.52 ± 16.90 [a]	56.24 ± 16.15	48.98 ± 16.84 [a]	43.36 ± 15.62 [ab]	54.18 ± 16.09
ε_e, %	33.95 [25.66, 39.81]	25.92 [17.13, 34.17] [a]	29.90 [24.61, 43.41]	21.89 [16.82, 33.97] [a]	22.57 [11.92, 31.03] [ab]	29.75 [21.78, 38.27]
ε_a, %	25.31 [18.39, 30.24]	22.20 [15.73, 27.68] [a]	23.60 [17.90, 28.27]	22.08 [15.05, 29.62]	19.82 [13.86, 25.67]	23.40 [16.46, 29.76]
SRs, 1/s	3.04 ± 0.95	2.62 ± 0.83 [a]	2.87 ± 0.74	2.65 ± 0.15	2.34 ± 0.80 ab	2.83 ± 0.78
SRe, 1/s	-2.62 [-3.62, -2.04]	-2.21 [-3.05, -1.46] [a]	-2.63 [-3.65, -2.09]	-2.41 [-2.97, -1.66]	-1.64 [-2.31, -1.15] [ab]	-2.59 [-3.32, -1.82]
SRa, 1/s	-2.97 [-3.77, -2.42]	-2.70 [-3.53, -1.89]	-2.95 [-3.48, -2.47]	-2.74 [-3.84, -1.68]	-2.24 [-3.25, -1.58]	-2.93 [-3.70, -2.18]

Values are presented as the mean ± standard deviation, number (percentage), or median [interquartile range].
CTD, connective tissue disease; LV, left ventricular; LVEF, left ventricular ejection fraction; LVEDVi, left ventricular end-diastolic volume index; LVESVi, left ventricular end-systolic volume index; LVSVi, left ventricular stroke volume index; RV, right ventricular; RVEF, right ventricular ejection fraction; RVEDVi, right ventricular end-diastolic volume index; RVESVi, right ventricular end-systolic volume index; RVSVi, right ventricular stroke volume index; GLS, global longitudinal strain; GCS, global circumferential strain; GLSR, global longitudinal strain rate; GCSR, global circumferential strain rate; RA, right atrial; RAEF, right atrial emptying fraction; RAV, right atrial volume; ε_s, right atrial reservoir strain; ε_e, right atrial conduit strain; ε_a, right atrial booster strain; SRs, reservoir strain rate; SRe, conduit strain rate; SRa, booster pump strain rate; a, $P < 0.05$ versus controls; b, $P < 0.05$ versus patients in the first tertile; c, $P < 0.05$ versus patients in the second tertile.

4 Discussion

To our knowledge, this is the first study to show the relationship between SUA levels and CMR-FT-derived right atrial and ventricular strain parameters in CTD patients. This study found that CTD patients had significant right cardiac remodeling and higher SUA levels compared to controls, and the SUA levels independently correlated with ε_e and RV GCS. Therefore, the SUA levels may be a potential risk factor of right cardiac chamber remodeling.

4.1 Right cardiac chamber remodeling in patients with CTD

The 2021 European League Against Rheumatism recommendations highlight the importance of cardiovascular risk management in patients with CTD (23). Until now, left heart involvement in patients with CTD has been extensively explored. CTD often combined with a variety of common complications under the influence of systemic proinflammatory state and other factors such as genetic susceptibility, including left heart disease, pulmonary fibrosis, or PH—all of these can affect RV afterload. Adaptive RV remodeling may occur in the early stages of the disease to overcome increased RV afterload and ensure sufficient stroke volume. With the progression of the disease, these compensatory mechanisms gradually become inadequate, leading to RV excessive dilatation, dysfunction, and, eventually, right heart failure (24). Although RVEF typically indicates a poor prognosis, the decreased RVEF will not appear until the late stage of heart failure. CMR-FT has the potential to detect myocardial function abnormalities earlier in the disease. In patients with CTD, there are significant correlations between reduced RVEF and impaired RV GCS and RV GLS. RV GCS remains impaired even in CTD patients with preserved RVEF. These findings were consistent with those of Wu et al. (5). Athletes' hearts also had a similar RV mechanical pattern in a recent study, with lower RVEF and RV GCS but preserved or even higher RV GLS compared with sedentary controls (25). These changes might be related to modified RV myocardial fiber orientation. Physiologically, RV contraction mainly depends on longitudinal shortening (26). The relative dominance of the circumferentially oriented fibers can be observed when chronically pressure-loaded (27). In patients with PH, the transverse wall motion of the mid-RV is significantly reduced and has stronger relationship with RVEF than longitudinal motion (28). Pettersen et al. (29) also demonstrated a predominant circumferential over longitudinal free wall shortening in patients with a systemic RV.

Furthermore, RV diastolic dysfunction may precede systolic dysfunction in diseases with chronic RV pressure overload. RA, mainly through increased dimension (higher RA reservoir function) and contractility (higher RA booster pump function), maintains RV filling of the stiffened ventricle, whereas passive emptying (conduit function) is the first to decline due to the reduced RA–RV pressure gradient (30, 31). The increased $RAEF_t$ and $RAEF_a$ and decreased RAV_{min} index may play as a compensatory mechanism in CTD patients with preserved RVEF. RA dilatation indicated the presence of RV diastolic stiffness (32). Excessive RA dilation and stiffness occur as the disease progresses, leading to deterioration of RA phasic functions and atrioventricular uncoupling. In the present study, the RA strain parameters significantly decline in CTD patients without overt RA dilation and dysfunction. Moreover, our findings indicate that ε_s was positively correlated with RVEF as well as negatively correlated with RAV_{min} index in patients with CTD, consistent with that observed in PH (33, 34). Therefore, RA strain parameters may be more sensitive and earlier predictors of RV overload in patients with CTD.

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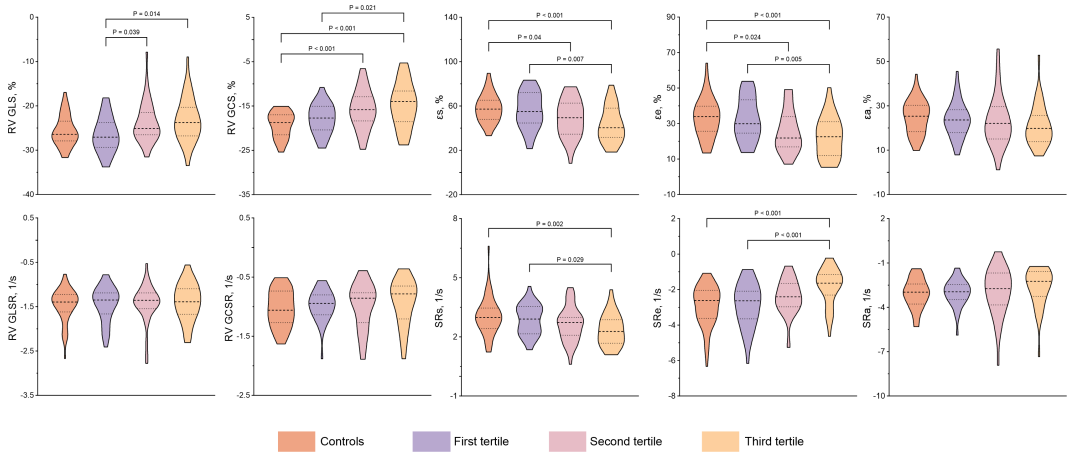


FIGURE 2
Comparison of RV and RA strain and strain rate between CTD subgroups by the tertiles of SUA and controls. CTD, connective tissue disease; SUA, serum uric acid; RV, right ventricular; GLS, global longitudinal strain; GLSR, global longitudinal strain rate; GCS, global circumferential strain; GCSR, global circumferential strain rate; ε_s, right atrial reservoir strain; ε_e, right atrial conduit strain; ε_a, right atrial booster strain; SRs, reservoir strain rate; SRe, conduit strain rate; SRa, booster strain rate.

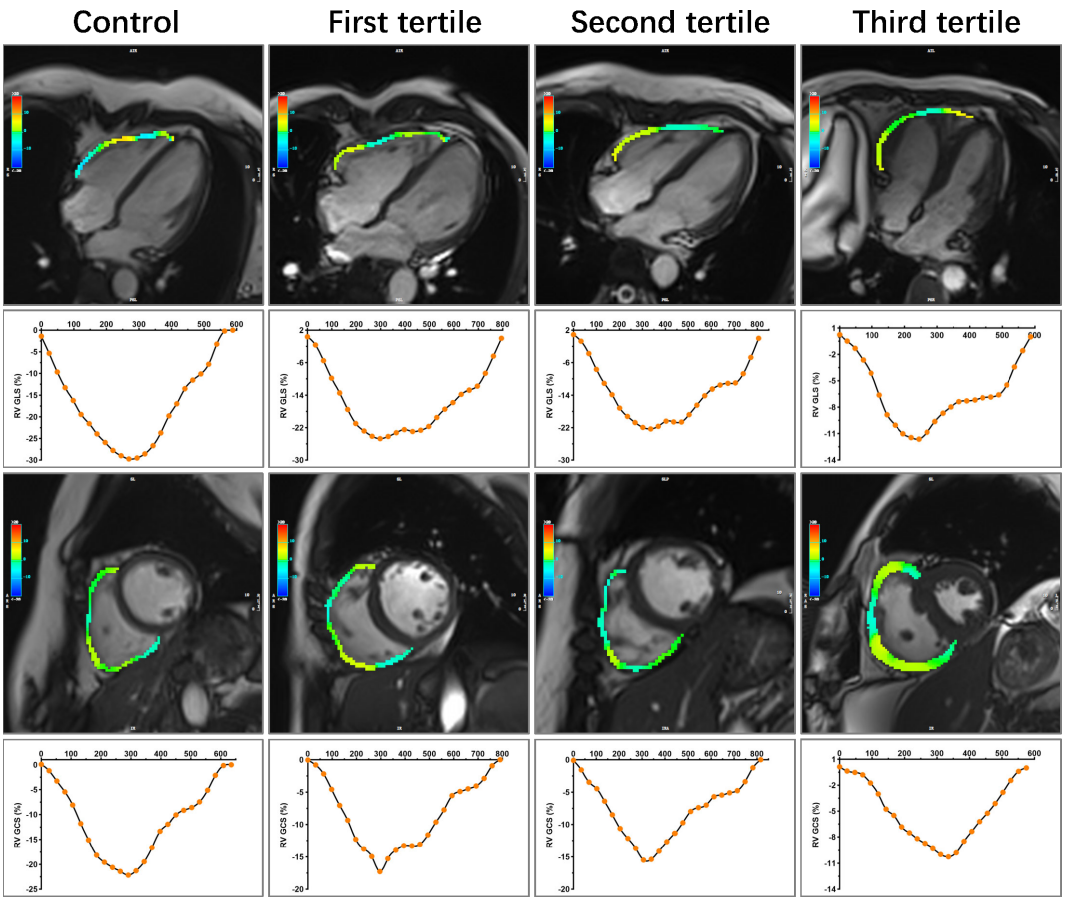


FIGURE 3
Representative images of RV longitudinal and circumference strain in control and CTD subgroups by the tertiles of SUA. A 47-year-old female served as control. A 50-year-old female with SLE was in the first tertile of SUA, and the disease duration at the time of CMR was 168 months. SUA level = 166 umol/L, sPAP = 51.24 mmHg. A 49-year-old female with SLE was in the second tertile of SUA, and the disease duration at the time of CMR was 120 months. SUA level = 279 umol/L, sPAP = 45.96 mmHg. A 49-year-old female with SLE was in the third tertile of SUA, and the disease duration at the time of CMR was 216 months. SUA level = 427 umol/L, sPAP = 85.00 mmHg. RV, right ventricular; CTD, connective tissue disease; SUA, serum uric acid; SLE, systemic lupus erythematosus; sPAP, systolic pulmonary arterial pressure; GLS, global longitudinal strain; GCS, global circumferential strain.

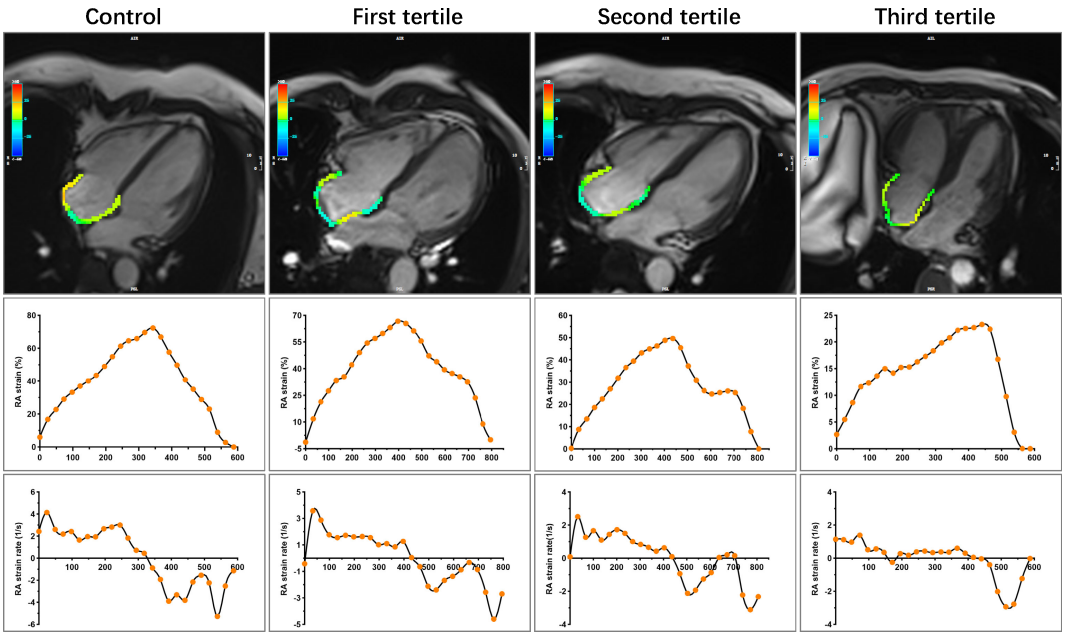


FIGURE 4
Representative images of RA strain and strain rates in control and CTD subgroups by the tertiles of SUA. Other details are as shown in Figure 3. CTD, connective tissue disease; SUA, serum uric acid; RA, right atrial.

4.2 Association between SUA and right cardiac chamber remodeling

The present results show that patients with CTD had significantly elevated SUA levels compared with the controls, and right cardiac chamber remodeling is more severe in CTD patients with higher SUA level subgroups. However, the precise mechanisms underlying the relationship between elevated SUA and right cardiac chamber remodeling found in this study are still unclear and remain to be elucidated.

The elevated SUA levels may lead to right cardiac chamber remodeling by promoting the increased RV afterload in patients with CTD. The RV is very sensitive to afterload change compared with the left ventricle. In the present study, nearly half of the patients were cases combined with pulmonary involvement, which had a higher percentage in the third tertile. Several studies found that the SUA levels were significantly higher in CTD patients with PH than in those without PH (11–36). Although Castillo-Martínez et al. (10) reported that the baseline SUA levels were similar whether SLE patients had PH or not, the steady hyperuricemia was associated with the development of PH in SLE patients without PH at baseline. In CTD-PAH, the baseline SUA levels also had a positive correlation with pulmonary vascular resistance, and steady hyperuricemia may predict a worsened clinical prognosis (12). The elevated SUA level may promote pulmonary vascular remodeling through increased oxidative stress, depletion of nitric oxide, endothelial dysfunction, vasoconstriction, and proliferation of vascular smooth muscle cells (7). Although Savale et al. (37) did not find the influence of hyperuricemia in pulmonary vascular remodeling, UA metabolism was also disturbed in the remodeled pulmonary vascular walls in PAH. Moreover, Wang et al. (38) observed that the SUA levels were higher in rheumatoid arthritis patients with ILD than in patients without ILD. The SUA levels were positively correlated with KL-6, a biomarker of ILD. They further explored the involvement of UA in the pathogenesis of ILD through *in vitro* cellular experiments and found that UA can induce the epithelial-to-mesenchymal transition in alveolar epithelial cells, which is a critical step in the progression of ILD.

Inflammation may play a key role between elevated uric acid and right heart remodeling. Elevated SUA levels may enhance the inflammatory response and thus affect right cardiac chamber remodeling in patients with CTD. Our data shows that patients in the third tertile had significantly impaired RV GLS and RV GCS as well as RA reservoir and conduit strain parameters compared with those in the first tertile, and RV GLS was also significantly lower in the second tertile—even the first and second tertiles of SUA were in the normal reference range. There was independent association between SUA levels and RV GCS and ε_e after adjustment for pulmonary fibrosis, PH, RVEF, and other potential confounding factors. Chronic long-term inflammation is one of the main pathological features in patients with CTD and be considered to have a contributing role in cardiovascular involvement as well as is one of the most important causes of elevated SUA levels. Increased SUA can, in turn, exacerbate the inflammatory response, resulting in a vicious cycle (39). Even though SSc-PAH patients have similar pulmonary vascular resistance with idiopathic pulmonary hypertension patients, there was a worse RV function and prognosis in the former (40). The primary myocardial involvement derived by inflammation may be one of the potential causes (41). However, none of these mechanisms is independent. They reinforce and interact with each other, ultimately leading to right cardiac chamber remodeling.

TABLE 3 Univariable and Multivariable linear regression analyses of serum uric acid and cardiac magnetic resonance-derived parameters on right ventricular (RV) and right atrial (RA) strain parameters in patients with connective tissue disease.

	RV (β)				RA (β)					
	GLS, %		GCS, %		ε_s, %		ε_e, %		ε_a, %	
	Univariable	Multivariable	Univariable	Multivariable	Univariable	Multivariable	Univariable	Multivariable	Univariable	Multivariable
SUA, umol/L	0.260[a]	0.127	0.285[a]	0.207[a]	-0.270[a]	-0.163	-0.286[a]	-0.212[a]	-0.107	–
eGFR, mL/min/1.73m^2	-0.086	–	-0.139	–	0.050	–	0.185		-0.152	–
RVEF, %	-0.629[a]	-0.502[a]	-0.644[a]	-0.665[a]	0.459[a]	0.259[a]	0.463[a]	0.358[a]	0.210[a]	-0.008
RVEDVi, mL/m^2	0.564[a]	–	0.415[a]	–	-0.343[a]	–	-0.281[a]	–	-0.243[a]	–
RVESVi, mL/m^2	0.678[a]	–	0.583[a]	–	-0.433[a]	–	-0.407[a]	–	-0.238[a]	–
RVSVi, mL/m^2	-0.062	–	-0.212[a]	–	0.063	–	0.183	–	-0.127	–
RV mass index, g/m^2	0.527[a]	0.206	0.355[a]	-0.114	-0.432[a]	-0.182	-0.367[a]	-0.082	-0.289[a]	-0.219
RAEFt, %	-0.384[a]	–	-0.169	–	0.608[a]	–	0.416[a]	–	0.537[a]	–
RAEFp, %	-0.402[a]	–	-0.271[a]	–	0.406[a]	–	0.493[a]	–	0.080	–
RAEFa, %	-0.217[a]	–	-0.033	–	0.485[a]	–	0.190	–	0.613[a]	–
RAV_{max} index, mL/m^2	0.204[a]	–	0.264[a]	–	-0.238[a]	–	-0.162	–	-0.210[a]	–
RAV_{min} index, mL/m^2	0.317[a]	0.032	0.224[a]	0.021	-0.493[a]	-0.318[a]	-0.359[a]	-0.190	-0.407[a]	-0.318[a]
$RAV_{pre\text{-}A}$ index, mL/m^2	0.311[a]	–	0.307[a]	–	-0.364[a]	–	-0.325[a]	–	-0.223[a]	–
R^2		0.501		0.471		0.382		0.297		0.248

Multivariable analyses were adjusted to all variables without collinearity and univariable p-value <0.05 as well as age, sex, hyperlipidemia, disease duration, pulmonary fibrosis, and PH, pulmonary hypertension. Other abbreviations are as shown in Tables 1 and 2.
β, value of standardized coefficients.

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TABLE 4 Intra- and inter-observer variabilities of cardiac magnetic resonance-derived right ventricular (RV) and right atrial (RA) strain parameters.

	Intra- observer (n = 40)		Inter-observer (n = 40)	
	ICC	95% CI	ICC	95% CI
RV strain parameters				
GLS, %	0.930	0.872 – 0.962	0.911	0.838 – 0.952
GCS, %	0.959	0.919 – 0.979	0.936	0.882 – 0.965
GLSR, 1/s	0.795	0.645 – 0.886	0.764	0.598 – 0.868
GCSR, 1/s	0.878	0.766 – 0.936	0.807	0.666 – 0.893
RA strain parameters				
ε_s, %	0.933	0.876 – 0.964	0.892	0.805 – 0.941
ε_e, %	0.919	0.853 – 0.956	0.875	0.777 – 0.932
ε_a, %	0.947	0.903 – 0.972	0.936	0.883 – 0.966
SRs, 1/s	0.903	0.822 – 0.948	0.855	0.741 – 0.921
SRe, 1/s	0.850	0.736 – 0.918	0.830	0.702 – 0.906
SRa, 1/s	0.939	0.885 – 0.967	0.917	0.850 – 0.955

Other abbreviations are as shown in Table 2.
ICC, intra-class correlation coefficient; CI, confidence interval.

In addition, both SUA levels and right heart remodeling are affected by exercise as a confounding factor. On the one hand, low- and moderate-intensity exercise can improve hyperuricemia (42), but high-intensity exercise may increase uric acid production and reduce excretion, leading to increased SUA (43, 44). On the other hand, long-term and intensive endurance exercise has potential effects on the heart, especially the right side, and even on pulmonary circulation (45). However, only one patient in our cohort has a history of long-term and high-intensity exercise and stopped training in the last 4 years. The effect of exercise was not discussed separately in this study. Nonetheless, previous studies suggested that exercise therapy was beneficial to CTD patients and moderates the side effects of high-dose glucocorticoid treatment (46–48). The influence of exercise on right heart remodeling and finding an appropriate kinesitherapy in CTD patients are worth exploring.

5 Limitation

There are some limitations in this study. First, this was a single-center study with a relatively small sample size. This study did not discuss the different types of CTD separately due to the limitations of the sample size, despite which is a heterogeneous group of diseases. Compared with IIM, other CTDs more likely to combine with PH and myocardial involvement may have more obvious right cardiac chamber remodeling and higher SUA levels. However, the common medications used in CTD patients are similar, including glucocorticoids and immunosuppressants, and based on the different symptoms performed personalized treatment. Second, causality cannot be determined due to the cross-sectional design; longitudinal or interventional studies should be conducted to further explore the causal relationship between SUA levels and right heart remodeling. Third, although we observed a correlation between SUA levels and LV mass index similar to previous studies in other diseases (49–51), we did not investigate the correlation further for the reason that to we focused on the right cardiac chamber remodeling in present study. The relationship between SUA levels and myocardial fibrosis, edema, and microvascular perfusion in patients with CTD has also not been investigated in this study due to the same reason. Subsequent studies need to focus on these aspects. Finally, given the lack of follow-up data on patients with CTD, further follow-up studies should be performed to explore the prognostic value of SUA levels and right heart strain parameters.

6 Conclusion

In summary, SUA may be a potential risk factor of right cardiac chamber remodeling and is independently associated with impaired ε_e and RV GCS in patients with CTD, which might be used as a non-invasive and low-cost indicator to determine subclinical right cardiac chamber remodeling.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Huazhong University of Science and Technology, Tongji Medical School Affiliated Tongji Hospital Medical Ethics Committee. The studies were conducted in accordance with the local legislation and institutional requirements. The human samples used in this study were acquired from primarily isolated as part of your previous study for which ethical approval was obtained. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements. Written informed consent was not obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article because this study protocol was approved by the Institutional Review Board of our hospital (TJ-IRB20230914), and the requirement for written informed consent was waived due to the retrospective design.

Author contributions

YT: Methodology, Writing – original draft. ZY: Writing – original draft, Writing – review & editing. JW: Methodology, Writing – original draft, Writing – review & editing. DT: Methodology, Writing – review & editing. YL: Methodology, Writing – review & editing. CX: Methodology, Writing – review

& editing. LH: Supervision, Writing – review & editing. LX: Supervision, Writing – review & editing.

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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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1351197/full#supplementary-material

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EDITED BY
Gabor Czibik,
Queen Mary University of London,
United Kingdom

REVIEWED BY
Hoda Elkafas,
University of Illinois Chicago, United States
Bo Sun,
First Affiliated Hospital of Zhengzhou University, China

*CORRESPONDENCE
Xuan Wang
wangxuan2024001@163.com
Xingjun Han
hanxingjun1228@163.com

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Uterine leiomyoma causes an increase in systolic blood pressure: a two-sample Mendelian randomization study

Hui Xu[1,2], Yuxia Ma[3], Yi Long[4], Ren Liu[5], Ziyang Cheng[2], Xiuzhen Xie[1], Xingjun Han[6*] and Xuan Wang[6*]

[1]Obstetrics and Gynecology Department, The Second Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Jinan, China, [2]The First Clinical College, Shandong University of Traditional Chinese Medicine, Jinan, China, [3]College of Acupuncture, Moxibustion and Tuina, Shandong University of Traditional Chinese Medicine, Jinan, China, [4]Shandong Provincial Traditional Chinese Medicine Data Center Management Office, The Second Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Jinan, China, [5]Medical Affairs Office, The Fifth Affiliated Hospital Sun Yat-sen University, Zhuhai, China, [6]Disease Prevention Center, The Second Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Jinan, China

Objectives: Hypertension and hypertensive disorders of pregnancy (HDP) are common diseases in women at different stages, which affect women's physical and mental health, and the impact of the latter on the offspring cannot not be ignored. Observational studies have investigated the correlation between uterine leiomyoma (UL) and the above conditions, but the relationship remains unclear. In this study, we employed two-sample Mendelian randomization (MR) analysis to assess the association between UL and hypertension, HDP, as well as blood pressure.

Methods: We collected genetic association data of UL (35,474 cases), hypertension (129,909 cases), HDP (gestational hypertension with 8,502 cases, pre-eclampsia with 6,663 cases and eclampsia with 452cases), systolic blood pressure (SBP) and diastolic blood pressure (DBP) (both 757,601 participants) from published available genome-wide association studies (GWAS). The single nucleotide polymorphisms (SNPs) associated with UL phenotype were used as instrumental variables, and hypertension, three sub-types of HDP, SBP and DBP were used as outcomes. The inverse-variance weighted (IVW) method was employed as the primary method of causal inference. Heterogeneity was assessed using Cochran's Q test, and sensitivity analyses were conducted using MR-Egger regression and MR pleiotropy residual sum and outlier (MR-PRESSO) tests to evaluate the pleiotropy of instrumental variables. PhenoScanner search was used to remove confounding SNP. Robustness and reliability of the results were assessed using methods such as the weighted median and weighted mode.

Results: The IVW analysis revealed a positive correlation between genetically predicted UL and SBP [odds ratio (OR)= 1.67, 95% confidence interval (CI):1.24~2.25, P = 0.0007], and no statistical association was found between UL and hypertension, HDP, or DBP. The MR-Egger regression suggested that the above causal relationships were not affected by horizontal pleiotropy. The weighted median method and weighted model produced similar results to the IVW.

Conclusion: Based on large-scale population GWAS data, our MR analysis suggested a causal relationship between UL and SBP. Therefore, women with UL, especially pregnant women, should pay attention to monitoring their blood pressure levels. For patients with hypertension who already have UL, interventions for UL may serve as potential therapeutic methods for managing blood pressure.

KEYWORDS

uterine leiomyoma, hypertension, hypertensive disorders of pregnancy, Mendelian randomization, causal relationship

Introduction

Hypertension is a common chronic disease. It is estimated that the number of global adults with hypertension is approaching 1 billion in 2000, and is projected to rise to 1.56 billion by 2025 (1), seriously affects people's physical and mental health. Hypertensive disorder of pregnancy (HDP) has a high prevalence globally, affecting 3% to 5% of pregnant women worldwide. Nearly one-third of hospitalized women die from this disease, making it the leading cause of maternal mortality (2, 3). Chronic hypertension increases the incidence of pregnancy-related diseases and adverse pregnancy outcomes in women, such as hypertensive disorders of pregnancy, intrauterine growth restriction, preterm birth, and stillbirth (4, 5). Uterine leiomyoma (UL) is a common benign tumor in women of childbearing age, and it is associated with adverse pregnancy outcomes, including preterm delivery and placental abruption (6, 7). The prevalence of UL is difficult to accurately estimate, and some patients are severely underestimated due to clinical asymptomatic status. Statistically, the prevalence of UL ranges from 4.5% and 68.6% in different countries (8).

Over the years, many studies have investigated the relationship between UL and cardiovascular diseases (9–15), but the conclusions of the studies have not yet been unified. For example, Chen et al. (9) conducted a cross-sectional study and meta-analysis involving 8,401 patients, showing a significant correlation between UL and hypertension. In contrast, the Northern Finland Birth Cohort study of 3,635 participants analyzed the situation from birth to 46 years and found no association between UL and hypertension (14). Additionally, hormonal changes often occur during pregnancy, and estrogen and progesterone often play a key role in the development of UL (16). A study in the United States showed that UL is relatively common during pregnancy, with significant differences in prevalence among different ethnic groups (17). It is worth noting that a recent study found that UL in early pregnancy may increase the risk of HDP (18). Due to inconsistent results from observational studies and the limitations in causal inference that exist in traditional observational studies, such as confounding factors and reverse causality, it is necessary to further investigate the relationship between UL and hypertension and HDP.

Mendelian randomization (MR) is a research method in genetic epidemiology that evaluates the causal relationship between exposure and outcome with the help of genetic variants, such as single nucleotide polymorphisms (SNPs), which are used as instrumental variables. Since genes are randomly allocated at the time of conception and are not affected by external environment or social factors, MR can avoid the confounding effects and reverse causality that exist in the observational studies, making it a relatively precise epidemiological method (19, 20). Genome-wide association study (GWAS) is a method used to detect gene variations associated with complex human diseases or traits, and its objective is to reveal the impact of genetic variations on the risk of complex diseases, thereby providing new clues and strategies for prevention, diagnosis, and treatment (21). In this study, we explored the relationships between UL and hypertension, gestational hypertension, pre-eclampsia, and eclampsia using a two-sample MR study through GWAS summary data, and further investigated the association between UL and systolic blood pressure (SBP) and diastolic blood pressure (DBP).

Materials and methods

Data sources

The genetic instruments for UL were obtained from the published GWAS meta-analysis of UL in 2019 (22), which is publicly available. The GWAS meta-analysis included the Women's Genome Health Study, Northern Finnish Birth Cohort, QIMR Berghofer Medical Research Institute, UK Biobank (UKBB), and the cohort studies from 23andMe. The study population included 35,474 cases and 267,505 controls, and all individuals were of European descent.

The genetic data for hypertension were obtained from the published GWAS of age-related diseases in 2021 (23), which extracted information from UKBB and contained 129,909 hypertension cases and 354,689 controls. There exist some overlap between the UL dataset and the hypertension dataset, accounting for up to 41.80% of the samples. However, the

calculated probability of type I error due to this overlap was 0.05, with potential bias less than 1%, which can be considered negligible.

The genetic data for HDP were obtained from the FinnGen R9 biobank published in May 2023. We chose three sub-types: gestational hypertension, pre-eclampsia, eclampsia. The study population was all of European ancestry.

The genetic data for SBP and DBP were selected from the International Blood Pressure Consortium and UKBB with a total of 757,601 participants of European ancestry (24) (Table 1). Similarly, there was some overlap between the exposure dataset and the current dataset (26.74% of the samples), but the potential bias due to this overlap was calculated to be negligible, less than 1%.

Study design

We tested the causal relationship between the exposure (UL) and each outcome (hypertension, the three sub-types of HDP, SBP, and DBP) using MR analysis. To make reasonable interpretations of MR analysis, three core assumptions must be satisfied (25). Firstly: Association - The genetic variations are strongly associated with exposure. Secondly: Independence - The genetic variations are independent of the confounders that affect the association between exposure and outcome. Thirdly: Exclusion - The genetic variations only affect the outcome through exposure (Figure 1).

Selecting methods for instrumental variables

Genetic variations, SNPs, were used as instrumental variables, which were extracted from relevant GWAS (Table 1). The selected instrumental variables needed to satisfy the following conditions: (1) $P < 5\times10^{-8}$, SNPs were significantly correlated with UL at the genome-wide level; (2) $R^2 = 0.001$, genetic distance=10,000kb, remove linkage disequilibrium; (3) SNPs were not significantly associated with the outcome, setting $P = 5\times10^{-5}$; (4) SNPs did not have palindromic structures; (5) F>10, SNPs with an F-statistic less than 10 were excluded to avoid bias brought by weak instrumental variables. Weak instrumental variables are associated with exposure but have low explanatory capacity of exposure, providing little statistical power to test the hypothesis, which may lead to inaccurate estimation of causal effects and increased type I error probability (26). The strength of instrumental variables can be quantitatively evaluated using the F-statistic. The F-statistic for individual SNPs is calculated using the formula: $F = \beta^2/se^2$ (27). According to traditional experience, $F > 10$ is less affected by the bias caused by weak instrumental variables (28).

Statistical analysis

Using the Steiger Test to detect the presence of reverse causation for instrumental variables (29). The inverse-variance weighting (IVW) method is used as the main method for causal inference. This method assumes that all SNPs are valid and exist no pleiotropy, providing a well statistical power, but when SNPs have pleiotropy, the results may be biased (30, 31). Cochran' s Q test is used to assess heterogeneity among selected SNPs. The Q statistic is a weighted sum of squared deviations standardized by study variance. P<0.05 suggests heterogeneity, and a random effects model is used to assess causal associations, otherwise a fixed effects model is used (32). Because of the impact of pleiotropy on estimated association effects, MR-Egger regression is used to test for pleiotropy. If the intercept of the MR-Egger regression model is not zero (P<0.05), it indicates the presence of gene pleiotropy (33). The robustness of the results is analyzed using MR-Egger method, weighted median method and weighted mode. The MR-Egger method is mainly used for MR causal inference when there is potential pleiotropy (33). The weighted median method requires that at least 50% of the weights come from valid instrumental variables. It is the best choice when there is heterogeneity but no pleiotropy (34). The weighted model identifies multiple variables as valid instrumental variables to detect similar causal effects (35). When the main method (IVW) results are significant (P<0.05) and the other three methods agree with IVW, it can be considered that there is a causal relationship. Additionally, the MR pleiotropy residual sum and outlier (MR-PRESSO) method is used. If

TABLE 1 Sample sources in MR studies.

Trait	Sample size	Ancestry	Year	Website
Uterine leiomyoma	302,979	European	2019	https://www.nature.com/articles/s41467-019-12536-4
Hypertension	484,698	European	2021	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7610725/
Gestational [pregnancy-induced] hypertension	202,768	European	2023	https://storage.googleapis.com/finngen-public-data-r9/summary_stats/finngen_R9_O15_GESTAT_HYPERT.gz
Pre-eclampsia	200,929	European	2023	https://storage.googleapis.com/finngen-public-data-r9/summary_stats/finngen_R9_O15_PREECLAMPS.gz
Eclampsia	194,718	European	2023	https://storage.googleapis.com/finngen-public-data-r9/summary_stats/finngen_R9_O15_ECLAMPSIA.gz
Systolic blood pressure	757,601	European	2018	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6284793/
Diastolic blood pressure	757,601	European	2018	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6284793/

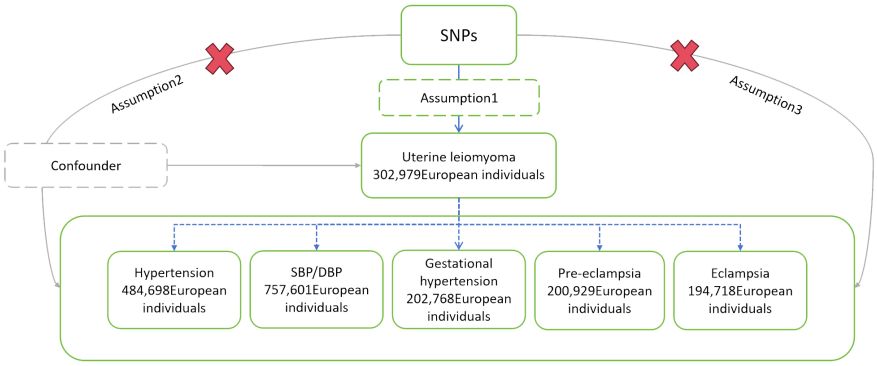


FIGURE 1
Schematic diagram of MR analysis. SNPs, single nucleotide polymorphisms; SBP, systolic blood pressure; DBP, diastolic blood pressure.

outliers are found, they are excluded and the causal association is re-estimated (36). To minimize the interference of horizontal pleiotropy on the results, each SNP was manually searched one by one in the human genotype-phenotype database PhenoScanner V2 (37) to identify and exclude risk factors shared with UL, hypertension and HDP, such as body mass index (BMI) (38, 39), waist circumference (40). Subsequently, SNPs with genome-wide significant associations ($P<5\times10^{-8}$) were selected, and causal inference was conducted anew.

Because this study has three subtypes of HDP, Bonferroni correction is used in order to reduce the probability of false-positive results. When $P<0.017$ (0.05/3), it indicates a significant causal relationship. The above methods are all performed using R 4.2.3. Statistical analysis was processed using the R Package Two Sample MR (v 0.5.8). The removal of outliers was conducted using the R Package MRPRESSO (v 1.0). And data visualization was conducted using the R Package forestploter (v 1.1.1) and CMplot(v 4.5.1).

Result

Incorporated instrumental variables

After selecting and harmonizing these instrumental variables (Figure 2), 24 SNPs were used for UL-hypertension MR-analysis, 25 SNPs were used for UL- three types of HDP MR-analysis, and UL-SBP and UL-DBP MR-analysis respectively included 21 SNPs and 18 SNPs (Those selected SNPs can be seen through Supplementary Tables 1-4). All instrumental variables passed the Steiger Test and no reverse causation was detected.

Causal link between UL and outcomes

The Cochran' s Q test showed significant heterogeneity in the outcomes of hypertension, SBP, and DBP, so a random effects model was used for causal inference. No heterogeneity was found in the outcomes of gestational hypertension, pre-eclampsia, and eclampsia ($P>0.05$), so a fixed effects model was used. The P-values of the MR-Egger intercept test for each outcome did not show evidence of pleiotropy (Table 2).

Genetic prediction suggested that UL may increase the risk of hypertension, gestational hypertension, pre-eclampsia, eclampsia, SBP and DBP, but none of these reached statistical significance. MR-Egger method, weighted median method and weighted model suggested that the causal association between UL and each outcome are consistent with IVW. It is worth mentioning that the MR-Egger analysis showed a significant association between UL and DBP [odds ratio, (OR)=1.62, 95% CI: 1.05~2.48, $P=0.04$]. However, the MR-Egger method is used for causal inference when there is potential pleiotropy, and the IVW method did not suggest a causal relationship, so it cannot be concluded that there is a causal association between UL and DBP (Table 3).

After the MR-PRESSO test, no outliers were found in the MR analysis of UL and the three subtypes of HDP. However, there existed outliers in the MR analysis of UL and hypertension (rs116251328, rs4325427, rs72709458), SBP (rs10508765, rs117245733, rs2131371, rs58415480, rs78378222), and DBP (rs117245733, rs35446936). After searching through the PhenoScanner V2 database for all instrumental variables, we identified and filtered out two SNPs (rs78378222, rs116251328), that had been found to be associated with BMI related phenotypes. No SNP had been found to be associated with phenotypes related to waist circumference. After removing these SNPs, the causal inference was re-conducted, and the main results are shown in Figure 3. The causal inference results of UL and hypertension and DBP were basically consistent with the results before removing the outliers. Interestingly, the IVW method of causal inference of UL and SBP suggested a positive casual relationship (OR =1.67, 95% CI: 1.24~2.25, $P=0.0007$). The MR-Egger method (OR=2.38, 95% CI: 1.03~5.46, $P=0.06$), weighted median method (OR=1.94, 95% CI: 1.40~2.70, $P=8.34\times10^{-5}$), and weighted model (OR=2.10, 95% CI: 1.33~3.33, $P=0.006$). All had consistent results with the IVW method. The Cochrane's Q test showed $P=0.06$, and the P-value of the MR-Egger intercept test was 0.39, indicating that there was no heterogeneity or pleiotropy. The scatter plot and Manhattan plot are shown in Figures 4 and 5. It can be considered that there is a positive causal relationship between UL and SBP.

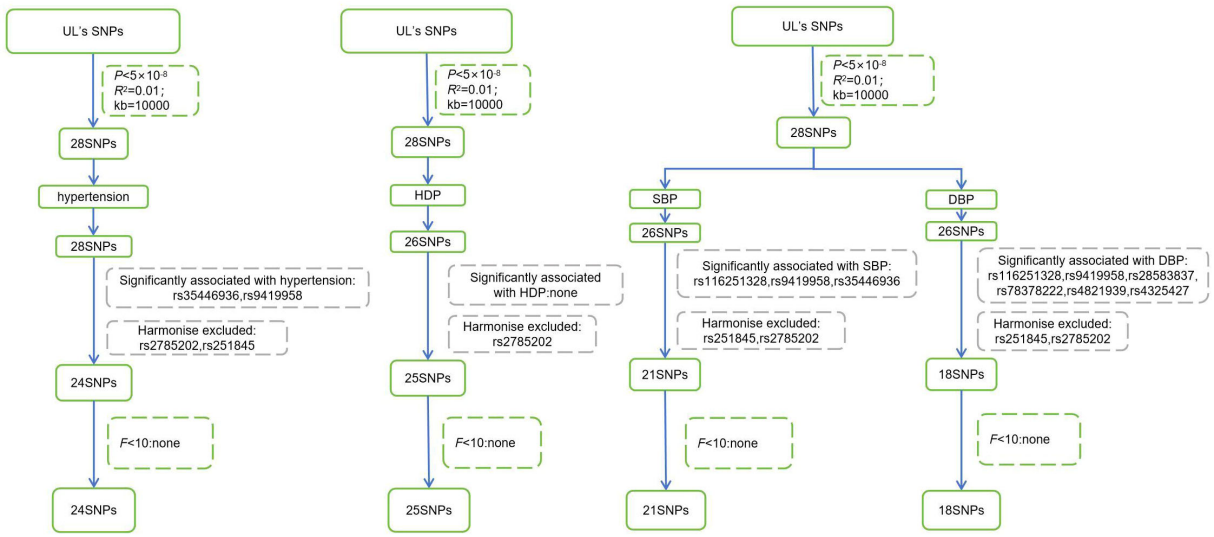


FIGURE 2
Steps for selecting instrumental variables. UL, Uterine leiomyoma; SNPs, single nucleotide polymorphisms; HDP, Hypertensive disorder of pregnancy; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Discussion

This study used large-scale GWAS data from public databases to explore the causal relationship between UL and each outcome (hypertension, HDP, SBP, and DBP) using two-sample MR methods. This analysis found that the occurrence of UL was positively associated with the risk of elevated SBP. No clear evidence of a causal relationship was found between UL and other outcomes.

UL is a common benign tumor in gynecology with a morbidity rate of up to 68.6% in certain area (8), while hypertension affects over 1 billion people worldwide (1). Both diseases have caused a significant medical burden globally. The relationship between UL and cardiovascular diseases has been studied for years (9–15), but the research conclusions remain controversial. Previous studies have reported that women with UL have higher SBP levels than women without UL (9, 10, 15), and similar results have been observed in studies of pregnant women (18). It is consistent with the results of our study, indicating that UL has certain effect on raising blood pressure. Another recent study has found that after surgical removal of UL, patients' SBP decreased (41), further indicating a possible link between UL and SBP. This study did not find a statistical association between UL and hypertension, which is consistent with the results of Uimari et al. (14) and Laughlin-Tommaso et al. (15). This may be due to differences in blood pressure baselines among different populations in different samples, and the hypertensive effect of UL may not yet meet the diagnostic criteria for hypertension in different populations, leading to variance in study results.

The pathophysiological connection between UL and hypertension remains unclear, yet they share structural similarities. Uterine leiomyoma is typically a benign tumor caused by the growth of smooth muscle cells, while hypertension is also associated with abnormalities in vascular smooth muscle (42). Therefore, some scholars have proposed that the proliferation of uterine smooth muscle is similar to the changes of atherosclerotic (43). The study by Hoag et al. (44) has found that the expression level of creatine kinase (CK) in uterine leiomyoma tissue was higher than that in adjacent uterine muscle tissue, and CK could provide ATP for vascular smooth muscle contraction (45). Additionally, higher CK activity is associated with increased arterial contractility (46). It has also been found that angiotensin II receptors type 1 and type 2 are expressed in both the myometrium and uterine fibroids (47). It is well known that angiotensin II plays an important role in the development of hypertension, so the involvement of the renin-angiotensin-aldosterone system may also explain, to some extent, the pathophysiology of these two diseases. The angiotensin-converting enzyme inhibitors can inhibit the production of angiotensin II, which have been commonly used in the treatment of hypertension. A cohort study analyzed 353,917 participants has found that the use of angiotensin-converting enzyme inhibitors could reduce the risk of UL, which seems to further support the link between UL and hypertension (48). Besides, UL may also stimulate

TABLE 2 Results of heterogeneity and pleiotropy tests for instrumental variables.

Outcome	heterogeneity test		pleiotropy test
	Q	*P*	*P*
Hypertension	63.49	<0.01	0.69
Gestational hypertension	23.05	0.52	0.91
Pre-eclampsia	29.42	0.20	0.81
Eclampsia	14.47	0.94	0.28
Systolic blood pressure	78.10	<0.01	0.97
Diastolic blood pressure	41.72	<0.01	0.13

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TABLE 3 UL's causal inference results with each ending.

Outcome	Method	nSNP	*beta*	*se*	OR(95%CI)	*P*
Hypertension	Inverse variance weighted	24	<0.01	<0.01	1.01(1.00-1.02)	0.07
	MR Egger	24	0.01	<0.01	1.01(0.99-1.03)	0.26
	Weighted median	24	<0.01	<0.01	1.00(0.99-1.01)	0.51
	Weighted mode	24	<0.01	<0.01	1.00(0.99-1.01)	0.88
Gestational hypertension	Inverse variance weighted	25	0.03	0.05	1.03(0.94-1.13)	0.57
	MR Egger	25	0.02	0.10	1.02(0.84-1.23)	0.87
	Weighted median	25	0.03	0.07	1.03(0.90-1.17)	0.67
	Weighted mode	25	0.03	0.08	1.03(0.88-1.21)	0.69
Pre-eclampsia	Inverse variance weighted	25	0.04	0.06	1.04(0.93-1.17)	0.50
	MR Egger	25	0.01	0.13	1.01(0.79-1.29)	0.92
	Weighted median	25	0.02	0.08	1.02(0.88-1.19)	0.76
	Weighted mode	25	0.02	0.08	1.02(0.87-1.20)	0.81
Eclampsia	Inverse variance weighted	25	0.22	0.20	1.25(0.85-1.84)	0.26
	MR Egger	25	0.63	0.42	1.88(0.83-4.26)	0.15
	Weighted median	25	0.37	0.29	1.45(0.83-2.55)	0.19
	Weighted mode	25	0.37	0.38	1.44(0.69-3.02)	0.34
Systolic blood pressure	Inverse variance weighted	21	0.10	0.20	1.11(0.74-1.65)	0.62
	MR Egger	21	0.03	0.46	1.03(0.42-2.57)	0.94
	Weighted median	21	0.22	0.18	1.24(0.87-1.78)	0.24
	Weighted mode	21	0.65	0.51	1.92(0.70-5.26)	0.22
Diastolic blood pressure	Inverse variance weighted	18	0.17	0.09	1.18(0.98-1.42)	0.08
	MR Egger	18	0.48	0.22	1.62(1.05-2.48)	0.04
	Weighted median	18	0.10	0.09	1.11(0.93-1.32)	0.25
	Weighted mode	18	0.09	0.11	1.10(0.88-1.36)	0.42

nSNP, numbers of single nucleotide polymorphisms; OR, odds ratio; CI, confidence interval.

smooth muscle proliferation and vasoconstriction through various growth factors such as insulin-like growth factor-1 and platelet-derived growth factor (12). Further research is needed to explore the biological roles of the identified risk locipotential biological mechanisms connecting risk gene loci with UL and SBP.

The strength of this study is that it first analyzed the relationship between UL and blood pressure using the MR method. However, there are also some limitations. Firstly, despite various sensitivity analyses conducted, it should be noted that the existence of horizontal pleiotropy cannot be fully excluded, which may introduce bias into the results. Secondly, the genetic variants used in this study reflect the impact of UL on blood pressure, but further analysis of the impact of UL's size, number, and location on blood pressure is lacking. Thirdly, the population of this study was

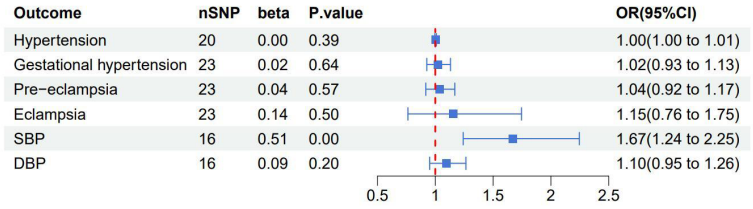


FIGURE 3
Forest plot of the results of the IVW method research between uterine leiomyoma and each outcome after excluding outliers and confounding SNPs associated with BMI related phenotypes. SBP, systolic blood pressure; DBP, diastolic blood pressure.

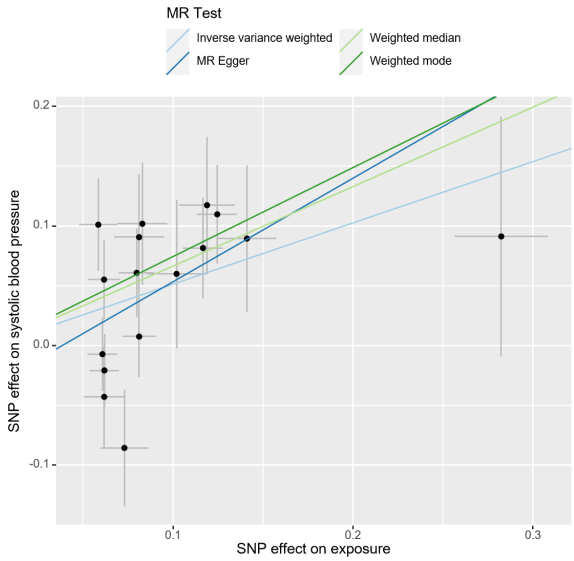


FIGURE 4
Scatter plot of the association of UL and SBP. The slope of the straight line indicates the magnitude of the causal association. UL, uterine leiomyoma; SBP, systolic blood pressure; SNP, single nucleotide polymorphisms; MR, Mendelian randomization.

derived from European ancestry. While this reduces population stratification bias, it may not be as reliable when extrapolated to other ethnic groups. Given that GWAS data for African populations are limited, and the sample size and case numbers of GWAS data for Asian populations are relatively small, future studies could pay more attention to expanding GWAS databases for Asians and Africans to further investigate the relationship between uterine leiomyomas and blood pressure in other populations. Besides, we hope that future researchers can conduct higher-quality and more detailed genome-wide association studies to identify genetic loci that affect the location, number, and size of uterine fibroids, so as to further explore the impact of different types of uterine fibroids on blood pressure.

The findings of this study provide robust causal evidence for the association between UL and blood pressure. This implies that UL is not merely a locally growing benign tumor, but it may also have certain impacts on systemic physiological indicators. Pulgar has indicated that UL might be a risk factor for the development of HDP (49). Consequently, in clinical practice, doctors need to pay closer attention to the blood pressure status of UL patients, particularly pregnant women combined with UL, and consider whether to reassess the patient's treatment plan. For hypertensive patients who already diagnosed with UL, if there is a case of elevated blood pressure, after ruling out other potential causes for the increase, doctors should be on high alert and consider whether interventional treatment for UL is necessary to prevent further elevation of blood pressure.

However, it is essential to recognize that while Mendelian randomization studies can provide robust causal evidence, their results still need to be validated in larger-scale clinical trials. Therefore, future research should aim to further confirm this discovery and explore the underlying biological mechanisms, in

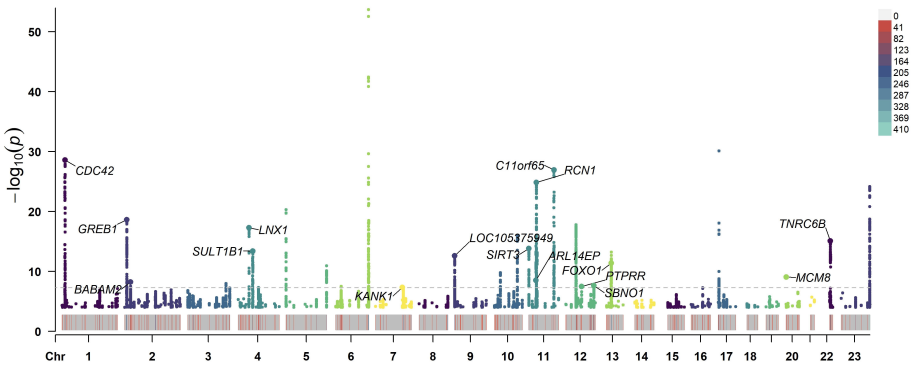


FIGURE 5
Manhattan plot of −log10 values using GWAS summary statistics of UL and SBP. The loci of 16 SNP that were significantly associated with UL and SBP is annotated in the plot. The horizontal axis represents the chromosome number. The dashed line indicates the $P<5\times10^{-8}$ threshold. GWAS, genome-wide association study; UL, uterine leiomyoma; SBP, systolic blood pressure; SNP, single-nucleotide polymorphism.

order to provide more precise and effective strategies for the treatment of UL, hypertension, or HDP patients. More rigorous and comprehensive prospective large-scale longitudinal cohort studies can be conducted to clinically validate the relationship between UL and hypertension and HDP, and further analyze whether the size, number, and location of UL have different impacts on blood pressure. It can also be investigated whether there is a change in blood pressure after treatment for UL. This may reduce the incidence and prevalence of hypertension and HDP in patients with UL.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ebi.ac.uk/gwas/studies, GCST90038604, https://gwas.mrcieu.ac.uk/, ieu-b-38, https://gwas.mrcieu.ac.uk/, ieu-b-39.

Ethics statement

Ethical approval was not required for studies on human participants, as only published GWAS summary statistics and ethically approved data obtained from FinnGen R9 biobank were used. These studies were conducted in accordance with local legislative and institutional requirements. The human samples used in this study were obtained from previous studies that had obtained ethical approval. No written informed consent from the subjects or their legal guardians was required for participation in this study.

Author contributions

HX: Writing – review & editing, Visualization, Writing – original draft, Resources, Formal analysis, Data curation. YM: Resources, Writing – review & editing, Funding acquisition. YL: Writing – review & editing, Supervision, Project administration, Formal analysis. RL: Funding acquisition, Writing – review & editing. ZC: Methodology, Data curation, Conceptualization, Writing – review & editing. XX: Supervision, Writing – review & editing. XH: Supervision, Writing – review & editing, Funding acquisition. XW: Supervision, Project administration, Writing – review & editing, Funding acquisition.

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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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1373724/full#supplementary-material

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EDITED BY
Eduardo Hertel Ribeiro,
Escola Superior de Ciências da Santa Casa de Misericórdia de Vitória (EMESCAM), Brazil

REVIEWED BY
Alberto Maino,
Azienda Provinciale per i Servizi Sanitari (APSS), Italy
Andrea Maria Maresca,
University of Insubria, Italy

*CORRESPONDENCE
Leonardo A. Sechi
sechi@uniud.it

†These authors have contributed equally to this work and share first authorship

‡These authors have contributed equally to this work and share last authorship

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Daytime plasma cortisol and cortisol response to dexamethasone suppression are associated with a prothrombotic state in hypertension

Gabriele Brosolo[1†], Andrea Da Porto[2†], Luca Bulfone[1], Antonio Vacca[1], Nicole Bertin[3], Cinzia Vivarelli[1], Cristiana Catena[1‡] and Leonardo A. Sechi[1,2,3*‡]

[1]Internal Medicine and European Hypertension Excellence Center, Department of Medicine, University of Udine, Udine, Italy, [2]Diabetes and Metabolism Unit, Department of Medicine, University of Udine, Udine, Italy, [3]Thrombosis and Hemostasis Unit, Department of Medicine, University of Udine, Udine, Italy

Background and aims: A prothrombotic state was demonstrated in patients with Cushing's syndrome and is involved in the development and progression of cardiovascular and renal damage in hypertensive patients. This study was designed to examine the relationships between cortisol secretion and the hemostatic and fibrinolytic systems in hypertension.

Methods: In 149 middle-aged, nondiabetic, essential hypertensive patients free of cardiovascular and renal complications, we measured hemostatic markers that express the spontaneous activation of the coagulation and fibrinolytic systems and assessed daily cortisol levels (8 AM, 3 PM, 12 AM; area under the curve, AUC-cortisol) together with the cortisol response to dexamethasone overnight suppression (DST-cortisol).

Results: Plasma levels of D-dimer (D-dim), prothrombin fragment 1 + 2 (F1 + 2), and von Willebrand factor (vWF) were progressively and significantly higher across tertiles of AUC-cortisol and DST-cortisol, whereas no differences were observed in fibrinogen, tissue plasminogen activator, plasminogen activator inhibitor-1, antithrombin III, protein C, and protein S. D-dim, F1 + 2, and vWF were significantly and directly correlated with age and both AUC-cortisol and DST-cortisol. Multivariate regression analysis showed that both AUC-cortisol and DST-cortisol were related to plasma D-dim, F1 + 2, and vWF independently of age, body mass index, blood pressure, and renal function.

Conclusion: Greater daily cortisol profile and cortisol response to overnight suppression are independently associated with a prothrombotic state in hypertensive patients and might contribute to the development of organ damage and higher risk of cardiovascular complications.

KEYWORDS

coagulation, cortisol, d-dimer, dexamethasone, fibrinogen, fibrinolysis, prothrombin fragment 1 + 2, von Willebrand factor

Introduction

Epidemiological evidence indicates that the coagulation system plays an important role in the pathophysiology of atherosclerosis (1), and increased incidence of cardiovascular events has been associated with elevated circulating levels of markers of hemostatic activation (2–4). A prothrombotic state is characterized by intrinsic subclinical activation of the hemostatic system and is a well-recognized risk factor for cardiovascular events in the general population (5, 6) and in patients with hypertension (7, 8) and early renal failure (9). In hypertension, a prothrombotic state is associated with subclinical changes of the arterial tree (10–12), impaired left ventricular diastolic properties (13), and intrarenal hemodynamic changes (14) that are associated with decreased glomerular filtration rate (15). Furthermore, a link of the prothrombotic state with an activated renin-angiotensin-aldosterone system has been demonstrated in hypertension (16, 17) suggesting interaction of the hemostatic system with blood pressure regulatory mechanisms.

A prothrombotic state can be detected in many disease states including those characterized by exogenous or endogenous hypercortisolism (18). In patients with Cushing's syndrome, elevated circulating levels of a multitude of hemostatic markers were demonstrated, together with evidence of an impaired fibrinolytic system (19–21). Interestingly, initial studies reported activation of the hemostatic system also in patients with subclinical Cushing's syndrome (22, 23) such as those with incidentally detected adrenal masses in the absence of overt clinical features of hypercortisolism (24). Along these lines, studies conducted in other groups of patients have reported observations that might suggest the existence of a relationship between circulating glucocorticoids and different components of the hemostatic and fibrinolytic systems (25–27).

Although patients with essential hypertension have cortisol secretory rates and response to overnight dexamethasone suppression (DST) within the physiologic range, minor differences in circulating cortisol levels might have some impact on the coagulation system. To date, no information is available on the possible relationship of circulating cortisol with the hemostatic-fibrinolytic balance in patients with hypertension. This information would be relevant for better understanding of mechanisms that contribute to the development and progression of hypertensive organ damage.

The aim of this study was to test the hypothesis that cortisol secretory rates and response to DST are associated with changes in the activity of the coagulation system. We examined the relationship of cortisol daily production and response to DST with a broad panel of hemostatic markers in nondiabetic patients with hypertension who were free of major cardiovascular and renal complications.

Materials and methods

Patients

Patients with grade 1/grade 2 essential hypertension who consecutively presented at the Hypertension Clinic of our department from January 2021 to December 2021 were included in a cross-sectional study. All patients were white Caucasian, lived in the North-East of Italy, and were representative of the hypertensive population of this regional territory (28). Blood pressure was measured in patients who remained supine for at least 15 minutes using an automatic tool equipped with appropriately sized cuffs (Omron M6, OMRON Healthcare Co., Kyoto, Japan), obtaining 3 separate readings. Diagnosis of hypertension was done after measurements obtained in at least 3 separate visits, according to current guidelines (29). We excluded patients with: age <18 or >80 years; body mass index (BMI) >40 kg/m^2; pregnancy or use of estrogens; any type of treatment with corticosteroids; history of alcohol abuse; grade 3 hypertension and secondary hypertension; diabetes; major depressive disorders; 24-hour creatinine clearance <60 ml/min/1,73 m^2; use of any type of drugs that could interfere with the hemostatic system; history of recent illness and acute or chronic inflammatory conditions; history of cerebrovascular, ischemic heart, or peripheral artery disease. Causes of secondary hypertension were ruled out according to current guidelines (29) as previously reported (30). Cushing's syndrome was excluded by measurement of daily plasma cortisol (8 AM, 3 PM, 12 AM), 24-hour free urinary cortisol, and plasma cortisol after an overnight DST with 1 mg dexamethasone, according to guidelines (31). When plasma cortisol after DST was >50 nmol/L (15 patients) a 2 mg/48-hour dexamethasone test was done to confirm suppression of cortisol below that level. All patients underwent either MRI or CT of adrenals to exclude presence of incidentalomas. Diabetes was excluded by measurement of fasting blood glucose and glycated hemoglobin, and by a standard oral glucose tolerance test (32). Smokers were defined if they smoked for more than 5 years and did not quit more than 1 year before examination. A standardized questionnaire was used to assess daily alcohol intake (33). The study was conducted following the statements of the Declaration of Helsinki and was approved by the Institutional Review Board of the Department of Medicine. All patients gave their informed consent.

Laboratory tests

Blood was collected by venipuncture without venous stasis in the early morning after an overnight fast. Plasma was separated and stored at -80°C until processing. Glucose, total and high-density lipoprotein cholesterol, triglycerides were measured with chemical methods in automated devices, as previously reported (34). Low-density lipoprotein level was calculated by the Friedewald formula. Glomerular filtration rate was assessed by duplicate measurements of 24-hour creatinine clearance. Coagulation parameters were measured in plasma as previously described (35). In brief, fibrinogen was assayed in an automatic coagulometer by a functional test, D-dimer was assayed immunoenzymatically, prothrombin fragment 1 + 2 (F1 + 2) and tissue-plasminogen activator (tPA) by an enzyme-linked immunosorbent assay, plasminogen activator inhibitor-1 (PAI-1) by immunoassay, antithrombin III (AT-III), protein C, protein S, and von Willebrand factor (vWF) by functional chromogenic assays. Plasma cortisol was measured by immunoassay (Electro

Chemiluminescence ECLIA, Elecsys cortisol II, Roche Diagnostics, Basel, Switzerland) with an intraassay and interassay coefficient of variation of 1.7% and 2.3%, respectively, and the lowest detection limit of 1.5 nmol/L. The area under the curve of daily cortisol (8 AM, 3 PM, 12 AM; AUC-cortisol) was calculated by the trapezoidal rule (36). Duplicate 24-hour urinary collections were obtained for measurement of free cortisol excretion with a direct chemiluminescence technique (ADVIA Centaur Cortisol Immunoassay System, Siemens Healthcare, Milan, Italy; detection range, 14–2069 nmol/l) and the average value was considered.

Statistical analysis

The Kolmogorov-Smirnov test was used to determine normality of distribution of the variables included in the study. Normally distributed variables are expressed as mean ± standard deviation and skewed variables as median [interquartile range]. Categorical data are expressed as absolute number and percentage. For statistical reasons patients were grouped in tertiles of either AUC-cortisol or DST-cortisol and two-way ANOVA and the Kruskal-Wallis test were used for comparisons among groups with normal or skewed variable distribution, respectively. The Pearson's chi-square test was used to compare frequency distributions. The relationships between different variables were examined by linear regression analysis, and correlation was expressed by the correlation coefficient *r*. In this analysis, variables with skewed distribution were log transformed. Multivariate regression analysis was performed to determine which variables were independently associated with hemostatic markers. A P value of less than 5% was considered to indicate statistical significance. All data analyses were performed using Stata 12.1 (StataCorp LP, College Station, TX, USA).

Results

One-hundred-forty-nine patients (age, 48±13 years; 77 males, 72 females) with essential hypertension were recruited for data analysis. Forty (27%) patients had BMI >30 of whom 21 (14%) had grade 1 (BMI 30–35) and 19 (13%) grade 2 obesity. Sixty-two (42%) patients had never been treated with antihypertensive agents and the remaining 87 (58%) who were taking an average of 1.3 antihypertensive agents (angiotensin-converting enzyme inhibitors or angiotensin receptor antagonists, 39%; calcium-channel blockers, 38%; diuretics, 19%; beta-blockers, 19%; alpha-blockers, 7%) had their drugs withdrawn for at least two weeks before the study.

For statistical reasons, patients were grouped according to tertiles of AUC-cortisol and DST-cortisol. The clinical characteristics of the study patients are summarized in Table 1 together with general biochemistries. Across AUC-cortisol and DST-cortisol tertiles, no significant differences were observed in demographic and anthropometric characteristics, systolic and diastolic blood pressure, plasma glucose and glycated hemoglobin, plasma lipids, electrolyte levels, renal function, and C-reactive protein. No differences were also observed in frequency of previous use of antihypertensive drugs. In particular, no significant differences were observed from patients previously treated with diuretics and the remaining patients. Table 2 summarizes hormonal measurements of the study patients showing that plasma cortisol levels measured at 8 AM, 3 PM, and 12 AM increased significantly across AUC-cortisol and DST-cortisol tertiles, whereas plasma ACTH, active renin and aldosterone, and daily urinary excretion of epinephrine, norepinephrine, and dopamine did not differ among groups. Table 3 shows the hemostatic variables showing that plasma D-dimer, F1 + 2, and vWF levels were significantly and progressively higher across both AUC-cortisol and DST-cortisol tertiles. No significant differences among groups were observed in fibrinogen, t-PA, PAI-1, AT-III, and protein C and protein S.

Analysis of univariate correlations (Table 4) showed that D-dimer, F1 + 2, and vWF were significantly and directly correlated with patients' age, AUC-cortisol (Figure 1) and DST-cortisol (Figure 2). No further significant relationship of plasma cortisol levels was observed with the other hemostatic markers. F1 + 2 was also inversely correlated with 24-hour creatinine clearance while fibrinogen, tPA and PAI-1 were directly correlated with BMI. Only fibrinogen was directly correlated with systolic blood pressure. None of the cortisol and hemostatic variables was correlated with C-reactive protein.

Multivariate regression analysis was conducted including different hemostatic markers as the dependent variables and age, sex, systolic blood pressure, creatinine clearance, and either AUC-cortisol (Model 1) or DST-cortisol (Model 2) as the independent variables, respectively (Supplementary Material). In both models, age was independently and directly related with F1 + 2 and vWF, and sex was independently related with log D-dimer. Both AUC-cortisol and DST-cortisol were significantly and independently correlated with log D-dimer (β-coefficient 0.001, P=0.029; β-coefficient 0.003, P<0.001; respectively), F1 + 2 (β-coefficient 0.267, P=0.002; β-coefficient 1.217, P<0.001; respectively), and vWF (β-coefficient 0.106, P=0.035; β-coefficient 0.877, P<0.001; respectively).

Discussion

A prothrombotic state is associated with major cardiovascular events in hypertension, and many factors can contribute to hemostatic activation in hypertensive patients (37). Evidence previously obtained in patients with Cushing's syndrome indicates that excess cortisol could contribute to a hypercoagulable state. We tested the hypothesis that even minor differences in regulation of cortisol secretion are associated with a prothrombotic state in patients with essential hypertension. Results show that plasma levels of D-dimer, F1 + 2, and vWF are progressively greater with increasing levels of plasma cortisol daily profile and response to DST. Both AUC-cortisol and DST-cortisol are significantly correlated with plasma D-dimer, F1 + 2, and vWF levels independently of age, BMI, blood pressure, and renal function. These findings indicate that differences in regulation of cortisol production within the physiologic

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TABLE 1 Clinical Characteristics and biochemical variables of hypertensive patients who were grouped according to either tertiles of the area under the curve (AUC) of daily plasma cortisol or plasma cortisol after dexamethasone suppression (DST).

Variables	All patients (n=149)	AUC Tertile I (n=49)	AUC Tertile II (n=50)	AUC Tertile III (n=50)	DST Tertile I (n=49)	DST Tertile II (n=50)	DST Tertile III (n=50)
Clinical characteristics							
Age, years	48 ± 13	48 ± 13	48 ± 13	49 ± 13	46 ± 10	48 ± 13	50 ± 15
Males, n (%)	77 (52)	22 (45)	28 (56)	27 (54)	27 (55)	25 (50)	25 (50)
Body mass index, kg/m^2	28.8 ± 5.8	28.2 ± 4.6	29.2 ± 6.4	29.1 ± 5.9	29.8 ± 6.4	28.5 ± 5.5	28.2 ± 4.9
Systolic BP, mm Hg	154 ± 17	154 ± 17	155 ± 18	152 ± 17	151 ± 17	154 ± 16	156 ± 19
Diastolic BP, mm Hg	95 ± 11	96 ± 9	94 ± 9	95 ± 14	96 ± 8	95 ± 13	93 ± 12
Previous drug use, n (%)	87 (58)	26 (53)	31 (62)	30 (60)	31 (63)	26 (52)	30 (60)
Alcohol intake, g/day	6 ± 12	5 ± 8	9 ± 14	8 ± 18	6 ± 16	5 ± 11	6 ± 10
Smokers, n (%)	29 (19)	8 (16)	11 (22)	10 (20)	10 (20)	11 (22)	8 (16)
Biochemical variables							
Glucose, mg/dL	89 ± 11	87 ± 9	90 ± 11	91 ± 12	88 ± 9	89 ± 10	91 ± 14
Glycated hemoglobin, %	5.6 ± 0.4	5.6 ± 0.5	5.5 ± 0.3	5.7 ± 0.5	5.5 ± 0.4	5.6 ± 0.4	5.7 ± 0.5
Triglycerides, mg/dL	116 ± 66	101 ± 57	124 ± 61	123 ± 78	124 ± 72	107 ± 51	118 ± 73
Total cholesterol, mg/dL	195 ± 40	193 ± 40	190 ± 37	200 ± 42	194 ± 41	197 ± 35	192 ± 44
HDL-cholesterol, mg/dL	55 ± 16	55 ± 15	55 ± 18	56 ± 15	53 ± 14	56 ± 16	56 ± 17
LDL-cholesterol, mg/dL	115 ± 35	117 ± 34	111 ± 34	117 ± 37	116 ± 30	117 ± 26	113 ± 39
GFR, ml/min/1.73 m^2	103 ± 26	106 ± 25	106 ± 26	98 ± 25	105 ± 31	104 ± 23	100 ± 23
Sodium, mmol/L	141 ± 2	142 ± 2	142 ± 3	141 ± 2	141 ± 2	141 ± 2	141 ± 2
Potassium, mmol/L	4.06 ± 0.42	4.10 ± 0.43	4.06 ± 0.31	4.01 ± 0.49	4.14 ± 0.40	4.04 ± 0.32	3.99 ± 0.50
C-reactive protein, mg/L	1.6 [0.7–3.7]	1.5 [0.8–3.1]	1.3 [0.6–3.1]	1.9 [0.9–4.1]	1.7 [0.8–3.9]	1.4 [0.7–2.5]	1.8 [0.8–4.0]

Values are expressed as mean ± SD. Median and interquartile range in square brackets are shown for variables with skewed distribution. Two-way ANOVA and the Kruskal-Wallis test were used for comparisons among groups with normal or skewed variable distribution, respectively. The Pearson's chi-square test was used to compare frequency distributions. BP, blood pressure; HDL, high-density lipoproteins; LDL, low-density lipoproteins; GFR, glomerular filtration rate as assessed by 24-hour creatinine clearance.

range might contribute to a prothrombotic state in patients with hypertension.

Hypercortisolism is associated with high risk of cardiovascular morbidity and mortality that might be related to a multiplicity of factors (38) including a prothrombotic state. After initial studies that suggested presence of a hypercoagulable state in patients with Cushing's syndrome (39), spontaneous activation of the hemostatic system was consistently shown in patients with hypercortisolism. Increased circulating levels of fibrinogen (19, 20, 40, 41), D-dimer (20, 23, 40), vWF (20, 42–44), AT-III (19–21, 44, 45), protein C-protein S complex (21, 22, 41, 45), and PAI-1 (19, 20, 41) were reported together with changes of additional coagulation factors and hemostatic tests (19–21, 41, 43, 46) in several cross-sectional comparisons of patients with Cushing's syndrome with healthy subjects. Some studies suggested that these changes could be more relevant in patients with ACTH-producing adenomas (44) and increased levels of procoagulants and antifibrinolytics were reported also in children with Cushing's syndrome that resolved after surgical treatment (45). Reversal of hypercoagulability was reported also in adults after surgical resolution of disease (43, 46), although this was not confirmed in other studies (40, 41). Interestingly, hemostatic changes were reported also in patients with excess plasma cortisol or incidentally detected adrenal masses in the absence of overt clinical features of hypercortisolism (22, 23), further supporting the hypothesis of a contribution of cortisol levels to regulation of coagulation and fibrinolysis (18). In our highly selected hypertensive patients who had physiologic suppression of plasma cortisol after DST, cortisol levels were significantly and independently associated with markers of intrinsic hemostatic activation. This study expands to a physiologic range of hormonal secretion the evidence of the potential role of cortisol in causing a prothrombotic state.

Due to its high prevalence in the general population, arterial hypertension is considered the leading cardiovascular risk factor. In patients with essential hypertension, we have previously demonstrated that a prothrombotic state contributes to the development of target organ damage and thereby might increase cardiovascular morbidity and mortality (7, 9–15). In fact, substantial experimental and clinical data indicate that platelets and coagulation cascade are important determinants of both

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TABLE 2 Hormonal variables of hypertensive patients who were grouped according to either tertiles of the area under the curve (AUC) of daily plasma cortisol or plasma cortisol after dexamethasone suppression (DST).

Variables	All patients (n=149)	AUC Tertile I (n=49)	AUC Tertile II (n=50)	AUC Tertile III (n=50)	P	DST Tertile I (n=49)	DST Tertile II (n=50)	DST Tertile III (n=50)	P
Cortisol 8 AM, nmol/L	433 ± 148	366 ± 123	451 ± 131	487 ± 164	<0.001	383 ± 139	430 ± 131	486 ± 158	0.002
Cortisol 3 PM, nmol/L	230 ± 108	164 ± 83	206 ± 54	316 ± 112	<0.001	208 ± 99	218 ± 99	259 ± 119	0.043
Cortisol 12 AM, nmol/L	114 ± 85	78 ± 45	124 ± 105	145 ± 87	<0.001	81 ± 54	122 ± 112	136 ± 73	0.004
AUC-cortisol, nmol/L/d	241 ± 77	163 ± 30	234 ± 23	324 ± 58	<0.001	236 ± 85	234 ± 68	253 ± 78	0.023
U cortisol, nmol/d	719 ± 353	714 ± 373	697 ± 313	745 ± 378	0.792	683 ± 336	724 ± 354	749 ± 375	0.649
DST-cortisol, nmol/L	24 [17–33]	21 [17–32]	25 [18–31]	27 [18–44]	0.026	15 [14–17]	24 [21–28]	43 [33–52]	<0.001
ACTH, pg/mL	21 ± 17	19 ± 17	21 ± 17	23 ± 17	0.506	21 ± 10	22 ± 20	21 ± 19	0.944
Active renin, mUI, mL	8.0 [3.8–17.6]	7.8 [3.2–20.7]	7.8 [3.6–11.1]	9.4 [4.6–17.8]	0.659	9.1 [3.3–17.8]	9.9 [4.2–19.0]	7.7 [3.2–12.1]	0.563
Aldosterone, pg/mL	131 ± 80	131 ± 66	121 ± 74	141 ± 95	0.454	141 ± 91	127 ± 64	125 ± 84	0.561
U epinephrine, nmol/d	22[13–35]	22[11–35]	24[14–34]	21[14–37]	0.904	22[13–33]	24[13–43]	20[13–35]	0.309
U norepinephrine, nmol/d	218 [147–321]	201 [140–266]	238 [139–349]	218 [159–305]	0.278	234 [154–386]	211 [143–326]	211 [128–239]	0.650
U dopamine, nmol/d	1736 [1224–2221]	1770 [1226–2134]	1844 [1398–2376]	1590 [1097–2208]	0.138	1840 [1356–2437]	1806 [1207–2413]	1587 [988–2062]	0.518

Values are expressed as mean ± SD. Median and interquartile range in square brackets are shown for variables with skewed distribution. Two-way ANOVA and the Kruskal-Wallis test were used for comparisons among groups with normal or skewed variable distribution, respectively. U, 24-hour urinary excretion.

atherogenesis and atherothrombosis (1). The hemostatic system exerts several actions on the vasculature that could influence the structure of the arterial wall and presumably the progression of atherosclerotic lesions. Hemostatic components have been implicated in causing disruption of endothelial lining, leukocyte recruitment, oxidative stress, vascular inflammation, migration of vascular smooth muscle cells, apoptosis, and angiogenesis (47, 48). For these reasons, identification of conditions that may contribute to a prothrombotic state in hypertension would be relevant as an issue for possible preventive interventions.

Many factors could cause activation of the hemostatic system in hypertension and the present study demonstrates that even minor

TABLE 3 Hemostatic variables of hypertensive patients who were grouped according to either tertiles of the area under the curve (AUC) of daily plasma cortisol or plasma cortisol after dexamethasone suppression (DST).

Variables	All patients (n=149)	AUC Tertile I (n=49)	AUC Tertile II (n=50)	AUC Tertile III (n=50)	P	DST Tertile I (n=49)	DST Tertile II (n=50)	DST Tertile III (n=50)	P
Fibrinogen, mg/dL	344 ± 97	335 ± 92	344 ± 78	353 ± 118	0.656	346 ± 94	338 ± 87	348 ± 111	0.866
D-dimer, ng/mL	206 [172–306]	185 [150–276]	206 [171–275]	253 [203–356]	0.018	191 [148–257]	198 [161–273]	266 [210–349]	0.001
F1 + 2, pmol/L	208 ± 78	182 ± 63	215 ± 82	226 ± 84	0.014	167 ± 59	225 ± 72	230 ± 87	<0.001
vWF antigen, %	123 ± 45	112 ± 38	124 ± 40	135 ± 51	0.034	99 ± 35	129 ± 35	141 ± 53	<0.001
t-PA, ng/mL	5.8 [4.2–7.4]	5.9 [3.9–7.3]	5.8 [3.9–7.7]	6.1 [4.5–7.4]	0.696	5.9 [4.8–7.3]	5.0 [4.0–7.0]	6.4 [3.7–8.4]	0.451
PAI-1, ng/mL	11.4 [5.7–18.1]	10.2 [4.3–18.9]	11.2 [7.0–17.7]	14.0 [6.6–17.9]	0.529	13.8 [8.0–24.6]	11.4 [5.8–16.7]	9.7 [5.4–17.9]	0.100
Antithrombin III, %	102 ± 16	100 ± 12	106 ± 22	100 ± 11	0.095	101 ± 13	101 ± 11	104 ± 22	0.562
Protein C, %	111 ± 22	112 ± 20	109 ± 20	113 ± 25	0.635	115 ± 23	110 ± 18	108 ± 25	0.275
Protein S, %	95 ± 23	92 ± 19	93 ± 18	98 ± 30	0.381	99 ± 28	94 ± 21	91 ± 19	0.220

Values are expressed as mean ± SD. Median and interquartile range in square brackets are shown for variables with skewed distribution. Two-way ANOVA and the Kruskal-Wallis test were used for comparisons among groups with normal or skewed variable distribution, respectively. F1 + 2, prothrombin factor 1 + 2; vWF, von Willebrand factor; t-PA, tissue plasminogen activator; PAI-1, plasminogen activator inhibitor-1.

TABLE 4 Univariate correlations of clinical and hormonal variables with the hemostatic markers.

Variables	Fibrinogen		D-dimer		F1 + 2		vWf		tPA		PAI-1	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Age	0.088	0.286	0.166	0.043	0.280	<0.001	0.274	<0.001	0.124	0.131	0.072	0.384
BMI	0.232	0.004	0.114	0.165	-0.067	0.416	-0.053	0.520	0.170	0.038	0.338	<0.001
Systolic BP	0.219	0.007	0.104	0.206	-0.032	0.703	0.058	0.479	-0.012	0.888	0.056	0.495
Diastolic BP	0.037	0.652	-0.084	0.311	-0.152	0.066	-0.103	0.211	-0.066	0.425	-0.018	0.829
GFR	-0.159	0.053	-0.106	0.197	-0.165	0.044	-0.110	0.180	-0.004	0.965	-0.086	0.298
Cortisol 8 AM	0.032	0.710	0.093	0.278	0.040	0.639	0.100	0.243	-0.042	0.624	-0.022	0.796
Cortisol 3 PM	0.030	0.761	0.025	0.799	0.209	0.031	0.182	0.059	-0.078	0.424	0.126	0.195
Cortisol 12 AM	0.070	0.474	0.158	0.105	0.175	0.073	0.311	0.001	0.067	0.494	-0.103	0.291
AUC-cortisol	0.045	0.589	0.194	0.018	0.265	0.001	0.178	0.030	0.040	0.625	0.051	0.535
DST-cortisol	0.031	0.705	0.281	<0.001	0.317	<0.001	0.376	<0.001	0.018	0.829	-0.014	0.870
Urinary cortisol	-0.152	0.064	-0.134	0.104	0.120	0.146	-0.065	0.434	0.152	0.065	0.044	0.595

BMI, body mass index; BP, blood pressure; GFR, glomerular filtration rate as assessed by 24-hour creatinine clearance; AUC-cortisol, area under the curve of daily plasma cortisol (8 AM, 3 PM, 12 AM); DST-cortisol, plasma cortisol after dexamethasone suppression test.

differences in regulation of cortisol secretion are associated with a prothrombotic state as defined by higher levels of D-dimer, F1 + 2, and vWF. Measurements of fibrin D-dimer, the principal breakdown fragment of fibrin, and F1 + 2 that is released when coagulation factor Xa converts prothrombin to thrombin provide a reliable estimation of the overall state of activation of the coagulation pathways. On the other hand, vWF enhances adhesion of platelets to subendothelial collagen and increases the stability of coagulation factor VIII in the hemostatic cascade. Older age, obesity, and impaired renal function are associated with a prothrombotic state, and this is why statistical analysis was corrected for these variables showing that D-dimer, F1 + 2, and vWF were all independently related to both AUC-cortisol and DST-cortisol. Thus, differences in cortisol secretion within a physiological range could contribute to a prothrombotic state and thereby to hypertensive organ damage. Also, to this point it should be noticed that minor differences in regulation of cortisol secretion were previously found to be associated with impaired glucose metabolism in nondiabetic hypertensive patients (36) and greater left ventricular mass (30), suggesting additional mechanisms that might mediate detrimental cardiovascular effects of cortisol in essential hypertension.

Mechanisms that could link excess cortisol secretion to the hypercoagulable state are mostly speculative. Cortisol-induced up-regulation of gene transcription of multiple coagulation factors with intrinsic activation of the hemostatic cascade is the most likely mechanism. Activation of markers of the overall activity of the coagulation system such as D-dimer and F1 + 2 would support this possibility. On the other hand, observation of significantly increased levels of vWF might suggest that the prothrombotic state caused by increased circulating cortisol could be related to an enhanced metabolic function of endothelial cells. This was also suggested by Fatti et al. who reported increased levels of additional markers of endothelial activation (thrombin-antithrombin complex and plasmin-antiplasmin complex) in patients with Cushing's syndrome (43). Hypercortisolism may also affect the multimeric structure of vWF causing an overexpression of abnormally high molecular weight multimers, capable of inducing spontaneous platelet aggregation (42).

Limitations to the present study need to be considered. First, the cross-sectional design limits the possibility to establish causality of the relationship between cortisol secretion and the prothrombotic state, although independence of this relationship from confounders in the multivariate analysis would suggest so. However, statistical adjustments cannot account for all biological and pathophysiological variables. Also, the possibility that plasma cortisol levels and prothrombotic markers causally affect one another cannot be excluded. Second, use of a selected clinic sample of white Caucasian hypertensive patients might limit the possibility to extend the present findings to a broader context. Third, inclusion of a significant proportion of patients who had been treated with antihypertensive drugs might have affected the results. However, it must be noticed that no differences were found in either hemostatic markers or cortisol measurements between these patients and those who were treatment naïve, nor differences were observed among patients who were treated with different categories of antihypertensive drugs. Last, due to the relatively small size of this study, the possibility that associations are merely due to chance should be considered.

Conclusion

This study demonstrates for the first time that even minor differences in daily cortisol secretion and cortisol levels after overnight suppression are independently associated with markers of a prothrombotic state in patients with essential hypertension who are free of major cardiovascular and renal complications. These

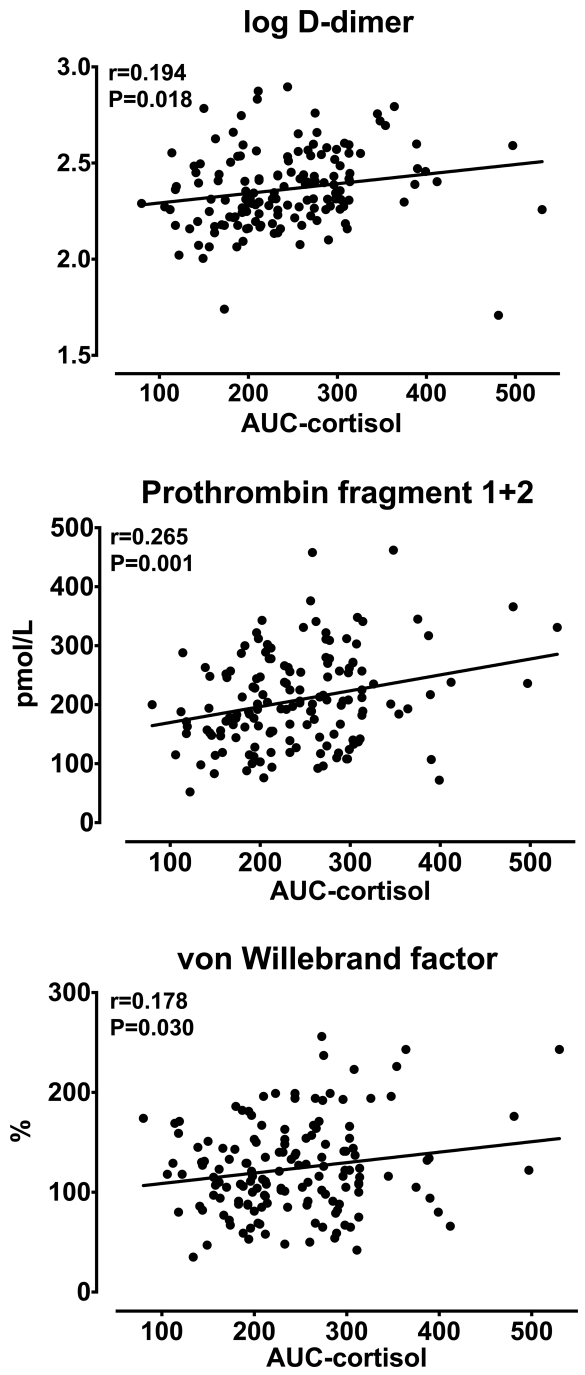


FIGURE 1
Relationships between the area under the curve (AUC) of daily (8 AM, 3 PM, 12 AM) plasma cortisol and log-transformed D-dimer, prothrombin fragment 1 + 2, and von Willebrand factor.

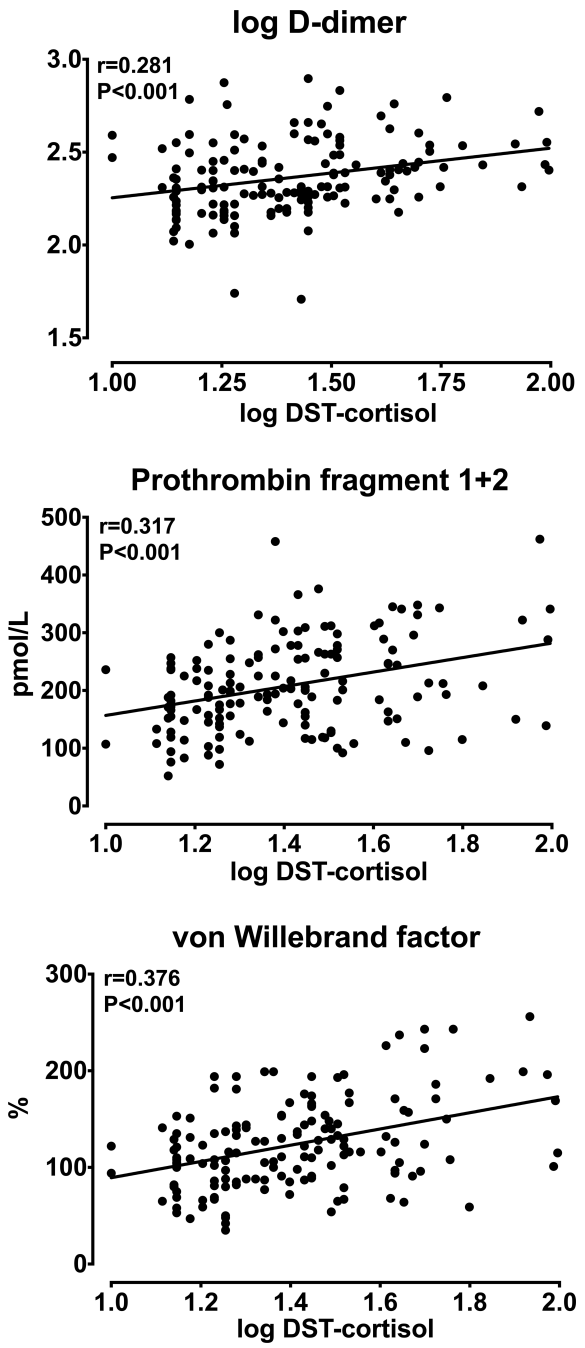


FIGURE 2
Relationships between log-transformed plasma cortisol measured after a 1 mg overnight dexamethasone suppression test (DST-cortisol) and log-transformed D-dimer, prothrombin fragment 1 + 2, and von Willebrand factor.

findings expand this evidence beyond the boundary of clinical and subclinical Cushing's syndrome. Results also provide better knowledge of factors that might contribute to develop a prothrombotic state in hypertension, thereby increasing target organ damage and, in turn, the risk of cardiovascular events. This study might have important clinical implications opening new paths to the possibility to identify those hypertensive patients at higher risk of development of subclinical organ damage. Detection of cortisol levels in the upper limit of normality in conjunction with markers of hemostatic activation could be useful to guide physicians toward more aggressive treatment and control of blood pressure and additional risk factors. Also, these findings open a window to the possibility to effectively prevent hypertensive organ damage through specific interventions on cortisol production and hemostatic system. Possible benefits of these interventions will have to be tested in future studies.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Institutional Review Board - Department of Medicine - University of Udine. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

GB: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing – original draft. AD: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing – original draft. LB: Data curation, Investigation, Methodology, Software, Supervision, Validation, Visualization, Writing – review & editing. AV: Data curation, Investigation, Methodology, Software, Supervision, Validation, Visualization, Writing – review & editing. NB: Data curation, Investigation, Supervision, Validation, Visualization, Writing – review & editing. CV: Data curation, Investigation, Supervision, Validation, Visualization, Writing – review & editing. CC: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. LS: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

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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.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1397062/full#supplementary-material

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EDITED BY
Gabor Czibik,
Queen Mary University of London,
United Kingdom

REVIEWED BY
Aleksandra Jotic,
University of Belgrade, Serbia
Alina Yu Babenko,
Almazov National Medical Research Centre, Russia

*CORRESPONDENCE
Shihan Wang
wangshihan91@126.com

†These authors have contributed equally to this work and share first authorship

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Long-term effects of different hypoglycemic drugs on carotid intima-media thickness progression: a systematic review and network meta-analysis

Qianyu Lv[1†], Yingtian Yang[1†], Yanfei Lv[2], Qian Wu[1], Xinzheng Hou[1], Lanlan Li[1], Xuejiao Ye[1], Chenyan Yang[1] and Shihan Wang[1*]

[1]Guang'anmen Hospital, China Academy of Chinese Medical Sciences, Beijing, China, [2]School of Management, Fudan University, Shanghai, China

Objective: The progression of carotid intima-media thickness (cIMT) can partially predict the occurrence of future cardiovascular events. This network meta-analysis compared the effects of 14 antidiabetic drugs (acarbose, alogliptin, exenatide, glibenclamide, glimepiride, ipragliflozin, metformin, nateglinide, pioglitazone, rosiglitazone, sitagliptin, tofoglifozin, troglitazone, voglibose) on the progression of cIMT.

Method: PubMed, EMBASE, Cochrane Library, and Web of Science were searched to screen all clinical trials of treatment of cIMT with hypoglycemic agents before March 1, 2024. The differences in the changes in cIMT between the treatment group and control group were evaluated.

Result: After screening 8395 citations, 25 studies (6675 patients) were included. The results indicated that exenatide had the best efficacy in slowing down cIMT progress, and exenatide [MD=-0.13,95%CI (-0.25, -0.01)], alogliptin [MD=-0.08,95%CI (-0.13, -0.02)] and metformin [MD=-0.05, 95%CI (-0.09, -0.02)] are more effective than placebo.

Conclusion: Long-term treatment of exenatide, alogliptin, and metformin may be more effective than other hypoglycemic drugs in slowing the progression of cIMT.

Systematic Review Registration: https://www.crd.york.ac.uk/PROSPERO/, identifier CRD42024519474.

KEYWORDS

atherosclerosis, intima-media thickness, antidiabetic drug, cardiovascular, diabetes

Introduction

Atherosclerosis (AS) plays a crucial role in the pathogenesis of cardiovascular diseases, which may ultimately lead to atherosclerotic cardiovascular disease (ASCVD), posing significant threats to human health. In this pathological process of atherosclerosis formation, excessive lipid deposition and macrophage transformation into foam cells lead to the increase in intima-media thickness (IMT) (1). In fact, IMT can assess the extent of atherosclerosis in the whole human body (2), especially the carotid intima-media thickness (cIMT), serves as a crucial indicator for cardiovascular disease, whose progression can predict future cardiovascular events to a certain extent (3–5). Dysglycemia is generally considered to be an important risk factor for AS (6). Some traditional hypoglycemic drugs(HD) have shown good cardiovascular safety (7–9), especially the clinical application of novel types of hypoglycemic drugs, including dipeptidyl peptidase-4 inhibitor (DPP-4i), glucagon like peptide-1 receptor agonists (GLP-1 RA), sodium-glucose cotransporter-2 inhibitor (SGLT-2i), peroxisome proliferator-activated receptor gamma (PPARγ) agonist, has ushered in a new era of diabetes treatment. These drugs are increasingly recognized to have multiple effects beyond lowering blood glucose, some of which may contribute to clinical practice in the treatment of cardiovascular diseases (10–13), SGLT-2i and GLP-1 RA are also included in the guidelines as a recommended drug for patients with diabetes and at high risk for cardiovascular events (14, 15). Some clinical trials have conducted to explore the efficacy of one or more HDs and reported the changes of cIMT, however, the impact of different HDs on cIMT has not yet been identified. A network meta-analysis (NMA) can simultaneously compare the effects of multiple interventions because of the lack of direct comparisons between these drugs (16). Therefore, we extracted data from relevant studies to conduct a NMA to assess the relative effectiveness of different HDs in cIMT changes, and to provide evidence for clinical application.

Methods

Study design and registration

We developed a detailed search strategy following Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (17), the research protocol was registered with PROSPERO(CRD42024519474).

Study selection

A comprehensive search was conducted using four databases (PubMed, Embase, Cochrane Library, and Web of Science), for all studies on the relationship between HDs and cIMT. The search strategy was implemented by multiplying the search formulas for HDs and carotid intima-media thickness/atherosclerosis. The search period was from the establishment of the database to March 1, 2024. The complete search strategy is given in additional file: Appendix S1.

Inclusion and exclusion criteria

Identified studies were enrolled based on the following inclusion criteria: (a) to evaluate the therapeutic effect of various HDs in retarding cIMT; (b) recruited patients with Type 1 diabetes mellitus(T1DM), type 2 diabetes mellitus(T2DM) or impaired glucose tolerance (IGT) or impaired fasting glucose(IFG) or coronary heart disease (CVD) or metabolic syndrome (MS) or abnormal glucose metabolism(AGM);(c) recruited patients were at least 18 years of age; (d) the duration of treatment is at least one year; (e) had a placebo (PLC) or comparative drug control group to compare the outcomes.

Studies containing the following items were excluded: (a) was not a randomized controlled trial (RCT) trial; (b) a combination of drugs was included in the treatment or control group; (c) treatment duration is less than one year; (d) the study did not provide sufficient information about the dataset; (e) animal experiments, review, protocol, case report, *post hoc* analysis; (f) non-English language literature.

Data extraction and quality assessment

Firstly, the possibly relevant research was imported into EndNote X9, after removing duplicates, independent literature data extraction is conducted by two reviewers, the title and abstract are reviewed to exclude unqualified literature, and then the full text evaluation is conducted. This NMA mainly extracted the basic information of the included literature (name and country of the main author, publication year), basic characteristics of the study population (number of cases, age, sex, disease situation, basic treatment), and outcome indicators (changes of the cIMT).

Literature quality was assessed using the RoB (18) in the following five bias domains: randomization process, allocation concealment, intervention blinding of participants and investigators, missing outcome data, outcome measurement and selection of reported results. An algorithm was employed to estimate the overall risk of bias, i.e., low risk, some concerns, or high risk. The above bias risk assessment was independently conducted and cross-checked by two reviewers. In case of disagreement, a third reviewer was involved to discuss and determine the evaluation result.

Abbreviations: AS, atherosclerosis; ASCVD, atherosclerotic cardiovascular disease; AGM, abnormal glucose metabolism; CI, confidence interval; cIMT, carotid intima-media thickness; DDP-4i, dipeptidyl peptidase-4 inhibitor; DIC, deviation information criterion; GDM, gestational diabetes mellitus; GLP-1 RA, Glucagon like peptide-1 receptor agonists; HD, hypoglycemic drugs; HDL-C, high density lipoprotein cholesterol; IFG; Impaired fasting glucose; IGT, Impaired glucose tolerance; IL-1, interleukin-1; IL-6:interleukin-6;IMT, intima-media thickness; LDL-C, low-density lipoprotein cholesterol; MD, mean difference; MS, Metabolic syndrome; NMA, network meta-analysis; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-analyses; PPARγ, peroxisome proliferator-activated receptor gamma; RCT, randomized controlled trial; SGLT-2i, sodium-glucose cotransporter -2 inhibitor; SUCRA, Surface Under the Cumulative Ranking curve. TC, total cholesterol; TG, triglyceride.

Data analysis

The statistical model of Bayesian framework was constructed using JAGS software 4.3.1 (gemtc package 0.8–2 and rjags package 4–10) (Rstudio, Boston, MA, USA), cIMT(mm) was used as continuous variable, while mean difference (MD) and 95% credible interval (CI) were used as effect indexes. A random effects model with four Markov chains, each chain generating 50,000 iterations (burn-in period of 20,000 iterations). was used for the outcome indicator. The convergence of iterations was monitored using plots and the Gelman–Rubin–Brooks statistic (19). Model consistency was assessed using the deviation information criterion (DIC), with differences in DIC < 5 points indicating good consistency and consistency modeling was used (20). Heterogeneity was estimated using the I^2 statistic with I^2 values < 25% indicating low heterogeneity, 25 to 75%, moderate heterogeneity, and > 75%, high heterogeneity (21). Publication bias was assessed by funnel plots. Network plots and funnel plots were drawn by Stata SE 15.0 (Stata Corp, College Station, Texas, USA). In addition, we also comparing the therapeutic effect of HDs using a Surface Under the Cumulative Ranking curve (SUCRA) (22), the closer SUCRA was to 100, the better the therapeutic effect. What is more, we performed sensitivity analyses to examine the influence of individual studies on the total merged effects to assess the stability of the conclusion, and publication bias was analyzed by funnel plot using Stata16.0 software.

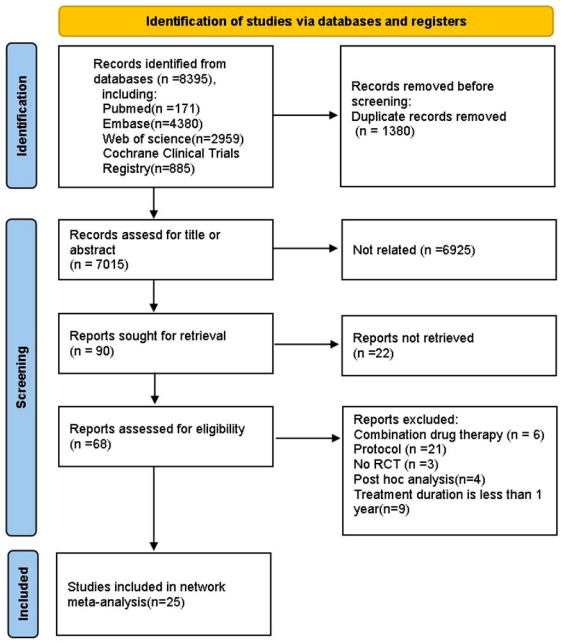


FIGURE 1
Flow chart of literature screening in the NMA.

Results

Data characteristics

We finally identified 8395 studies through an extensive search, after removing 1380 duplicates, we reviewed the titles and abstracts of the remaining articles based on eligibility criteria. 6959 studies were excluded because they were not relevant to our NMA, original articles were not found for 22 articles, 6 papers reported the combined use of drugs, 21 protocols, 3 studies were not RCT, 4 were *post hoc* analyses, 9 studies had a treatment duration less than one year. Ultimately, 25 studies (12, 23–46) were included in the NMA, and the literature screening process is shown in the (Figure 1). Our NMA included 14 HDs (acarbose, alogliptin, exenatide, glibenclamide, glimepiride, ipragliflozin, metformin, nateglinide, pioglitazone, rosiglitazone, sitagliptin, tofoglifozin, troglitazone, voglibose and PLC). A total of 6720 patients with complete outcome data were included in the NMA. Among them, there were 2491 patients with pre-diabetes, while the number of patients with diabetes was 3980. The main basic characteristics of the included patients are shown in Table 1.

Quality assessment

The quality assessment results showed that the overall quality of the included literatures was high (Figure 2).

NMA outcomes

The NMA network diagram involved conducting direct and indirect comparisons among a total of 14 drugs (Figure 3), DIC comparison results showed good agreement (DIC, 102.6 VS. 103.0). According to the NMA, exenatide [MD=-0.13,95%CI (-0.25, -0.01)] was found to be the most promising drug for slowing the progression of cIMT comparing to PLC, followed by alogliptin [MD=-0.08,95%CI (-0.13, -0.02)] and metformin [MD=-0.05, 95% CI (-0.09, -0.02)], the forest diagram is shown in Figure 4.

What's more, the results of our pairwise comparison analysis among HDs indicate that exenatide[MD=-0.23, 95%CI (-0.39, -0.08)], alogliptin[MD=-0.18,95%CI (-0.3, -0.06)], metformin [MD=-0.16, 95%CI (-0.27, -0.05)], nateglinide[MD=-0.14,95%CI (-0.27, -0.01), pioglitazone[MD=-0.11, 95%CI (-0.21, -0.01)], rosiglitazone[MD=-0.12, 95%CI (-0.23, -0.02)], sitagliptin[MD=-0.13, 95%CI (-0.24, -0.02)] was more effective than glibenclamide; exenatide[MD=-0.14, 95%CI (-0.27, -0.01)] and alogliptin[MD=-0.08, 95%CI (-0.16, -0.01)] was superior to that of tofoglifozin. However, voglibose demonstrated inferior efficacy compared to exenatide [MD=0.23, 95%CI (0.39, 0.07), alogliptine[MD=0.17, 95%CI (0.3, 0.05)], metformin [MD=0.15, 95%CI (0.27, 0.04)] and sitagliptin[MD=0.13, 95%CI (0.24, 0.01)].

The drug with the highest SUCRA value was exenatide(SUCRA 94.9%), followed by alogliptin(SUCRA 86.9%) and metformin (SUCRA 81.2%), PLC has a SUCRA values with 31.7%, Table 2 shows the results of the SUCRA analysis.

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TABLE 1 Main basic characteristics of the included patient.

Author(year)	Country	Size	Male(%)	Age	Drug vs. Comparator	Duration(year)	Study population
Hanefeld et al. (23)	Germany	115	70(60.8)	55.3 ± 7	Acarbose vs.PLC	3.9	IGT
Nakamura et al. (24)	Japan	45	25(55.6)	64.6 ± 9.5	Pioglitazone vs. Glibenclamide vs.Voglibose	1	T2DM
Sidhu et al. (25)	England	92	41(85.9)	62.5 ± 8	Rosiglitazone vs. PLC	1	CVD
Xiang et al. (26)	America	192	0(0)	34.6 ± 6.8	Troglitazone vs. PLC	2	GDM history
Hodis et al. (27)	America	276	230(67.4)	52.5 ± 9	Troglitazonevs. PLC	2	T2DM
Mazzone et al. (28)	America	361	111 (63.7)	66.3 ± 8	Pioglitazone vs. Glimepiride	1.5	T2DM
Hedblad et al. (29)	England	555	255(45.9)	66.5 ± 7	Rosiglitazone vs. PLC	1.1	T2DM
Mita et al. (30)	Japan	70	33(47.1)	61.5 ± 7	Nateglinide vs. PLC	1	T2DM
Lonn et al. (32)	Canada	1425	638(44.78)	53.9 ± 10.8	Rosiglitazone vs. PLC	1	IFG
Koyasu et al. (33)	Japan	81	74 (91.4)	66.3 ± 8	Acarbose vs. PLC	1	CVD
Yamasaki et al. (35)	Japan	186	117(62.9)	56.9	Pioglitazone vs. PLC	4	T2DM
Yasunari et al. (34)	Japan	48	12(25)	56.7 ± 10.4	Pioglitazone vs. PLC	4	T2DM
Saremi et al. (36)	America	382	175(45.8)	53.5 ± 11.5	Pioglitazone vs. PLC	2.3	IGT
Patel et al. (37)	America	219	74(33.8)	53.6 ± 11	Acarbose vs. PLC	5	IFG
Ishikawa et al. (38)	Japan	76	65(85.5)	71.4 ± 8	Sitagliptin vs. PLC	2.2	CVD
Mita et al. (39)	Japan	341	199(58.4)	64.6 ± 9.5	Alogliptin vs. PLC	2.2	T2DM
Mita et al. (39)	Japan	282	165 (60.2)	63.7 ± 9.8	Sitagliptin vs. PLC	2.2	T2DM
Oyama et al. (41)	Japan	442	297 (67.2)	69.3 ± 9	Sitagliptin vs. PLC	2	T2DM
Katakami et al. (42)	Japan	339	198(58.4%)	61.1 ± 9.5	Tofoglifozin vs. PLC	2.2	T2DM
Zhang et al. (43)	China	59	33(55.9)	58.4 ± 12.8	Exenatide vs. PLC	1.1	T2DM
Oyama et al. (31)	Japan	84	30(35.7)	60 ± 5	Acarbose vs. PLC	1	T2DM
Tanaka et al. (12)	Japan	464	161 (69.4)	68 ± 5.9	Ipragliflozin vs. PLC	2	T2DM+CVD
Meaney et al. (44)	Mexico	58	17(29.3)	49 ± 9	Metformin vs. PLC	1	MS
Petrie et al. (45)	England	428	175(40.89%)	55.5 ± 8.6	Metformin vs.Acarbose	3	T1DM
Lin et al. (46)	China	100	58(58%)	68.1 ± 7.8	Metformin vs.Acarbose	1	AGM

AGM, coronary heart disease; CVD, coronary heart disease; GDM, gestational diabetes mellitus; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; MS, metabolic syndrome; T1DM, Type 2 diabetes mellitus; T2DM, Type 2 diabetes mellitus.

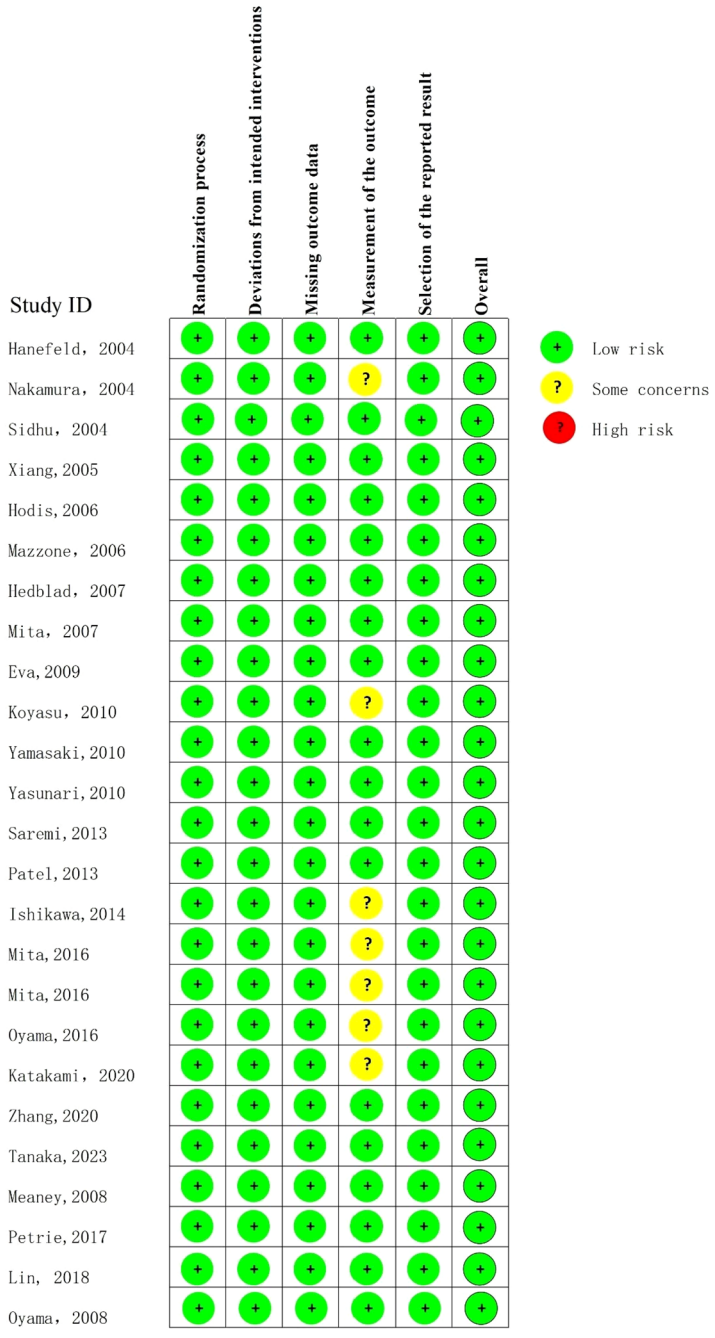


FIGURE 2
Risk of bias traffic light plot of ROB 2 assessments.

Sensitivity analysis

A sensitivity analyses was conducted by systematically excluding individual studies and performing an additional meta-analysis with each study removed. The impact of each exclusion on the pooled MD was evaluated. Based on the results of the sensitivity analysis, we found that none of the studies significantly influenced the overall effect, indicating the robustness of our NMA. Results of sensitivity analyses are provided in Appendix S2.

Bias of publication

There was no significant publication bias in the funnel plot of bias analysis drawn for cIMT (Figure 5).

Discussion

The presence of abnormal blood glucose levels significantly exacerbates the morbidity and mortality associated with

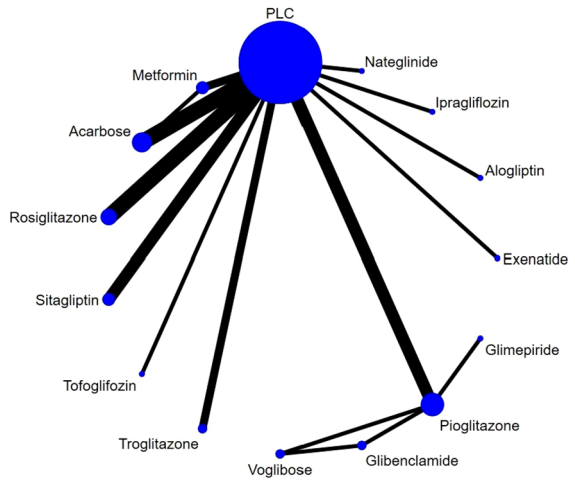


FIGURE 3
Network plot of clinical trials on HDs or PLC patients. Nodes stand for the comparison between treatments and the size is proportional to the number of subjects. The width of the lines is proportional to the number of trials per pair of interventions.

atherosclerosis (47, 48). It is of great interest to reduce the occurrence of cardiovascular events through controlling the risk factors. We compared the treatment effects of long-term usage of 14 types of HD on cIMT in individuals with and at high risk for diabetes in our NMA. The results of our NMA indicate that exenatide may be the optimal treatment option, followed by alogliptin and metformin.

Exenatide is a short-acting analogue of the Glucagon-like peptide-1(GLP-1). This type of medications worked by binding to the GLP-1 receptor, improving insulin sensitivity and insulin secretion function, and help to lower blood glucose levels (49–51). Recently, it has been discovered that the utilization of GLP-1

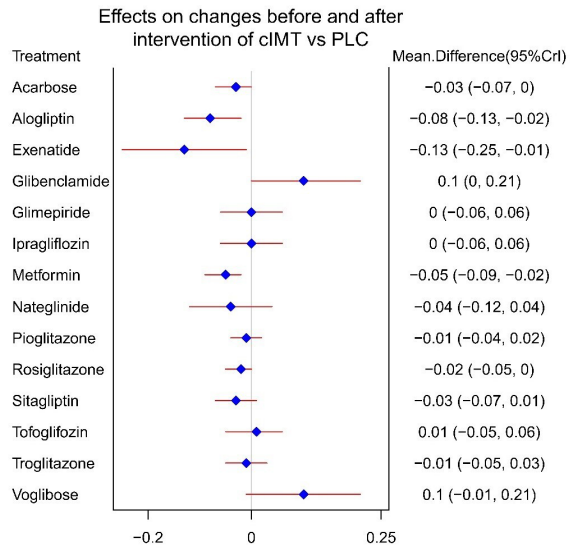


FIGURE 4
Forest plot showing the outcomes of NMA (relative differences of various HDs in reducing cIMT compared with PLC).

RA can not only effectively regulates blood glucose levels but also exerts significant effects on the modulation of blood lipids, blood pressure, and adipose content (52–54), all of which are recognized risk factors for AS (55–57). Thus, he protective effect of GLP-1 RA on the cardiovascular system may, in part, be attributed to its capacity for mitigating these risk factors. A large meta-analysis, involving 60,080 patients, reported that GLP-1 RA can reduce major adverse cardiovascular events (MACE) in type 2 diabetes patients with ASCVD by 14%, and lowers the risk of hospitalization due to heart failure (58). Our NMA suggests that exenatide, among the 14 drugs included, may be the best treatment for retarding the progression of cIMT. Dyslipidemia has been demonstrated to be a significant risk factor for the development of AS. Various studies have shown that exenatide significantly reduces serum total cholesterol(TC), triglyceride (TG), and low-density lipoprotein cholesterol(LDL-C) levels while increasing high density lipoprotein cholesterol (HDL-C) levels in patients with T2DM, and exhibiting favorable long-term effects (59–61). Koska et al. reported that once weekly administration of exenatide resulted in positive effects on blood glucose and body weight, as well as an appropriate increase in heart rate, which is also a risk factor for carotid atherosclerosis (62, 63). Several *in vivo* experiments suggest the potential mechanism by which exenatide improves IMT. Yang et al. (64) found that exenatide could inhibit oxidative stress and inflammation in mice, and improve the accumulation of plaque macrophages and osteopontin expression. Jansen et al. (65) demonstrated that exenatide improved abdominal fat deposition in high-fat fed mice. At the same time, there was study reported no statistically significant difference in the incidence of major adverse cardiovascular events with once-weekly exenatide versus PLC (66).

Only one GLP-A RA, exenatide, was included in our study. However, at the same time, some other types of GLP-A RA have also attracted our attention because of their cardiovascular protective effects (67, 68). Related to our study, a real-world study in patients with T2DM showed that the addition of liraglutide to metformin monotherapy significantly reduced cIMT, as well as serum TC, TG, and LDL-C (69). Another clinical study reported that 4 months of semaglutide injection treatment could also reduce cIMT to some extent (70). Unfortunately, these studies were not included in the NMA because they lacked a control group.

The NMA findings also indicate that alogliptin, a DDP-4i, achieved good performance in intervening cIMT progression. DPP-4i, belong to the group of incretin-based medications that act by stimulating the insulin secretion and inhibiting glucagon secretion in a glucose-dependent manner (71). A prospective clinical study observed that a 10-month treatment of alogliptin intervention can significantly reduce the volume of AS plaque and necrosis, as well as an increase in fibrosis volume compared to PLC (72). In addition, a mechanistic study had shown that alogliptin can inhibit the expression of inflammatory factors interleukin-1(IL-1) and Interleukin-6(IL-6) through a glucose-dependent or independent way (73). However, the extended study based on the SPEAD-A trial followed T2DM patients for 520 weeks and found that early use of alogliptin was not associated with a reduction in the risk of developing cardiovascular disease, which may be related to a relatively small number of events during follow-up (74). A clinical trial conducted by Barbieri et al. (75)

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TABLE 2 The rank of various of HDs and PLC on retarding the progression cIMT.

Treatment	SUCRA	RANK	Treatment	SUCRA	RANK
Exenatide	94.9%	1	Troglitazone	43.7%	9
Alogliptin	86.9%	2	Ipragliflozin	36.6	10
Metformin	81.2%	3	Glimepiride	35.3	11
Nateglinide	67.1%	4	PLC	31.8	12
Acarbose	63.1	5	Tofoglifozin	29.7	13
Sitagliptin	62.8	6	Voglibose	6.5%	14
Rosiglitazone	56.9%	7	Glibenclamide	5.1%	15
Pioglitazone	45.5%	8	–	–	–

showed that both 3 months of sitagliptin and vildagliptin treatment may possible to prevent AS progression in patients with T2DM by reducing inflammation and oxidative stress, moreover, compared with baseline, vildagliptin was more effective in reducing IMT than sitagliptin. It has also reported that vildagliptin is more effective than sitagliptin in controlling the mean 24-hour blood glucose level at a same dose (76), and vildagliptin also shows a superior effectiveness in improving oxidative stress and inflammatory markers, the variations in effectiveness among these drugs are likely attributed to their structural heterogeneity (77).

Our study also included three PPARγ agonists, pioglitazone, rosiglitazone and troglitazone, but no significant improvement was observed for any of them compared with PLC. Contrary to our study, a previous meta-analysis reported a statistically significant reduction in the progression of cIMT with the use of pioglitazone (78). At the same time, clinical studies and meta-analyses (79, 80) have reported that long-term use of rosiglitazone may increase the risk of cardiovascular mortality. Indeed, the administration of pioglitazone and rosiglitazone was associated with the adverse effect of fluid retention, which potentially contributed to or exacerbated congestive heart failure (81).

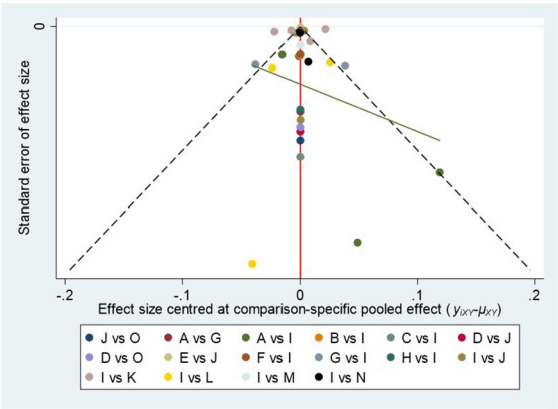


FIGURE 5
Plot of funnel (A, acarbose; B, alogliptin; C, exenatide; D, glibenclamide; E, glimepiride; F, ipragliflozin; G, metformin; H, nateglinide; I, PLC; J, pioglitazone; K, rosiglitaxone; L, sitagliptin; M, tofoglifozin; N, roglitaxone; O, voglibose).

Despite numerous reports demonstrating the cardiovascular benefits of SGLT-2i (82–84), we did not find significant effect of SGLT-2i on the progression of cIMT. This may be related to the limited number of corresponding clinical studies, as we were only able to retrieve data on two SGLT-2i, ipragliflozin and tofogliflozin.

So far, metformin is still the first-line drug for people with diabetes (85). The results of our NMA also demonstrated a significant improvement in cIMT with the long-term administration of metformin. This finding aligns with a meta-analysis conducted by Chen et al. (86), their study also showed that metformin exhibited a significant impact on cIMT only when the intervention exceeded 12 months. Therefore, we speculate that longer duration of drug therapy is likely to result in a more pronounced therapeutic effect.

Limitation

This study may represent the first NMA to compare and rank the effects of long-term use of different HDs on cIMT progression. This NMA may serve as a valuable reference for the utilization of cardiovascular protective drugs in individuals with diabetes or at risk of diabetes. However, the present NMA has some limitations. Firstly, limited number of studies and some small sample sizes may potentially affect the accuracy and applicability of the obtained results. Secondly, the research population in this NMA is based on type 1 diabetes patients, type 2 diabetes patients, pre-diabetes patients and coronary heart disease patients, which may lead to heterogeneity between the studies, so the interpretation of the results should be cautious. At last, we expect to see the performance of a variety of emerging HDs, such as DDP-4i of SGTL-2i, GLP-1 RA and PPARγ agonist. However, the available literature is currently limited. In conclusion, we anticipate to see more kinds of drugs and higher quality RCTs to verify our result in the future.

Conclusions

We employed a Bayesian NMA to assess the impact of prolonged utilization of various HD methods on retarding the progression of carotid intima-media thickness (cIMT). Long-term administration of exenatide, alogliptin and metformin may be most effective in retarding the progression of cIMT.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

QL: Conceptualization, Data curation, Formal Analysis, Methodology, Writing – original draft, Writing – review & editing. YY: Conceptualization, Data curation, Formal Analysis, Methodology, Writing – original draft, Writing – review & editing. YL: Data curation, Writing – original draft. QW: Data curation, Writing – original draft. XH: Formal Analysis, Writing – original draft. LL: Formal Analysis, Writing – original draft. XY: Data curation, Writing – original draft. CY: Data curation, Writing – original draft. SW: Conceptualization, Writing – review & editing.

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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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1403606/full#supplementary-material

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EDITED BY
Eduardo Hertel Ribeiro,
Escola Superior de Ciências da Santa Casa de Misericórdia de Vitória (EMESCAM), Brazil

REVIEWED BY
Catherine Bulka,
University of South Florida, United States
Claire Joanne Stocker,
Aston University, United Kingdom

*CORRESPONDENCE
Yongqiang Sun
qiang19850418@163.com

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U-shaped association between serum Klotho and all-cause mortality in US cardiovascular patients: a prospective cohort study

Shasha Liu[1], Zhanfang Zhu[2], Kai Yu[3], Wei Zhang[1], Jie Pu[1], Ying Lv[1], Zhiguo Tang[1], Fuqiang Liu[1] and Yongqiang Sun[1,4]*

[1]Department of Cardiology, Shaanxi Provincial People's Hospital, Xi'an, China, [2]Department of Internal Medicine, Xi'an Jiaotong University Hospital, Xi'an, China, [3]Department of Cardiology, Pucheng County Hospital, Weinan, China, [4]Department of Interventional Radiography, Shanxi Provincial People's Hospital, Xi'an, China

Background: Increased levels of serum Klotho have been associated with a reduced risk of several cardiovascular diseases (CVD). However, limited studies exist on the association between serum Klotho and mortality in patients with CVD.

Methods: We collected data from CVD patients in the National Health and Nutrition Examination Survey (NHANES) spanning 2007 to 2016. We linked NHANES data with the National Death Index to determine the survival status of participants. Univariate and multivariable Cox regression models were used to investigate the relationship between serum Klotho levels and mortality in CVD patients. The relationship between serum Klotho quartiles and mortality in CVD patients was visualized using Kaplan-Meier (KM) curves and restricted cubic spine. Finally, subgroup analyses were used to examine the association between serum Klotho and all-cause mortality in different populations.

Results: 1905 patients with CVD were finally enrolled in our study with a mean follow-up of 7.1 years. The average age of the participants was 63.4 years, with 58.40% being male. KM showed that lower Klotho levels were associated with lower survival rates. After adjusting for potential confounders, patients with higher serum Klotho levels had lower all-cause mortality (Q1: 1.00, Q2: 0.58 (0.42–0.80), Q3: 0.69 (0.47–1.01), and Q4:0.64 (0.45–0.92). However, the relationship between serum Klotho levels and cardiovascular mortality was not statistically significant. Dose-response analysis shows a U-shaped relationship between serum Klotho levels and all-cause mortality in patients with CVD (P nonlinear=0.002). Subgroup analysis indicated that participants with a history of hypertension had a higher risk of all-cause mortality in serum Klotho Q4 compared to Q1 (P trend <0.05).

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Conclusion: The relationship between serum Klotho levels and all-cause mortality in CVD patients exhibits a U-shaped association. The underlying mechanisms of this association need further investigation.

KEYWORDS

cardiovascular disease, serum Klotho, NHANES, all-cause mortality, National Health and Nutrition Examination Survey

Introduction

Cardiovascular disease (CVD) is a major cause of global disease burden and a key factor contributing to premature death and escalating healthcare costs (1, 2). CVD encompasses a range of conditions including heart failure, coronary heart disease, angina, heart attacks, and strokes. Identifying factors that predict mortality in patients with CVD is critical to promoting early prevention of the disease.

The Klotho protein is encoded by the Klotho gene, initially described for its anti-aging abilities. Mice lacking Klotho exhibit a range of syndromes resembling human aging, including shortened lifespan, infertility, vascular calcification, arteriosclerosis, skin atrophy, osteoporosis, and emphysema (3). Conversely, overexpression of Klotho can act as an anti-aging hormone (4). Two different types of Klotho proteins can be detected in humans: one located in the cytoplasmic membrane as a transmembrane protein, and the other as soluble Klotho, including soluble and secreted Klotho circulating in the blood (5). Numerous studies have shown that increased serum Klotho levels are closely associated with decreased cardiovascular risk. In middle-aged and elderly Americans, serum Klotho levels are negatively correlated with the presence of atrial fibrillation (6). Studies in diabetic mice have found that serum Klotho levels can improve diabetic cardiomyopathy and myocardial fibrosis (7, 8). In a large and diverse cohort, an independent inverse relationship was observed between serum Klotho levels and arterial stiffness indicator, pulse pressure (9). Serum Klotho, as a cardiac protector, can prevent the effects of aging on the heart and reduce the burden of CVD (10), but there is currently no research analyzing the relationship between serum Klotho levels and all-cause mortality and cardiovascular mortality in CVD patients.

By examining data from the National Health and Nutrition Examination Survey (NHANES), this study took into account potential confounding factors and investigated the relationship between serum Klotho levels and mortality in patients with CVD. This has important implications for the management of the health of patients with CVD.

Method

Study population

The data used in this study were obtained from the NHANES database (https://www.cdc.gov/nchs/nhanes/index.htm). NHANES

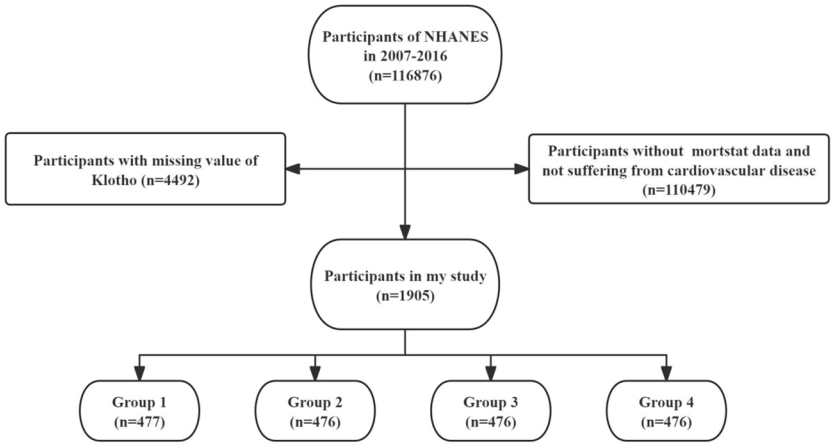


FIGURE 1
Flow chart of the study subjects.

is a vital national health and nutrition survey program conducted by the U.S. government. NHANES collects a vast amount of medical, nutritional, and health-related data through population surveys and physical examinations, providing a rich source of information for research in the field of public health. In this study, we used data from 5 cycles, involving a total of 116,876 participants from 2007 to 2016. After excluding individuals with missing mortality data and without cardiovascular disease (n = 110,479), and those with missing serum Klotho data (n = 4,492), a total of 1,905 participants with CVD were included in the final analysis. The selection process of this study is illustrated in Figure 1.

Outcome variable

CVD was defined as individuals who answered "yes" to the question: "Have you been told by a doctor or other health professional that you have congestive heart failure/CHD/myocardial infarction/stroke?". All-cause mortality and cardiovascular mortality were obtained from the National Death Index (NDI) database as of December 31, 2019.

Exposure variable

Participants' blood samples were collected in NHANES (National Health and Nutrition Examination Survey) and stored at -80°C until analyzed. Klotho concentrations in serum were measured using a commercially available ELISA kit (product offered by IBL International, Japan). The lower limit of detection for Klotho in this kit is typically set at 6 pg/mL prior to the assay, frozen serum samples were thawed and the manufacturer's protocol was followed. Each sample is usually assayed twice to ensure accuracy and reproducibility of results. Two concentration levels of quality control samples (low and high Klotho concentrations) were included in the assay. If a sample's duplicate assay value varies by more than 10%, that sample is subject to re-assay. If the value of the quality control sample is outside the range of two standard deviations from the known value, the result of the entire assay panel will be considered invalid and will need to be re-assayed. The final determination is expressed as the average of two measurements (11). For a detailed description of the Klotho detection method, please visit the NHANES website.

Covariates

We performed covariate inclusion through previous literature. Demographic, laboratory, physical examination, and questionnaire data were collected from the NHANES database. Age, poverty income ratio (PIR), and body mass index (BMI) were considered continuous variables (12, 13). Sex, race, smoking status, drinking status, education level, marital status, hypertension, and type 2 diabetes mellitus (DM) were considered categorical variables (14–16). Race was categorized as Mexican American, non-Hispanic Black, non-Hispanic White, other Hispanic, and other Race. Smoking status was categorized as former smoker, never smoker, and current smoker based on whether the participant had smoked 100 cigarettes in the past year. Drinking status was defined as non-drinker, low-to-moderate drinker, and heavy drinker. Education level was categorized as below high school, high school, and above high school. Marital status was classified as separated, married, and never married. Type 2 DM was defined according to the following criteria: (1) participants reporting a diagnosis of diabetes by a healthcare professional, (2) glycated hemoglobin testing 6.5% or higher, or (3) fasting blood glucose 126 mg/dL or higher. Hypertension was defined as self-reported hypertension, systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or use of antihypertensive medication.

Statistical analysis

Continuous variables were expressed as mean ± standard error and differences between groups were compared using weighted t-tests or one-way ANOVA. Categorical variables were expressed as frequencies and percentages, and differences between groups were compared using Chi-square tests. Univariate and multivariate Cox proportional hazards regression models were used to examine the association between serum Klotho levels and all-cause mortality in patients with CVD. In this study, we used three models: Crude model (unadjusted), Model 1 (adjusted for age, gender, and race), and Model 2 (further adjusted on the basis of model 2 for PIR, education level, BMI, hypertension, type 2 DM, drinking status, and smoking status). Kaplan-Meier (KM) curves were used to show the association between serum Klotho levels and all-cause mortality in patients with CVD when stratified by serum Klotho quartiles. In addition, restricted cubic spline curves (RCS) adjusted for confounders were utilized to show the dose-response relationship between serum Klotho levels and mortality in CVD patients. Finally, we also performed subgroup analyses and interaction tests to examine the relationship between serum Klotho levels and all-cause mortality in CVD populations with different characteristics.

All results of this study were based on clustering, stratification, and weighting calculations from the NHANES database and were analyzed using R software (version: 4.1.3) and the *survey* package (version: 4.4–1). A two-tailed p value of less than 0.05 was considered statistically significant.

Result

Baseline characteristics

A total of 1905 subjects were enrolled in this study, with an average age of 63.4 years, 58.40% were male, and a mean follow-up time of 7.1 years (range 0.1 to 13.2 years) (Table 1). Serum Klotho levels were categorized into four groups based on quartiles (Q1 ≤ 614.1, 614.1 < Q2 ≤ 763.4, 763.4 < Q3 ≤ 946.2, 946.2 < Q4, pg/mL). There were no significant differences among the four groups of participants in terms of age, sex, race, marital status, education level, BMI, PIR, smoking status, drinking status, type 2 DM, hypertension, or cardiovascular mortality (P>0.05). Participants in

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TABLE 1 Baseline characteristics of participants according to quartiles of Klotho.

	Klotho quartiles (pg/mL)					P-value
	Total (N=1905)	Q1 (N=477)	Q2 (N=476)	Q3 (N=476)	Q4 (N=476)	
Age, years	63.4(0.3)	63.7(0.7)	63.8(0.5)	63.4(0.6)	62.7(0.6)	0.498
Sex (%)						0.347
female	804(41.6)	201(42.3)	185(40.1)	195(38.3)	223(46.0)	
male	1101(58.4)	276(57.8)	291(59.9)	281(61.7)	253(54.1)	
Race (%)						0.420
Mexican American	224(4.9)	55(4.9)	54(4.6)	58(5.5)	57(4.5)	
non-Hispanic Black	436(11.6)	110(12.5)	102(10.6)	108(11.2)	116(12.3)	
non-Hispanic White	918(72.2)	220(67.7)	249(75.1)	230(73.4)	219(72.4)	
other Hispanic	196(3.9)	55(4.6)	42(2.9)	46(4.1)	53(4.3)	
other Race	131(7.4)	37(10.3)	29(6.9)	34(5.9)	31(6.6)	
Marital status, %						0.209
separated	642(27.1)	167(31.8)	152(22.8)	164(27.9)	159(26.4)	
married	1113(65.7)	268(62.0)	280(67.8)	278(64.3)	287(68.5)	
never married	150(7.2)	42(6.2)	44(9.4)	34(7.8)	30(5.0)	
Education level, %						0.278
below high school	319(9.4)	66(7.6)	86(8.6)	91(12.7)	76(8.5)	
high school	816(40.8)	220(43.2)	189(38.3)	207(39.6)	200(42.3)	
above high school	767(49.8)	190(49.2)	201(53.1)	176(47.6)	200(49.3)	
BMI, kg/m^2	31.5(0.2)	31.0(0.4)	31.6(0.4)	31.9(0.5)	31.4(0.5)	0.456
PIR	2.6(0.1)	2.4(0.1)	2.7(0.1)	2.6(0.1)	2.5(0.1)	0.182
Smoking status, %						0.100
former smoker	734(39.9)	187(39.3)	183(37.7)	194(45.9)	170(36.8)	
never smoker	688(34.8)	147(29.5)	174(38.1)	170(33.5)	197(37.7)	
current smoker	483(25.3)	143(31.2)	119(24.2)	112(20.6)	109(25.5)	
Drinking status, %						0.376
non-drinker	864(40.7)	223(47.2)	212(39.5)	213(42.6)	216(42.9)	
low-to-moderate drinker	732(43.7)	181(41.9)	179(46.3)	191(48.1)	181(47.7)	
heavy drinker	193(10.5)	45(11.0)	57(14.2)	43(9.3)	48(9.4)	
Type 2 DM, %						0.119
no	1026(59.6)	250(57.7)	287(66.1)	248(56.5)	241(57.7)	
yes	878(40.4)	227(42.3)	188(34.0)	228(43.6)	235(42.3)	
Hypertension, %						0.859
no	376(24.0)	88(22.2)	91(24.4)	102(24.1)	95(25.3)	
yes	1529(76.0)	389(77.8)	385(75.6)	374(76.0)	381(74.7)	
All-cause mortality, %						0.019
no	1355(75.9)	310(69.4)	353(80.1)	335(74.7)	357(79.0)	
yes	550(24.1)	167(30.6)	123(20.0)	141(25.4)	119(21.0)	

(Continued)

TABLE 1 Continued

	Klotho quartiles (pg/mL)					P-value
	Total (N=1905)	Q1 (N=477)	Q2 (N=476)	Q3 (N=476)	Q4 (N=476)	
Cardiovascular mortality, %						0.465
no	1746(93.2)	430(91.2)	438(93.8)	437(93.4)	441(94.2)	
yes	159(6.8)	47(8.8)	38(6.2)	39(6.6)	35(5.8)	

BMI, body mass index; DM, diabetes mellitus; PIR, poverty income ratio.
All values are expressed as a proportion (%) or mean ± standard deviation.

higher quartiles of serum Klotho exhibited a lower all-cause mortality(Q1: 30.63%, Q2:19.95%, Q3:25.35%, Q4: 20.98%, P <0.05).

Table 2 stratified the participants into two groups based on their survival status, with 1355 individuals in the survival group and 55 individuals in the non-survival group. The two groups differed significantly in terms of age, marital status, education level, PIR, smoking status, drinking status, type 2 DM, hypertension, and cardiovascular mortality (P<0.05). Compared with the survivor group, the non-survivor group had a higher percentage of patients who were older, had lower PIR and education level, smoked, non-drinking, type 2 diabetes, and hypertension. In addition, cardiovascular mortality was higher in the non-survivor group.

The relationship between serum Klotho and mortality in CVD patients

According to the KM curve stratified by quartiles based on serum Klotho levels, the all-cause mortality in the lowest quartile (Q1) was significantly higher than in the other three groups (P = 0.014), but there was no difference in cardiovascular mortality (P >0.05) (Figure 2).

Further multivariate COX regression was used to analyze the independent association between serum Klotho levels and mortality (Table 3). In CVD patients, an inverse association was observed between serum Klotho levels and all-cause mortality across quartiles (Table 3). Compared with Q1, all-cause mortality was significantly lower in those with higher serum Klotho levels in fully adjusted Model 2 (Q2: 0.58 (0.42–0.80), Q3: 0.69 (0.47–1.01), and Q4: 0.64 (0.45–0.92), and the test for trend was no difference in the quartiles. However, in the study of cardiovascular mortality, no significant association was found between quartiles in the fully adjusted Model 2 (P>0.05) (Table 4).

Dose-response relationship between serum Klotho and mortality

RCS was used to portray a dose-response association between serum Klotho levels and all-cause and cardiovascular mortality in patients with CVD (Figure 3). There was a significant nonlinear association between serum Klotho and all-cause mortality and an insignificant association with cardiovascular mortality (all-cause mortality: nonlinear P = 0.002, cardiovascular mortality: overall P>0.05). When serum Klotho levels were below 763.4 pg/mL, all-cause mortality decreased with increasing serum Klotho levels, and when Klotho was above 763.4 pg/mL, all-cause mortality increased with increasing serum Klotho levels.

Subgroup analysis

As depicted in Figure 4, a subgroup analysis was performed considering age, sex, BMI, drinking status, smoking status, hypertension, and type 2 DM. The inverse association between serum Klotho and all-cause mortality is maintained in most subgroups, and the interaction did not show differences between subgroups (P for interaction >0.05).

Discussion

Our study represents the first investigation into the relationship between Klotho levels and mortality among CVD patients within a large population. This extensive prospective cohort study analyzed data from 1905 CVD patients included in NHANES spanning from 2007 to 2016. Upon adjusting for confounding variables, we observed that higher all-cause mortality in CVD patients was linked to lower serum Klotho levels, a finding reinforced by KM curves. Notably, serum Klotho levels did not exhibit a statistically significant association with cardiovascular mortality in CVD patients. RCS curves revealed a U-shaped correlation between serum Klotho levels and all-cause mortality in CVD patients.

Serum Klotho, an aging-related protein, is characterized by a gradual decline in levels with advancing age (17). Studies have identified its protective effects against various degenerative diseases. Notably, Serum Klotho has exhibited kidney-protective properties in animal models of acute kidney injury and chronic kidney disease (18). Nephritis mice overexpressing Klotho transgene showed improved survival rates and kidney function, along with decreased morphological alterations in renal tubules and glomeruli (19). Additionally, the kidney-protective role of serum Klotho was evident in mice with renal fibrosis, where treatment with serum Klotho protein hindered the fibrotic process (20). Furthermore, Serum Klotho has demonstrated significant involvement in

TABLE 2 Characteristics of the study population according to their all-cause mortality.

	Total (N=1905)	Survivor (n = 1355)	Non-survivor (n = 550)	P-value
Age, years	63.4(0.3)	61.9(0.3)	68.0(0.4)	<0.001
Sex (%)				0.872
female	804(41.6)	595(41.5)	209(42.1)	
male	1101(58.4)	760(58.6)	341(57.921)	
Race (%)				0.286
Mexican American	224(4.9)	169(5.1)	55(4.2)	
non-Hispanic Black	436(11.6)	318(11.7)	118(11.1)	
non-Hispanic White	918(72.2)	604(71.1)	314(75.8)	
other Hispanic	196(3.9)	155(4.4)	41(2.7)	
other Race	131(7.4)	109(7.7)	22(6.2)	
PIR	2.6(0.1)	2.7(0.1)	2.1(0.1)	<0.001
Marital status, %				<0.001
separated	642(27.1)	421(24.2)	221(36.4)	
married	1113(65.7)	824(68.3)	289(57.6)	
never married	150(7.2)	110(7.5)	40(6.0)	
Education level, %				0.004
below high school	319(9.4)	210(8.2)	109(13.2)	
high school	816(40.8)	561(39.4)	255(45.0)	
above high school	767(49.8)	582(52.4)	185(41.8)	
BMI, kg/m^2	31.5(0.2)	31.5(0.2)	31.2(0.6)	0.605
Klotho, pg/mL	806.6(8.6)	814.4(9.0)	782.1(16.8)	0.065
Smoking status, %				0.006
former smoker	734(39.9)	488(37.7)	246(47.0)	
never smoker	688(34.8)	526(37.0)	162(27.9)	
current smoker	483(25.3)	341(25.4)	142(25.1)	
Drinking status, %				<0.001
non-drinker	864(40.7)	571(39.4)	293(54.3)	
low-to-moderate drinker	732(43.7)	561(49.0)	171(36.5)	
heavy drinker	193(10.5)	145(11.6)	48(9.2)	
Type 2 DM, %				<0.001
no	1026(59.6)	781(63.4)	245(47.7)	
yes	878(40.4)	573(36.6)	305(52.3)	
Hypertension, %				0.033
no	376(24.0)	281(25.8)	95(18.6)	
yes	1529(76.0)	1074(74.2)	455(81.4)	

BMI, body mass index; DM, diabetes mellitus; PIR, poverty income ratio.
All values are expressed as a proportion (%) or mean ± standard deviation.

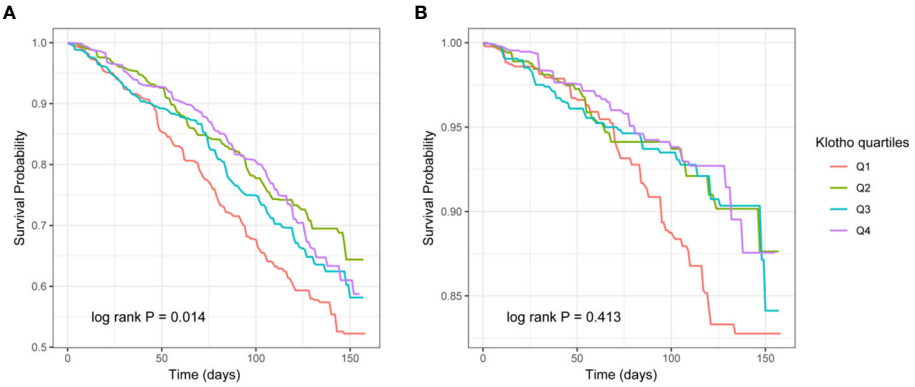


FIGURE 2
The Kaplan–Meier (KM) survival curves classified by Klotho quartiles (**A**: all-cause mortality, **B**: cardiovascular mortality).

TABLE 3 Association between Klotho and all-cause mortality in the CVD patients.

	Crude model		Model 1		Model 2	
	HR(95%CI)	P-value	HR(95%CI)	P-value	HR(95%CI)	P-value
Klotho quartiles						
Q1	ref		ref		ref	
Q2	0.63(0.44,0.88)	0.008	0.59(0.44,0.80)	<0.001	0.58(0.42,0.80)	<0.001
Q3	0.76(0.53,1.11)	0.158	0.75(0.52,1.09)	0.129	0.69(0.47,1.01)	0.054
Q4	0.64(0.46,0.88)	0.006	0.65(0.48,0.89)	0.007	0.64(0.45,0.92)	0.015
P for trend		0.047		0.068		0.069

Crude model: no adjusted;
Model 1: adjusted for age, sex, and race;
Model 2: adjusted for age, sex, race, PIR, education level, BMI, hypertension, type 2 DM, drinking status, and smoking status.
ref, reference; BMI, body mass index; DM, diabetes mellitus; PIR, poverty income ratio.

TABLE 4 Association between Klotho and cardiovascular mortality in the CVD patients.

	Crude model		Model 1		Model 2	
	HR(95%CI)	P-value	HR(95%CI)	P-value	HR(95%CI)	P-value
Klotho quartiles						
Q1	ref		ref		ref	
Q2	0.68(0.35,1.31)	0.246	0.63(0.34,1.18)	0.148	0.75(0.39,1.45)	0.397
Q3	0.69(0.38,1.26)	0.227	0.66(0.36,1.21)	0.178	0.66(0.33,1.35)	0.260
Q4	0.62(0.35,1.08)	0.090	0.62(0.36,1.07)	0.087	0.65(0.35,1.19)	0.158
P for trend		0.129		0.141		0.191

Crude model: no adjusted;
Model 1: adjusted for age, sex, and race;
Model 2: adjusted for age, sex, race, PIR, education level, BMI, hypertension, type 2 DM, drinking status, and smoking status.
ref, reference; BMI, body mass index; DM, diabetes mellitus; PIR, poverty income ratio.

degenerative lung diseases, with serum Klotho shielding the lungs from oxidative damage and cell apoptosis by enhancing the endogenous antioxidant capacity of pulmonary epithelial cells (21, 22). In the realm of cancer research, investigations have revealed an inverse relationship between serum Klotho levels and cancer risk (23). Additionally, serum Klotho has been suggested to function as a tumor suppressor gene by modulating cell metabolism and various carcinogenic pathways (24).

Experimental studies conducted on laboratory animals have highlighted the direct protective impact of serum Klotho on the

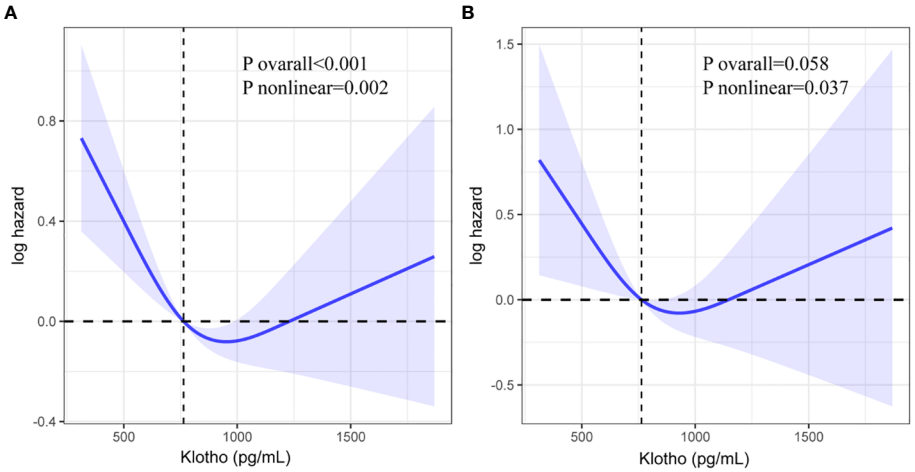


FIGURE 3
Restricted cubic spline (RCS) regression analysis of Klotho with all-cause mortality (**A**: all-cause mortality, **B**: cardiovascular mortality).

cardiovascular system. Notably, Klotho-deficient mice have demonstrated arterial wall calcification (3), a risk that can be mitigated by serum Klotho supplementation resulting in decreased blood phosphate levels (25). Additionally, cardiac dysfunction, as evidenced by hypertrophy and fibrosis in Klotho-deficient mice, can be ameliorated by supplementing serum Klotho (26, 27). Clinical investigations involving patients undergoing non-emergent coronary angiography revealed an association between lower levels of serum Klotho and the presence as well as the severity of coronary artery disease (28). Furthermore, studies involving hypertensive patients showed that lower serum Klotho levels were linked to an increased risk of all-cause mortality (29).

Our findings suggest that serum Klotho levels are associated with all-cause mortality but not cardiovascular mortality in a cardiovascular disease population. Similar results were obtained in a large-scale study involving hypertensive patients, where serum Klotho levels were linked to all-cause mortality in this population but not to cardiovascular mortality (29). While two independent studies identified serum Klotho as a predictive factor for cardiovascular mortality and all-cause mortality in chronic hemodialysis patients (30, 31), it is important to note that the cardiovascular death risk in this patient population is significantly elevated compared to the general population (32).

The observed negative association between serum Klotho levels and all-cause mortality in CVD patients can be elucidated through

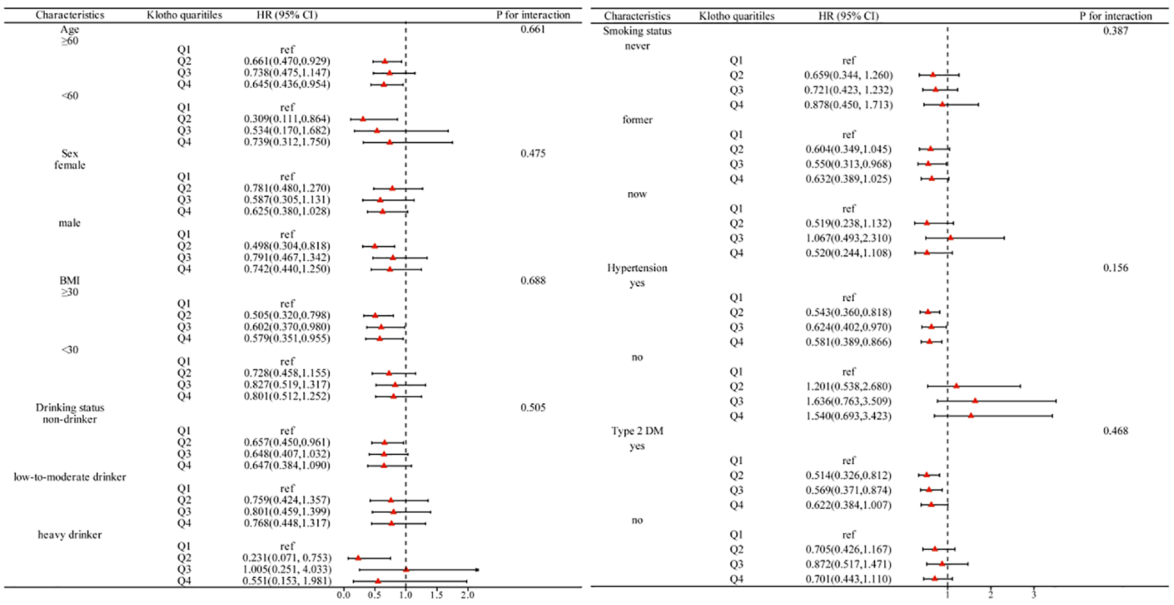


FIGURE 4
Subgroup analysis of the association between Klotho quartiles with all-cause mortality.

the mechanism of oxidative stress. The Klotho protein exhibits both antioxidant and anti-apoptotic properties, thereby exerting inhibitory effects on insulin, transforming growth factor-beta 1 signaling pathways, and the release of pro-inflammatory cytokine interleukin 6. These actions serve to inhibit oxidative stress, mitigate inflammation, and prevent fibrotic effects, ultimately contributing to a reduction in the risk of all-cause mortality (20, 33–37).

Our findings reveal a U-shaped relationship between serum Klotho levels and all-cause mortality in patients with CVD, a trend that aligns with previous research. Studies involving middle-aged and older adults have similarly reported an elevated risk of death for individuals at the extremes of Klotho levels (38). Furthermore, investigations in populations with type 2 DM and rheumatoid arthritis have also identified a U-shaped association between serum Klotho levels and the risk of mortality (39, 40). Given serum Klotho's crucial role in mineral regulation and inflammation prevention within the body (41), it is hypothesized that individuals with extreme serum Klotho levels may experience disruption in their physiological balance, subsequently increasing the risk of death.

While our research utilizing a nationally representative population provided valuable insights into the association between serum Klotho levels and the risk of death in CVD patients, it is important to acknowledge several limitations when interpreting the results. Firstly, serum Klotho levels were measured only once, and there may be other factors interfering with the accuracy of the measurement results. Secondly, the use of stored residual serum for measuring serum Klotho may introduce measurement bias stemming from sample quality issues. Thirdly, despite adjusting for confounding factors like vital signs, lifestyle habits, and comorbidities, the presence of residual confounders could potentially impact our outcomes. As a result, prospective studies are necessary to validate and confirm the findings presented in our research.

Conclusion

Our study conducted on a nationally representative sample of American CVD patients revealed a U-shaped relationship between serum Klotho levels and all-cause mortality in this specific population. These findings offer valuable insights for prognostic assessment and treatment strategies tailored to individuals with CVD.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by National Center for Health Statistics Ethics Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

SL: Writing – original draft. ZZ: Writing – original draft. KY: Writing – original draft. WZ: Writing – original draft. JP: Writing – original draft. YL: Writing – original draft. ZT: Writing – original draft. FL: Writing – original draft. YS: Writing – review & editing.

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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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EDITED BY
Daigo Sawaki,
Jichi Medical University, Japan

REVIEWED BY
Akito Nakagawa,
Nakagawa Clinic, Japan
Thomas E. Sharp III,
University of South Florida, United States

*CORRESPONDENCE
Shougang Sun
sunshg@lzu.edu.cn

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Association between serum osmolality and 28-day all-cause mortality in patients with heart failure and reduced ejection fraction: a retrospective cohort study from the MIMIC-IV database

Qi Zou, Jiazheng Li, Pengyang Lin, Jialiang Ma, Zhiliang Wei, Ting Tao, Guodong Han and Shougang Sun*

Department of Cardiology, Lanzhou University Second Hospital, Lanzhou, China

Background: Previous studies have not thoroughly explored the impact of serum osmolality levels on early mortality in heart failure and reduced ejection fraction (HFrEF) patients. The purpose of this study was to investigate the relationship between serum osmolality levels and early all-cause mortality in patients with HFrEF.

Methods: The open access MIMIC-IV database was the source of data for our study. We collected demographic data, vital signs, laboratory parameters, and comorbidities of the included patients and divided them into 3 groups based on their initial serum osmolality on admission, with the primary outcome being all-cause mortality within 28 days of admission. Smoothing Spline Fitting Curve, the Kaplan-Meier survival curve, and Threshold effect analysis were used to assess the relationship between serum osmolality and early mortality in HFrEF patients.

Results: A total of 6228 patients (55.31% male) were included. All-cause mortality within 28 days on admission was 18.88% in all patients. After adjusting for confounders, higher serum osmolality levels were independently associated with an increased risk of 28-days all-cause mortality compared with the reference group (Reference group Q2: 290–309 mmol/L, Q4: HR, 1.82 [95% CI 1.19–2.78] P<0.05, Q5: HR, 1.99 [95% CI 1.02–3.91] P<0.05). Smooth spline fitting revealed a U-shaped association between serum osmolality and 28-days all-cause mortality. Further threshold effect analysis results suggested that each unit increase in serum osmolality level was associated with a 2% increase in 28-days all-cause mortality when serum osmolality levels were ≥ 298.8 mmol/L (HR, 1.019 [95% CI 1.012–1.025] P<0.05).

Conclusion: A U-shaped correlation between initial serum osmolality and 28-days all-cause mortality in HFrEF patients was identified, revealing higher osmolality levels significantly increase mortality risk. These results underscore serum osmolality's critical role in early mortality among HFrEF patients, highlighting the need for further, larger-scale studies for validation.

KEYWORDS

serum osmolality, all-cause mortality, heart failure, U-shaped correlation, MIMIC-IV database

Introduction

Heart failure (HF) remains a leading cause of morbidity and mortality worldwide, presenting a substantial burden to healthcare systems (1). Despite advancements in therapeutic strategies, the prognosis for HF patients is often poor, with a high rate of hospital readmission and mortality within the first year following diagnosis (2).

Research indicates that serum osmolality, a crucial indicator of fluid and electrolyte equilibrium, may play an integral role in the pathophysiology of HF (3). In healthy individuals, serum osmolality is rigorously maintained, serving as a determinant of intracellular and extracellular water distribution (4). Elderly patients with HF frequently present with concurrent comorbidities, such as diabetes, hypertension, and renal disease, that can precipitate further perturbation of serum osmolality (5, 6).

Hyponatremia is recognized as a prognostic marker in HF, correlating with increased rates of hospitalization and mortality (7). Nevertheless, the prognostic value of serum osmolality remains insufficiently examined (8). Elucidating the association between serum osmolality and HF outcomes could enhance patient management and risk assessment. While a limited body of research suggests that atypical serum osmolality correlates with adverse HF prognoses, including heightened mortality and readmission rates, the specific relationship to short-term mortality, particularly within the first 28 days post-admission, is less understood (9, 10). This understanding is vital due to the acute nature of HF exacerbations and the imperative of early prognostic determinants in this phase.

Our study endeavors to elucidate the correlation between serum osmolality and 28-days all-cause mortality in heart failure and reduced ejection fraction (HFrEF) patients post-hospital admission. The findings may offer significant insights into the acute-phase prognostic relevance of serum osmolality in HFrEF, potentially informing clinical interventions and enhancing patient outcomes.

Methods

Study population

Our study was a retrospective observational analysis. The data was sourced from the Medical Information Mart for Intensive Care IV (MIMIC-IV) database. MIMIC-IV is a large, freely accessible database comprising de-identified health-related data associated with over forty thousand patients who stayed in critical care units of the Beth Israel Deaconess Medical Center between 2001 and 2019 (11). The database includes information such as demographic data, vital signs, laboratory parameters, comorbidities, and more. Author ZQ completed the "Protecting Human Research Participants" online course from the National Institutes of Health, granting them access to the dataset. The use of the MIMIC-IV database for research purposes has been approved by the Institutional Review Boards of the Massachusetts Institute of Technology and the Beth Israel Deaconess Medical Center, with a waiver of informed consent granted. We successfully gathered information on 6228 patients with HFrEF using PostgreSQL Structured Query Language based on International Classification of Diseases (ICD-9 and ICD-10) codes.

Inclusion and exclusion criteria

The prerequisites for participation were as follows: Patients diagnosed with HFrEF using ICD-9 and ICD-10 disease diagnosis codes in the MIMIC-IV database.

Patients with the following characteristics are excluded: (1) those under the age of 18; (2) those with an admission stay of fewer than 24 h; (3) those with leukemia and myelodysplastic syndrome; (4) those with missing baseline sodium, potassium, urea, and glucose at admission; and (5) data for which the calculated serum osmolality value is anomalous.

Data collection

Structured Query Language (SQL) for PostgreSQL (version 9.6) was used to extract baseline characteristics including gender, age, comorbidities (hypertension, diabetes mellitus, chronic obstructive pulmonary disease, renal disease, cerebrovascular disease, coronary artery disease) as well as laboratory markers and vitals extracted from the MIMIC-IV database for the first 24 hours after hospitalization. Serum osmolality was calculated using the

equation [2 × (Na^++ K^+) + (glucose/18) + (urea/2.8)]. In MIMIC-IV, variables with missing data are common, so missing values were estimated using regression imputation. Variables with more than 20% missingness were removed from the model to avoid bias that might result from directly filling in missing values. All screening variables had less than 20% missing values.

Variables

Initial serum osmolality on admission was calculated and recorded as a continuous variable for patients in this study. As a dichotomous variable, the all-cause death during the course of 28 days was tracked.

We obtained the ultimate outcome variable in accordance with research and guidelines that have been published. This analysis considered the following covariables as factors to consider: (1) demographic data; (2) vital signs; (3) laboratory parameters; and (4) comorbidities.

As a result, the fully adjusted model was developed using the variables below: (1) continuous variables (obtained at baseline): age; gender; heart rate; systolic blood pressure (SBP); diastolic blood pressure (DBP); respiratory rate; pulse oxygen saturation (SPO_2); anion gap; bicarbonate; creatinine; glucose; bun; calcium; sodium; potassium; platelet; aspartate transaminase (AST); albumin; urea; chloride; NT-proBNP; Hemoglobin; C-Reactive Protein (CRP); serum osmolality level and (2) categorical variables (obtained at baseline): chronic obstructive pulmonary disease (COPD); hypertension; coronary artery disease (CAD); cerebrovascular disease (CVD); diabetes mellitus; renal disease; atrial fibrillation (AF).

Statistical analysis

Continuous variables are presented as the mean ± standard deviation or median with interquartile range and were compared with Student's t-test. Categorical variables are presented as frequencies and percentages, and differences between groups were performed with a Pearson chi-square test or Fisher's exact test. The Lowess Smoothing technique was used to explore the relationship between osmolarity and mortality. To evaluate the incidence rate of primary outcome events among groups according to different levels of serum osmolality, we used Kaplan-Meier survival analysis, and discrepancies among groups were evaluated with log-rank tests. We used Cox proportional hazards models to estimate the hazard ratio (HR) and 95% confidence interval (CI) between serum osmolality and 28-days all-cause mortality, adjusted for multiple models. To avoid overfitting the model because of multicollinearity among variables, we also calculated the variance inflation factor (VIF). Variables with VIF ≥ 5 were excluded. Finally, clinically relevant and prognosis-associated variables were enrolled in the multivariate model: model 1: unadjusted; model 2: adjusted for age and sex; model 3: adjusted for age, sex, heart rate, SBP, DBP, respiratory rate, SPO2, anion gap, bicarbonate, creatinine, glucose, bun, calcium, sodium, potassium, platelet, AST, albumin, urea, chloride, NT-proBNP, hemoglobin, CRP, hypertension, diabetes mellitus, CAD, COPD, renal disease, CVD. Furthermore, a segmented regression model and logarithmic likelihood ratio test were used to analyze the threshold effect between serum osmolality levels and 28-days all-cause mortality.

We used R 4.1.3 (R Foundation for Statistical Computing, Vienna, Austria) and Stata 12.0 (Stata Corporation LLC, College Station, USA) for data analysis. A two-sided P-value < 0.05 was considered statistically significant for all analyses.

Results

A total of 6228 patients were included in this study, the mean age of the patients was 72.62 ± 13.44 and 3445 (55.31%) were male (Figure 1). The mean serum osmolality of all included patients was 304.271 ± 10.87. The all-cause mortality rate within 28 days of admission was 18.88% (Table 1).

Baseline characteristics

Table 1 lists the baseline characteristics of the study patients according to serum osmolality tertiles. Patients were categorized into three groups (G1: <290 mmol/L, G2: 290–310 mmol/L, G3: >310 mmol/L) based on admission serum osmolality level. The mean levels of serum osmolality in the three groups were G1: 285.51 ± 3.65, G2: 300.89 ± 5.28, G3: 317.98 ± 6.09. The patients in the group with higher serum osmolality were older, predominantly male, and had higher levels of SBP, respiratory rate, bun, creatinine, calcium, sodium, urea, chloride levels, and comorbidities with renal disease and diabetes mellitus were relatively more common. As serum osmolality increased, heart rate, DBP, and platelets showed a tendency to decrease, and comorbidities with hypertension were less common. 28-days all-cause mortality showed a significant incremental trend between serum osmolality groups. Overall, the 28-days all-cause mortality rate in the study population was 18.88%, and the difference in mortality rates between the three groups was statistically significant (18.30% vs. 14.94% vs. 28.26%, P < 0.001), suggesting that increased serum osmolality is associated with a higher risk of 28-days all-cause mortality.

Smoothing spline fitting curve

Figure 2 shows the relationship between initial serum osmolality on admission and 28-days all-cause mortality among HFrEF patients as determined using the Lowess Smoothing technique. The model produced a nonlinear relationship, with the lowest mortality rates at serum osmolality of approximately 290–299 mmoL/L. For our included patients, the results of Lowess Smoothing showed a U-shaped relationship between initial serum osmolality on admission and 28-days all-cause mortality (Figure 2).

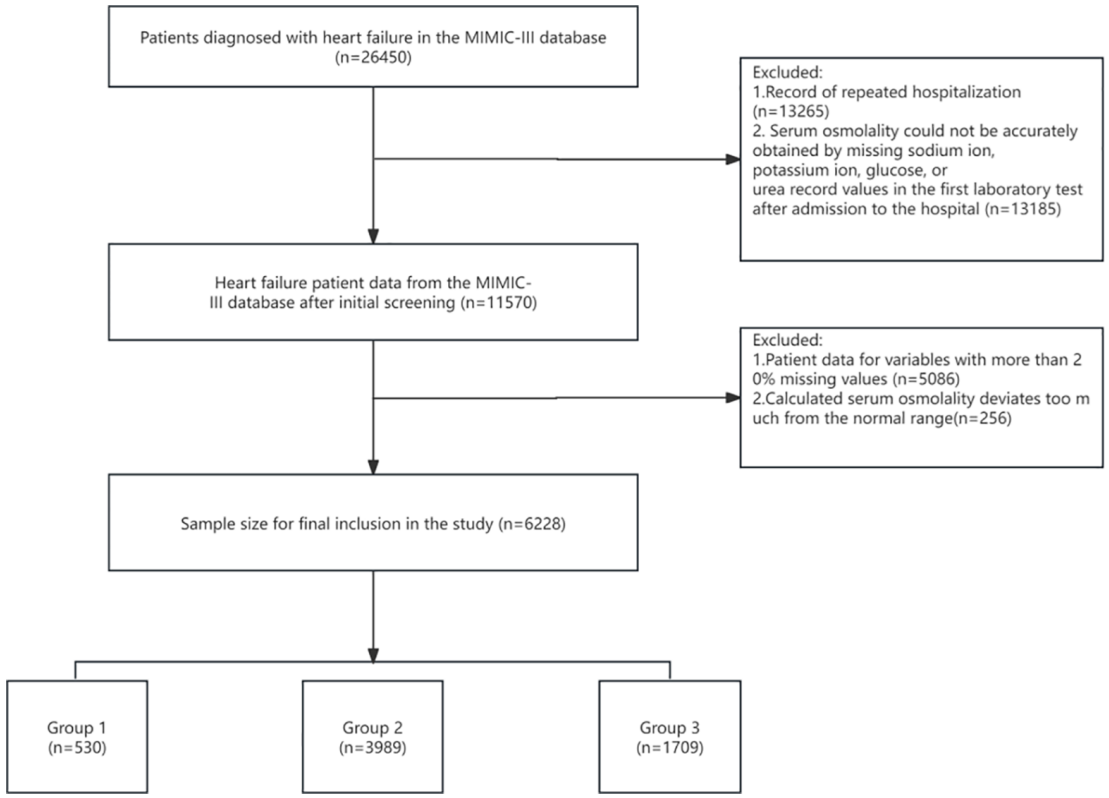


FIGURE 1
Flowchart of patient selection.

TABLE 1 Baseline characteristics (N = 6228).

Variable	Serum osmolality(mmoL/L)				P-value
	Total (N=6228)	G1 (<290 mmol/L) (N=530)	G2 (290-310 mmol/L) (N=3989)	G3 (>310 mmol/L) (N=1709)	
Baseline variables					
Age	72.62 ± 13.44	70.40 ± 13.79	71.63 ± 13.68	75.62 ± 12.24	<0.001
Gender					0.009
Male	3445 (55.31%)	288 (54.34%)	2158 (54.10%)	999 (58.46%)	
Female	2783 (44.69%)	242 (45.66%)	1831 (45.90%)	710 (41.54%)	
Vital signs					
Heart rate (bpm)	86.25 ± 18.32	88.35 ± 18.64	86.52 ± 18.09	84.96 ± 18.70	<0.001
SBP (mmHg)	119.53 ± 22.52	117.65 ± 21.93	119.09 ± 22.43	121.14 ± 22.84	0.001
DBP (mmHg)	64.38 ± 15.48	64.27 ± 15.18	64.59 ± 15.34	63.94 ± 15.90	0.307
Respiratory rate (bpm)	19.34 ± 5.23	19.30 ± 5.06	19.15 ± 5.24	19.78 ± 5.23	<0.001
SPO2 (%)	96.86 ± 2.88	96.81 ± 2.79	96.88 ± 2.87	96.82 ± 2.94	0.679
Laboratory parameters					
Anion gap (mmol/L)	14.34 ± 3.30	13.77 ± 3.31	14.03 ± 3.14	15.26 ± 3.50	<0.001
Bicarbonate (mmol/L)	25.47 ± 4.71	25.17 ± 4.22	25.61 ± 4.42	25.23 ± 5.43	0.003
Creatinine (mEq/L)	1.21 ± 0.53	0.98 ± 0.45	1.12 ± 0.47	1.50 ± 0.57	<0.001

(Continued)

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TABLE 1 Continued

Variable	Serum osmolality(mmoL/L)				P-value
	Total (N=6228)	G1 (<290 mmol/L) (N=530)	G2 (290-310 mmol/L) (N=3989)	G3 (>310 mmol/L) (N=1709)	
Laboratory parameters					
Glucose (mg/dL)	7.52 ± 3.45	6.40 ± 1.91	7.14 ± 2.78	8.75 ± 4.69	<0.001
BUN (mg/dL)	30.85 ± 19.38	18.93 ± 10.46	25.00 ± 13.14	48.21 ± 22.51	<0.001
Calcium (mmol/L)	8.61 ± 0.59	8.45 ± 0.57	8.62 ± 0.58	8.64 ± 0.62	<0.001
Sodium (mmol/L)	138.65 ± 4.30	132.02 ± 2.89	138.24 ± 3.25	141.66 ± 4.13	<0.001
Potassium (mmol/L)	4.22 ± 0.66	4.16 ± 0.63	4.17 ± 0.60	4.35 ± 0.76	<0.001
Platelet (10^9/L)	208.31 ± 83.64	212.55 ± 92.05	210.27 ± 82.63	202.41 ± 83.00	0.001
AST (IU/L)	36.14 ± 19.39	37.16 ± 20.01	35.96 ± 19.30	36.25 ± 19.39	0.187
Albumin (g/L)	28.44 ± 17.27	27.73 ± 16.25	28.45 ± 17.44	28.63 ± 17.19	0.487
Urea (mg/dL)	28.15 ± 14.55	18.93 ± 10.46	24.55 ± 11.96	39.41 ± 14.84	<0.001
Chloride (mmol/L)	101.67 ± 5.74	96.41 ± 5.09	101.52 ± 5.10	103.65 ± 6.22	<0.001
NT-proBNP (pg/mL)	7802.32 ± 8079.70	6503.74 ± 6857.10	6926.01 ± 7440.60	10250.43 ± 9266.06	<0.001
Hemoglobin (g/dl)	11.69 ± 2.27	11.67 ± 2.25	11.86 ± 2.25	11.27 ± 2.25	<0.001
C-Reactive Protein (mg/L)	61.79 ± 73.17	60.74 ± 63.50	59.38 ± 71.12	67.60 ± 80.47	0.267
serum osmolality (mmol/L)	304.271 ± 10.87	285.51 ± 3.65	300.89 ± 5.28	317.98 ± 6.09	<0.001
Comorbidity					
COPD	252 (4.05%)	23 (4.34%)	148 (3.71%)	81 (4.74%)	0.183
Hypertension	2833 (45.49%)	292 (55.09%)	1940 (48.63%)	601 (35.17%)	<0.001
CAD	3419 (54.90%)	273 (51.51%)	2194 (55.00%)	952 (55.71%)	0.232
CVD	318 (5.11%)	28 (5.28%)	196 (4.91%)	94 (5.50%)	0.642
Renal disease	762 (12.24%)	61 (11.51%)	409 (10.25%)	292 (17.09%)	<0.001
Diabetes mellitus	2490 (39.98%)	138 (26.04%)	1479 (37.08%)	873 (51.08%)	<0.001
Atrial fibrillation	3163 (50.79%)	265 (50.00%)	1974 (49.49%)	924 (54.07%)	0.006
Events					
28-days all-cause mortality	1176 (18.88%)	97 (18.30%)	596 (14.94%)	483 (28.26%)	<0.001

Association between initial serum osmolality and 28-days all-cause mortality in HFrEF

The independent correlation between initial serum osmolality level on admission and 28-days all-cause mortality in HFrEF patients is shown in Table 2. When serum osmolality was treated as a continuous variable, it was positively associated with 28-days all-cause mortality. In unadjusted Model I, each unit increase in serum osmolality level was associated with a 3% increase in the risk of 28-days all-cause mortality (HR, 1.03[95% CI 1.03–1.04] P<0.05). In Model II (adjusted for age and sex), the results remained significant (HR, 1.03[95% CI 1.02–1.03] P<0.05). In model III (adjusted for age, sex, and potential confounders), the results did not show significant changes (HR, 1.04[95% CI 1.02–1.06] P<0.05). To further explore the effect of serum osmolality, patients were divided into 5 groups according to the value of serum osmolality (see Table 2 for details), where the Q2 group (290–309 mmol/L) was used as the reference group, and the results of model I showed that higher initial serum osmolality on admission of the patients was associated with an increase in the 28-days all-cause mortality of the patients after admission, with a gradual increase in the HR starting from Q3 (HR, 1.83[95% CI 1.56 - 2.14] P<0.001) to Q5 (HR, 3.93[95% CI 2.58 - 6.00] P<0.001), and

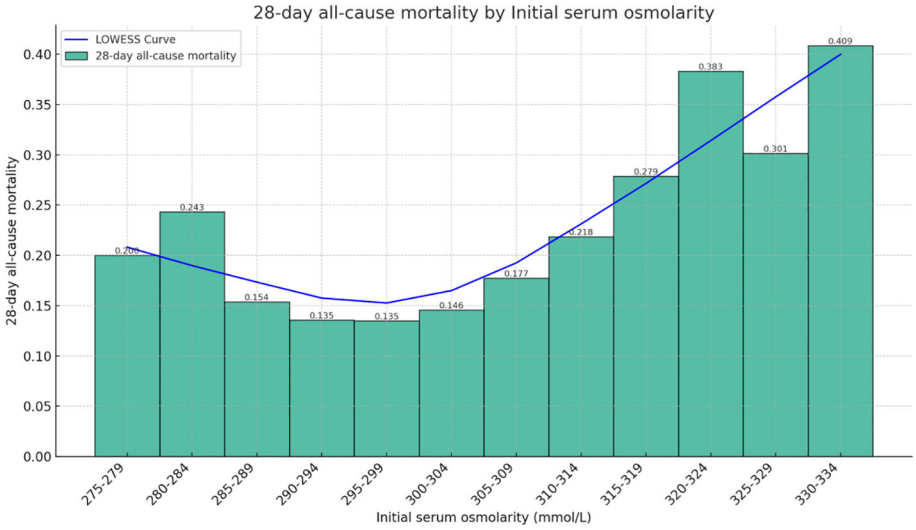


FIGURE 2
Relationship between serum osmolality and 28-days all-cause mortality in patients with HFrEF. The graph shows a nonlinear relationship.

the overall trend in adjusted model II and model III showed consistency with model I.

Survival analysis

Among the 6228 patients included in the study, 1176 (18.88%) died within 28 days of hospital admission. The 28-days all-cause mortality for Q1 (≤ 289 mmol/L), Q2 (290–309 mmol/L), Q3 (310–319 mmol/L), Q4 (320–329 mmol/L), and Q5 (≥ 330 mmol/L) were 18.30%, 14.94%, 24.31%, 39.86%, and 40.86%, respectively. Kaplan-Meier curves were constructed to visualize the association between serum osmolality quintiles and the 28-days all-cause mortality (Figure 3). During the short-term follow-up of 28 days, a statistically significant difference in mortality rates was observed between the groups. Patients had a higher short-term survival rate when serum osmolality was between 290–309 mmol/L, a lower survival rate within 1–14 days of admission when serum osmolality was between 320–329mmol/L, and a lower survival rate within 15–28 days of admission when serum osmolality was above 330mmol/L.

TABLE 2 Association between serum osmolality and 28-days all-cause mortality in patients with HFrEF.

Variables	Model I (HR,95% CI)	Model II (HR,95% CI)	Model III (HR,95% CI)
Serum osmolality	1.03(1.03, 1.04)*	1.03(1.02, 1.03)*	1.04(1.02, 1.06)*
Serum osmolality quintiles			
Q1 (≤289mmol/L)	1.28(1.01, 1.62)*	1.34 (1.05, 1.71)*	0.88 (0.30, 2.63)*
Q2 (290-309mmol/L)	Ref.	Ref.	Ref.
Q3 (210-319mmol/L)	1.83 (1.56, 2.14)*	1.64 (1.39, 1.93)*	1.61 (0.74, 3,52)*
Q4 (320-329mmol/L)	3.14 (2.55, 3.87)*	2.86 (2.31, 3.54)*	1.62 (0.40, 5.76)*
Q5 (≥330mmol/L)	3.93 (2.58, 6.00)*	3.84 (2.49, 5.92)*	1.79 (0.25, 12.70)*
P value for trend	<0.001	<0.001	<0.001

HR, hazard ratio; CI, confidence interval; Ref, reference; *P value <0.05.
Model I: adjusted for none.
Model II: adjust for age; sex.
Model III: adjusted for age, sex, heart rate, SBP, DBP, respiratory rate, SPO2, anion gap, bicarbonate, creatinine, glucose, bun, calcium, sodium, potassium, platelet, AST, albumin, urea, chloride, NT-proBNP, hemoglobin, CRP, hypertension, diabetes mellitus, CAD, COPD, renal disease, CVD, AF.

Threshold effect analysis for the relationship between serum osmolality levels and 28-days all-cause mortality

Through Lowess Smoothing analysis, it was found that the relationship between serum osmolality levels and 28-days all-cause mortality is U-shaped. Further analysis using a segmented regression model of serum osmolality levels and 28-days all-cause mortality identified a turning point at 298.8 mmol/L of serum osmolality. At a serum osmolality level of 298.8 mmol/L, the mortality rate for patients with HFrEF was the lowest (Figure 4). The results suggest that serum osmolality levels are associated with a doubled risk of 28-days all-cause mortality in patients with HFrEF after admission. For serum osmolality levels ≥298.8 mmol/L, each unit increase in serum osmolality level is associated with a 2% increase in mortality within 28 days of admission (HR, 1.019[95% CI 1.012–1.025]). The LRT test was significant (P = 0.002), indicating a non-linear association between serum osmolality levels and 28-days all-cause mortality (Table 3).

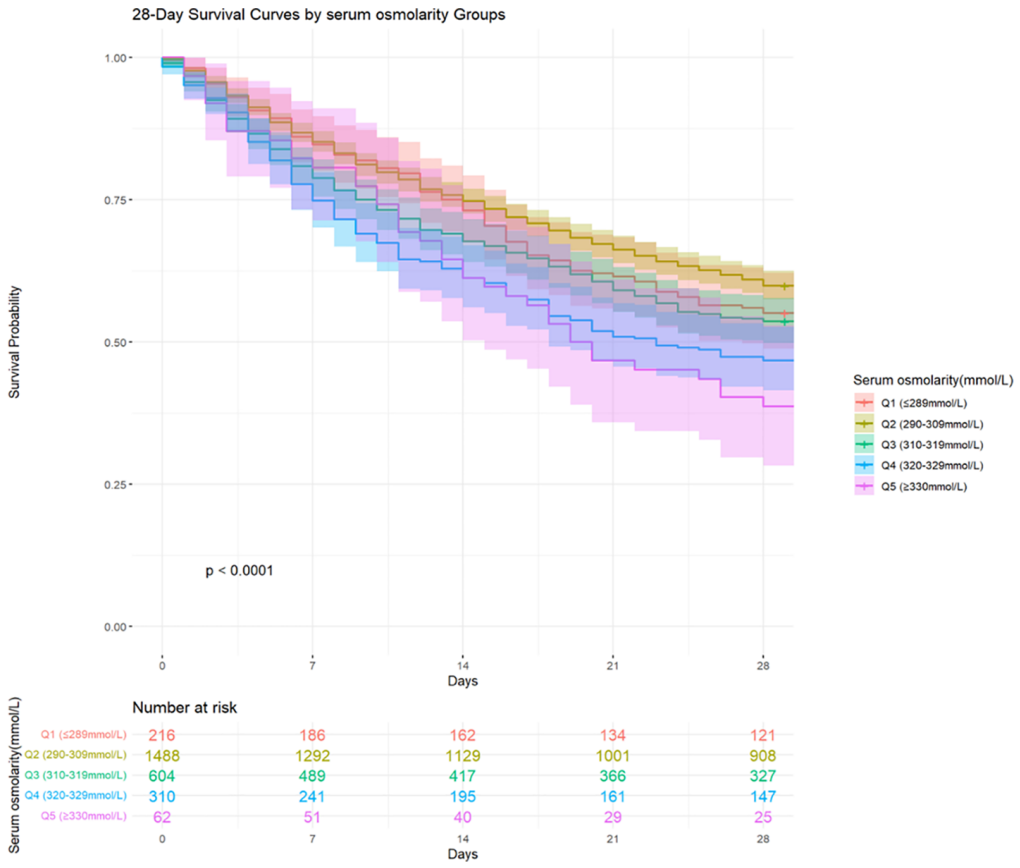


FIGURE 3
Kaplan–Meier survival analysis curves for 28-days all-cause mortality.

Discussion

To the best of our knowledge, this is the most comprehensive study utilizing the MIMIC-IV database to examine the link between serum osmolality imbalance and mortality among HFrEF patients. Our results reveal that elevated serum osmolality correlates with increased 28-days all-cause mortality in a U-shaped relationship, with the association persisting after adjusting for potential confounders.

Our analysis indicates that individuals with higher serum osmolality are typically older, possess more comorbidities, particularly diabetes and renal disease, and exhibit increased levels of glucose, urea, creatinine, and potassium. These findings align with those of José et al. Previous research has questioned the impact of serum osmolality variations on the short-term prognosis of older HFrEF patients, noting that the confluence of multiple underlying diseases and diverse laboratory index alterations exerts a more pronounced effect on short-term mortality, complicating the interpretation of serum osmolality's impact (8). To mitigate this complexity, we employed several models: Model I with no adjustments, Model II adjusted for age and gender, and Model III, which further adjusted for multiple confounders. The findings affirm that serum osmolality exerts a direct effect on 28-days all-cause mortality in HFrEF patients, independent of age, sex, comorbidities, and various laboratory measures. Having established the non-linear relationship between initial serum osmolality and 28-days all-cause mortality, we employed threshold effect modeling to corroborate the association. We observed that serum osmolality above 298.8 mmol/L significantly increases 28-days all-cause mortality, reinforcing the non-linear correlation with mortality. To further compare the sensitivity and specificity of serum osmolality in predicting short-term mortality in patients with HFrEF, we analyzed it using ROC curves to describe its AUC value and further compared it with the individual indices (Na+, K+, glucose, urea) used to calculate serum osmolality. In our study, we found that the AUC values of serum osmolality were greater than those of the individual markers (Figure 5), demonstrating superior sensitivity and specificity for predicting the short-term prognosis of patients with heart failure. This finding reinforces the need to monitor changes in serum osmolality in patients with severe HFrEF.

Previous research has consistently reported hyponatremia as the most prevalent electrolyte imbalance in hospitalized HF patients, typically precipitated by neurohormonal activation or pharmacological interventions (12). In HF patients, chronic activation of the renin-angiotensin-aldosterone system and the sympathetic nervous system in response to insufficient tissue perfusion promotes water and sodium retention. Moreover,

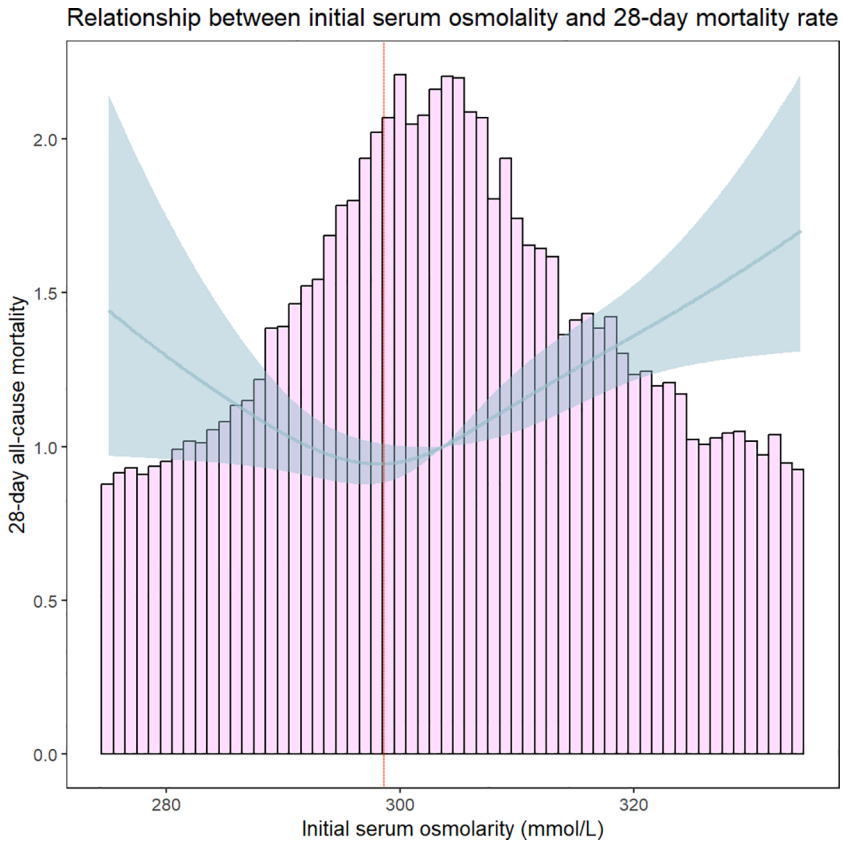


FIGURE 4
Threshold effect analysis of serum osmolality on 28-day all-cause mortality in Heart Failure Patients. The red dashed line indicates that 28-day all-cause mortality was lowest in HFrEF patients when serum osmolality was 298.8 mmol/L.

TABLE 3 Association between serum osmolality and 28-days all-cause mortality in patients with HFrEF.

Models	HR (95%CI)	P-value
Model I		
Fitting model by standard linear regression	1.011 (1.006-1.016)	<0.001
Model II		
Fitting model by two-piecewise linear regression		
Turning point (K1)		
<298.833	0.987 (0.972-1.002)	0.094
>298.833	1.019 (1.012-1.025)	<0.001
LRT test	0.002	

Data were presented as HR (95% CI) P-value; Model I, linear analysis; Model II, non-linear analysis. LRT test, Logarithmic likelihood ratio test ($p < 0.05$ means Model II is significantly different from Model I, which indicates a non-linear relationship).

arginine vasopressin, released due to low cardiac output, augments intravascular volume, contributing to hyponatremia development in HF patients (13). Numerous studies have confirmed the association of hyponatremia with increased morbidity and mortality in hospitalized HF patients (14–16). Peng et al. also identified a U-shaped relationship between serum sodium levels and all-cause mortality in HF patients (17). Serum osmolality, a more comprehensive measure, is influenced not only by sodium but also by glucose, urea, and potassium concentrations, which collectively regulate it within a narrow range of 275–295 mmoL/L (18). In our study, the higher osmolality group exhibited elevated glucose, urea, and potassium levels. The influence of serum osmolality on HFrEF patients has been a longstanding concern, with other studies highlighting low osmolality as a significant risk factor for increased morbidity and mortality. José et al. documented that high serum osmolality forecasts poorer outcomes in patients with decompensated HF over one year. However, their study did not elaborate on the impact of confounding factors (8).

Our investigation, focusing on short-term prognosis post-hospital admission (28 days), concurs with these observations,

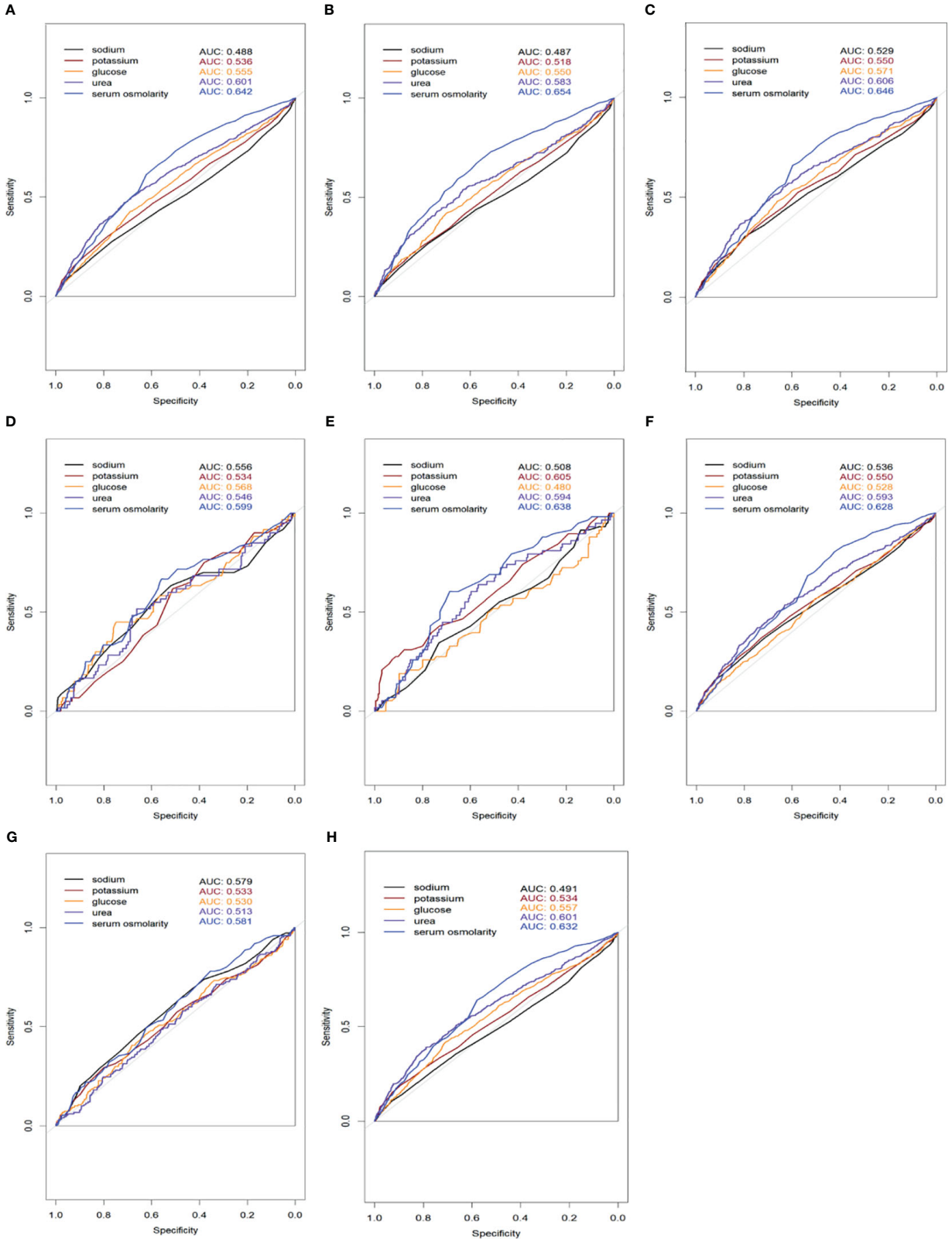


FIGURE 5
Receiver Operating Characteristic (ROC) curves comparing the sensitivity and specificity of serum osmolality and individual markers (sodium, potassium, glucose, urea) for predicting 28-days all-cause mortality in HFrEF patients. The Area Under the Curve (AUC) values for each marker are displayed in the legend. **(A)** All patients included in the study; **(B)** Patients with coexisting hypertension; **(C)** Patients with coexisting CAD; **(D)** Patients with coexisting CVD; **(E)** Patients with coexisting COPD; **(F)** Patients with coexisting diabetes mellitus; **(G)** Patients with coexisting renal disease; **(H)** Patients with coexisting AF.

further noting that higher serum osmolality is linked to increased short-term mortality, even after adjusting for confounders using various observational models. Alongside existing literature, the detrimental effects of serum osmolality on HF patients can be rationalized through several mechanisms. Firstly, antidiuretic hormone (AVP) secretion, regulated by extracellular fluid solute concentration, blood volume, cardiac filling pressure, and arterial pressure, is heightened in HF due to sympathetic and renin-angiotensin-aldosterone system activation, particularly during acute water overload. Elevated serum osmolality may intensify AVP release, with AVP adversely affecting cardiovascular dynamics and promoting HF progression (19, 20). Secondly, increased serum osmolality may disrupt venous reserve distribution. High arterial and non-visceral venous osmolality can shift fluid from venous reserves to the effective circulation, raising preload and leading to congestion. This may manifest as elevated pulmonary artery pressures and potentially exacerbate pulmonary edema, evidenced by a link between osmolality and increased extravascular lung water. Finally, elevated osmolality could impair renal function through several mechanisms. Heightened AVP levels may aggravate chronic kidney disease, while high serum osmolality may induce renal tubular injury and fibrosis via the sympathetic nervous system and aldose reductase pathway, culminating in oxidative stress (21, 22). Moreover, increased extracellular osmolality may prompt water excretion, resulting in cellular shrinkage and intracellular dehydration, precipitating local and systemic conditions detrimental to cellular integrity.

In summary, serum osmolality levels serve as a cost-effective and widely accessible metric to assess HF severity and prognosticate mortality risk. This study delineates the association between serum osmolality and 28-days all-cause mortality post-hospitalization, offering a clinical reference point based on serum osmolality levels. The significance of our research is manifold: (1) It elucidates a curvilinear relationship between serum osmolality and all-cause mortality within 28-days all-cause mortality in HF patients under intensive care; (2) It advances the development of short-term prognostic models based on serum osmolality levels for HF patients; (3) It distinguishes higher serum osmolality as an independent determinant of 28-days all-cause mortality, accounting for confounding variables,a contrast to prior studies. (4) The results have been substantiated through Kaplan-Meier survival curve analysis, enhancing the robustness of future research findings; (5)A threshold effect model was utilized to pinpoint the turning point where serum osmolality begins to significantly impact 28-days all-cause mortality; (6) Ethical integrity was maintained throughout the study, with no patients being harmed or ethical boundaries transgressed.

Our study is subject to several limitations: (1) It is confined to examining the relationship between initial serum osmolality and short-term mortality post-hospital admission, without accounting for the potential influence of post-admission medications, such as renin-angiotensin-aldosterone system (RAAS) inhibitors and diuretics, on serum sodium levels and, consequently, serum osmolality; (2) Serum osmolality was estimated using a formula rather than direct measurement, precluding the detection of "delta osmolality" or "osmolal gap". Although the formula was chosen with diligence, it may not accurately reflect true osmolality values; (3) The study relies on data from the MIMIC-IV database, which predominantly comprises patients with severe HFrEF, thus constraining the broader applicability of the findings; (4) The causal mechanisms linking serum osmolality with 28-days all-cause mortality were not explored, indicating the need for further investigative research.

Conclusion

Our findings reveal a U-shaped correlation between serum osmolality levels and 28-days all-cause mortality in HFrEF patients post-hospital admission, after accounting for confounding factors. This study enhances the understanding of serum osmolality's relevance in HFrEF cases. Given its simplicity, ease, and cost-effectiveness, serum osmolality measurement underscores the significance of osmolality management in aiding physicians to identify patients at high risk. Nonetheless, additional research is essential to ascertain whether interventions aimed at serum osmolality levels can favorably alter clinical outcomes in this cohort.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

QZ: Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. JL: Data curation, Writing – review & editing. PL: Data curation, Software, Writing – review & editing. JM: Software, Writing – review & editing. ZW: Conceptualization, Data curation, Writing – review & editing. TT: Conceptualization, Writing – review & editing. GH: Investigation, Writing – review & editing. SS: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Software, Supervision, Writing – original draft, Writing – review & editing.

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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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EDITED BY
Gabor Czibik,
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REVIEWED BY
Shinsuke Hidese,
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Silvia Alboni,
University of Modena and Reggio Emilia, Italy

*CORRESPONDENCE
Huifeng Zhang
zhfhaust@163.com

†These authors have contributed equally to this work and share first authorship

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The value of the platelet/high-density lipoprotein cholesterol ratio in predicting depression and its cardiovascular disease mortality: a population-based observational study

Huifeng Zhang[1*†], Ying Xu[2†] and Yaying Xu[3†]

[1]Department of Cardiovascular, The First Affiliated Hospital, and College of Clinical Medicine of Henan University of Science and Technology, Luoyang, China, [2]Department of Hematology, The First Affiliated Hospital, and College of Clinical Medicine of Henan University of Science and Technology, Luoyang, China, [3]Department of Endocrinology, The First Affiliated Hospital, and College of Clinical Medicine of Henan University of Science and Technology, Luoyang, China

Background: Cardiovascular disease (CVD) and depression have a bidirectional association, with inflammation and metabolic factors being common important triggers for both conditions. However, as a novel inflammatory and metabolic marker, platelet-to-HDL-C ratio (PHR) has not been established in relation to depression and cardiovascular disease.

Materials and methods: Participants aged 20 years and older were included in the 2005–2018 NHANES database. PHR was calculated as the ratio of platelet count (1000 cells/μL) to HDL-C (mmol/L). The Patient Health Questionnaire (PHQ-9) was used to diagnose depression, with a cutoff value of 10. Weighted logistic regression analysis and restricted cubic spline (RCS) analysis were employed to examine the association between PHR and depression-related features. Additionally, weighted COX regression and RCS were used to analyze the association of PHR with CVD mortality in patients with depression. Receiver operating characteristic curves were used to assess whether PHR had an advantage over HDL-C in predicting depression. Finally, the mediating role of PHR in the latest cardiovascular health indicator Life's Essential 8 and depression was explored.

Results: A total of 26,970 eligible participants were included, including 2,308 individuals with depression, representing approximately 160 million U.S. adults when weighted. After full adjustment, we estimated that the odds ratio (OR) of depression associated with a per standard deviation (SD) increase in PHR was 1.06 (95% CI: 1.01–1.12, P=0.03). The restricted cubic spline (RCS) analysis indicated a linear association (Nonlinear P=0.113). When PHR was divided into four groups based on quartiles and included in the model after full adjustment for depression risk factors, participants in quartile 2, quartile 3, and quartile 4 of PHR showed a trend of increasing risk of depression compared to the lowest quartile group (P trend=0.01). In addition, weighted COX regression and RCS revealed that a per SD increase in PHR was associated with a higher risk of CVD mortality among patients with depression (HR: 1.38, 95% CI: 1.05–1.81, P=0.02, Nonlinear

P=0.400). Subgroup analyses showed that current alcohol consumption enhanced the association between PHR and depression (P for interaction=0.017). Furthermore, the areas under the ROC curves (AUC) were 0.556 (95% CI, 0.544–0.568; P < 0.001) for PHR and 0.536 (95% CI, 0.524–0.549; P < 0.001) for HDL-C (P_{DeLong} = 0.025). Finally, mediation analysis indicated that PHR was an intermediate mechanism between LE8 and depression (mediation proportion=5.02%, P=0.02).

Conclusion: In U.S. adults, an increase in PHR linearly increases the risk of depression and CVD mortality among individuals with depression. Additionally, PHR has a better predictive advantage for depression compared to HDL-C. Furthermore, PHR significantly mediates the association between LE8 scores and depression.

KEYWORDS

high-density lipoprotein cholesterol, platelet/high-density lipoprotein cholesterol ratio, depression, NHANES, CVD mortality

1 Introduction

Depression is one of the most common mental disorders and is considered the most significant type among modern psychological disorders (1). Currently, approximately 1 in every 20 individuals is at risk of developing depression, and it is projected to become the leading burden of disease globally within the next five years (1, 2). In the United States, the prevalence of depression is even higher among adults, with approximately 1 in every 7 individuals affected by this condition (3). The comorbidity of depression and cardiovascular disease (CVD) has long been a matter of concern. As a maladaptive psychosocial disorder, depression influences the occurrence and progression of cardiovascular diseases, while CVD can also trigger and worsen depression (4–6). Therefore, there is a need for further exploration of this issue.

Research indicates that inflammation and metabolic factors are common mechanisms underlying both depression and cardiovascular disease. Dysregulation of the innate immune system and adaptive immune system occurs in patients with depression, affecting the treatment and prognosis of depression, including the response to antidepressant medications (7). Furthermore, many metabolic pathways, such as those involving alanine, aspartate, and glutamate metabolism, undergo changes in depression, involving pathways related to inflammation, neuroprotection, and energy metabolism (8). On the other hand, inflammation may serve as a stronger predictor of future cardiovascular events, such as heart attacks and strokes (9). Systemic chronic inflammation (SCI) is the pathological basis of CVD, and it is also an important factor contributing to increased incidence and mortality rates (10).

High-density lipoprotein cholesterol (HDL-C) promotes the efflux of dietary cholesterol through the reverse cholesterol transport pathway and can exert anti-inflammatory and antioxidant effects (11). However, based on current epidemiological evidence, the relationship between HDL-C and depression seems to be difficult to determine. HDL-C levels have been reported to be positively correlated, not correlated, and negatively correlated with depression (12–16). Furthermore, platelets produce a variety of immune effects through the production of numerous cytokines and chemokines after activation (17). Research indicates that patients with depression exhibit platelet overactivation and inflammatory responses (18). However, other studies suggest an association between depression and low platelet activation levels (19). Platelets are closely related to central serotonergic function and are considered a window into brain serotonergic function (20). Disruption of the serotonergic pathway may be associated with platelet aggregation dysregulation in depression and cardiovascular disease (21). These studies all suggest that a single indicator may have unreported deficiencies in predicting depression, and composite indicators may be more promising. The platelet-to-HDL-C ratio (PHR) was initially proposed by Jialal et al. and was considered an effective biomarker for predicting metabolic syndrome (MetS) (22). Since its proposal, research by Ni et al. (23) has indicated a positive non-linear correlation between PHR and the risk of kidney stones. Additionally, a study by Lu et al. (24) supports PHR as an effective marker for non-alcoholic fatty liver disease and liver fibrosis. Based on this, we speculate that, compared to HDL-C, the PHR ratio may be a valuable new predictive signal for depression.

In this study, weighted logistic regression, COX regression, and restricted cubic spline (RCS) regression models were constructed to investigate the association characteristics between PHR and the risk of depression as well as CVD mortality in depressed patients.

Receiver operating characteristic (ROC) curves were constructed to evaluate the ability of PHR and HDL-C to predict depression. Additionally, the study explored the mediating role of PHR in a new cardiovascular health (CVH) measure, life's essential 8 (LE8), in relation to depression.

2 Methods

2.1 Data source

This cross-sectional study investigated participants in the NHANES survey across 7 cycles from 2005 to 2018. NHANES utilizes a complex, multi-stage probability sampling design, including face-to-face interviews, physical examinations, and laboratory tests for data collection. The survey obtained ethical approval from the National Center for Health Statistics Ethics Review Board, and all participants provided written informed consent. Details are available at https://www.cdc.gov/nchs/nhanes/index.htm. Across the 7 cycles, there were a total of 70,190 participants. Participants were sequentially excluded as follows: 30,441 participants under 20 years old, 3,489 with missing platelet count data, 485 lacking HDL-C testing data, 3,226 with missing depression diagnosis information, and 5,579 participants with missing covariates, resulting in a final eligible sample of 26,970 participants (Supplementary Figure 1).

2.2 Platelet count/HDL-C (PHR)

The platelet count (PC) (1000 cells/μL) and HDL-C (mmol/L) are biochemical parameters obtained from blood samples collected from participants at the Mobile Examination Center (MEC). The Platelet-to-HDL-C ratio (PHR) was calculated as the ratio of PC to HDL-C (22). Due to the non-normal distribution of PHR values, a standardized transformation (Z-SCORE) was performed.

2.3 Depression

Depression was determined based on the Patient Health Questionnaire-9 (PHQ-9), which assesses depressive symptoms present in the past two weeks. The sum of the individual scores for each question (0–3 points) constitutes the depression score, with a total score ranging from 0 to 27 points. A higher score indicates more severe depression. Based on previous research, a PHQ-9 total score of ≥10 is defined as depression (25–27). This definition method of depression is widely used in clinical research, and its sensitivity and specificity are both 88% (25).

2.4 Confirming the death status

The National Death Index (NDI) provides death certificate data. NHANES survey data is linked to NDI data using probabilistic matching to ascertain participants' vital status. Participants for whom NDI matching was unsuccessful are presumed to be alive (28, 29). Follow-up duration is calculated in years based on the time interval between the date of death or December 31, 2019 (whichever came first) and the recruitment date. Cardiovascular disease (CVD) mortality is determined based on the guidelines of the Tenth Revision of the International Statistical Classification of Diseases and Related Health Problems (ICD-10), including 1) Diseases of heart (I00-I09, I11, I13, I20-I51); 2) Cerebrovascular diseases (I60-I69).

2.5 Covariates

1) Demographic characteristics: Age (<40 years, 40–60 years, ≥60 years), gender (male/female), race (Mexican American, non-Hispanic Black, non-Hispanic White, other Hispanic, other race - including multiracial), education level (below college, college and above), marital status (divorced/separated/widowed, married/cohabiting, never married) were self-reported at the time of the questionnaire. It also assessed the ratio of household income to poverty level (<1.3, 1.3–3.5, >3.5). 2) Lifestyle habits and physical measures: Alcohol consumption (never, former, current), smoking (never, former, current), total energy intake from the diet (categorized as high or low based on median values), Body Mass Index (BMI), defined as weight in kilograms divided by the square of height in meters (kg/m^2). 3) Comorbidities: Cancer, liver disease, cardiovascular disease (including coronary artery disease, congestive heart failure, myocardial infarction, stroke, and angina), chronic kidney disease (CKD) (diagnosed as A2, G3a and above for CKD) (30), diabetes, arthritis, thyroid disease. 4) Antidepressant medication use, categorized as non-use and use of antidepressants; antiplatelet medications (including aspirin, dipyridamole, cilostazol, clopidogrel, ticlopidine) and statins (atorvastatin, simvastatin, fluvastatin, lovastatin, nystatin, pravastatin, rosuvastatin, simvastatin).

2.6 Life's Essential 8

LE8 was calculated based on the research conducted by Donald et al. (31). Detailed scoring information for the 8 components can be found in Supplementary Tables 1 and 2. In brief, scores were assigned to the 8 sub-item components ranging from 0 to 100, and ultimately LE8 represented the arithmetic mean of these 8 components. The continuous LE8 scores were transformed into a categorical variable with three classes: 0–49 (low), 50–79 (moderate), and 80–100 (high) (32).

2.7 Statistical analysis

Following the complex sampling design principles adopted by NHANES, this study utilized Mobile Examination Center (MEC) weights (1/7 * WTMEC2YR) for subsequent analysis. Participants were divided into four groups based on PHR quartiles, with continuous variables analyzed using one-way ANOVA or

Kruskal-Wallis tests and results presented as means (standard errors) or medians (IQR); categorical variables were analyzed using chi-square tests and presented as counts (n) and percentages (%). To meet statistical requirements and facilitate interpretation, PHR was standardized (Z-SCORE) or categorized into four groups and included in weighted multivariable logistic regression and COX proportional hazards models to estimate the associations between exposure and outcome risks using odds ratios (ORs), hazard ratios (HRs), and 95% confidence intervals (CIs). Multiple models were constructed: Model 0, unadjusted; Model 1, adjusted for gender, age, race, and education; Model 2, further adjusted for marital status, household income to poverty ratio, alcohol consumption, smoking, and BMI; Model 3, further adjusted for comorbidities, antidepressant medication use, antiplatelet use and statins use. In the survival analysis model, due to the limited number of CVD-specific deaths among depressed survivors, we employed Least Absolute Shrinkage and Selection Operator regression (LASSO) to select important covariates and simplify the model. Collinearity was assessed using variance inflation factor (VIF), with all VIF values being below 10 in this study; Schoenfeld residuals were used to test the proportional hazards assumption. Subgroup analyses were conducted, exploring interactions between covariates and PHR using likelihood ratio tests. Exposure-response relationships between continuous PHR and risk of depression or CVD mortality risk among depressed patients were evaluated using restricted cubic splines (RCS) with 3 nodes (10th, 50th, and 90th percentile). Additionally, in the mediation analysis, the Bootstrap method was employed to estimate the mediation effect and its confidence intervals through 5000 repeated simulations (27, 33–35).

Several sensitivity analyses were conducted: 1) without considering weights; 2) multiple imputation of missing covariates; 3) additional adjustment for metabolic syndrome; 4) in the survival analysis, individuals who died within two years of follow-up were excluded.

All statistical procedures involved in this study were performed using R software version 4.3.1 (R Foundation for Statistical Computing). The "survey" package and "survival" package were used for weighted regression analysis; the "rms" package was utilized to build RCS regression models; the "mediation" package was employed for mediation analysis; the "mice" package was used for multiple imputation; the "glmnet" package was used to implement LASSO regression for selecting feature variables in survival analysis; and the "car" package was used to calculate variance inflation factors. In the present study, a two-tailed p-value < 0.05 was considered statistically significant.

3 Results

3.1 Population characteristics

A total of 26,970 eligible participants were included after strict exclusion criteria (Supplementary Figure 1), among whom 2,308 were diagnosed with depression, resulting in a weighted prevalence of 7.45%. Table 1 presents detailed data on PHR, HDL-C, and PC for all participants. Supplementary Table 3 presents the characteristics of each group based on quartiles. Compared to the lowest quartile group of PHR, individuals in the highest quartile group had an approximately 65% higher prevalence of depression, with more males, younger age, higher proportion of Mexican ethnicity, lower education level, higher unmarried rate, higher poverty level, higher past alcohol consumption and current smoking, higher overweight or obesity rate, and higher energy intake. In terms of comorbidities, the prevalence of cancer, thyroid disease, liver disease, arthritis, CVD, and CKD was lower, while the prevalence of diabetes was higher. Additionally, the individuals in the highest quartile group had a lower proportion of using statins. In the survival analysis, during a mean follow-up period of 7.57 years, until December 31, 2019, 289 deaths were identified among the 2,308 depressed patients, with 83 of them being CVD-specific deaths. Among individuals who died due to CVD-specific causes, there was a higher age and, higher proportion of low education level, and the Divorced/separated/widowed category was twice as high as the survivor group. The proportion of current alcohol drinkers was lower. Furthermore, there was a 2–3 times higher probability of comorbid arthritis, diabetes, CVD, CKD, and a higher proportion of statins and antiplatelet use (Supplementary Table 4).

3.2 Estimation of the association of PHR with depression and CVD mortality among depression patients

The results of weighted multivariable logistic regression are shown in Table 2. In the crude model (Model 0), a per SD increase in PHR [OR (95% CI): 1.20 (1.14, 1.25), P < 0.0001] was associated with an increased risk of depression. After fully adjusting for depression risk factors, we estimated that the odds ratios (ORs) of depression associated with a per SD increase in PHR were 1.06 (95% CI: 1.01–1.12, P = 0.03). When PHR was included in the model as a categorical variable and after full adjustment for confounders,

TABLE 1 The unweighted statistical values of PHR, HDL-C, and PC.

				Percentiles					
	Mean	SD	Min	P 1	P 25	P 50	P 75	P 99	Max
PC (1000 cells/μL)	248.3	66.386	11	118	204	241	285	443.31	1000
HDL-C (mmol/L)	1.367	0.415	0.16	0.67	1.06	1.29	1.6	2.61	5.84
PHR	184.328	81.813	9.244	69.604	141.606	184.328	238.542	449.559	1835.185

HDL-C, high-density lipoprotein cholesterol; PHR, platelet-to-high-density lipoprotein cholesterol ratio; PC, platelet count; SD, standard deviation.

TABLE 2 Weighted multivariate Logistic regression analysis for the risk of depression with PHR.

PHR		Model 0		Model 1		Model 2		Model 3	
Categorical		OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
	Quartile 1	ref		ref		ref		ref	
	Quartile 2	1.25(1.04,1.50)	0.02	1.27(1.06,1.52)	0.01	1.17(0.98,1.41)	0.09	1.20(0.99,1.46)	0.07
	Quartile 3	1.43(1.19,1.70)	<0.001	1.42(1.19,1.71)	<0.001	1.24(1.03,1.48)	0.02	1.24(1.03,1.50)	0.03
	Quartile 4	1.71(1.46,2.02)	<0.0001	1.67(1.41,1.98)	<0.0001	1.34(1.12,1.60)	0.002	1.31(1.10,1.56)	0.003
	p for trend		<0.0001		<0.0001		0.003		0.01
Continuous									
	per SD^{+}	1.20(1.14,1.25)	<0.0001	1.18(1.13,1.24)	<0.0001	1.09(1.03,1.15)	0.002	1.06(1.01,1.12)	0.03

Model 0: Not adjusted;
Model 1: Adjusted for age, gender, education attainment, and race;
Model 2: Further adjusted for marital status, poverty-income ratio, smoking and drinking status, BMI and total energy intake based on Model 1;
Model 3: Further adjusted for arthritis, thyroid problems, cancer, diabetes, liver diseases, CVD, CKD, statins, and antiplatelet based on Model 2.
PHR, platelet-to-high-density lipoprotein cholesterol ratio; BMI, body mass index; CVD, cardiovascular disease; CKD, chronic kidney disease; SD, standard deviation; OR, odds ratio; CI, confidence interval; ref, reference.

compared to the lowest quartile group, participants in quartile 2 [OR (95% CI): 1.20 (0.99, 1.46), P = 0.07], quartile 3 [OR (95% CI): 1.24 (1.03, 1.50), P = 0.03], and quartile 4 [OR (95% CI): 1.31 (1.10, 1.56), P = 0.003] showed a trend of increasing risk of depression (p trend = 0.01). Restricted cubic splines (RCS) demonstrated a linear relationship between PHR and depression (Nonlinear P = 0.113) (Figure 1).

Considering that there were only 83 cases of CVD-specific deaths, LASSO regression was used for variable selection and identified a set of important variables, including Age, race, BMI, DM, CKD, and CVD, totaling 6 factors (Figures 2A, B). A multivariable COX regression model was then constructed based on these variables. Schoenfeld residual tests in this study indicated that all models met the proportional hazards assumption. As shown in Table 3, weighted COX regression demonstrated that for PHR, in the unadjusted model (Model 0), per SD increase in PHR was not significantly associated with CVD mortality [HR (95% CI): 1.15 (0.85, 1.54), P = 0.36]; after adjusting for the above 6 factors, statins, and antiplatelet, we estimated that a per SD increase in PHR was associated with a higher risk of CVD mortality among depression survivors (HR: 1.38, 95% CI: 1.05–1.81, P = 0.02), and RCS fitted a linear dose-response relationship between PHR and CVD mortality (Nonlinear P = 0.400) (Figure 2C).

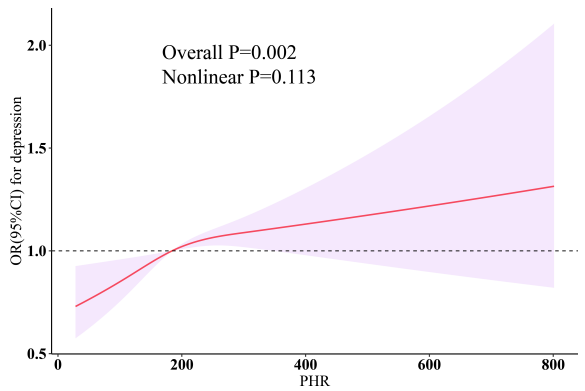


FIGURE 1
Dose-response relationship of PHR with depression. Three knots (10th, 50th, 90th percentiles) were selected for fitting the restricted cubic spline model, and the median value of PHR (184.328) was served as the reference point. All models were adjusted for age, sex, ethnicity, education, marital status, poverty-income ratio, total energy intake, BMI, smoking, drinking, arthritis, thyroid problems, cancer, diabetes, liver diseases, CVD, CKD, statins, and antiplatelet. HDL-C, high-density lipoprotein cholesterol; PHR, platelet-to-high-density lipoprotein cholesterol ratio; BMI, body mass index; CVD, cardiovascular disease; CKD, chronic kidney disease; OR, odds ratio; CI, confidence interval.

The areas under the ROC curves (AUC) were 0.556 (95% CI, 0.544–0.568; P < 0.001) for PHR and 0.536 (95% CI, 0.524–0.549; P < 0.001) for HDL-C (PDeLong = 0.025) (Figure 3).

3.3 Subgroup analysis and sensitivity analysis

The current alcohol consumption (P for interaction: 0.014) was observed to enhance the association between PHR and depression (Supplementary Figure 2). Additionally, the associations between PHR and CVD mortality in depression survivors were generally consistent across all subgroups (Supplementary Figure 3). Furthermore, the results of several sensitivity analyses were consistent with the main results (Supplementary Tables 5, 6).

3.4 Mediation analysis

As is shown in Figure 4, we estimated per grade increase in LE8 was significantly negatively correlated with PHR (β= -0.3495, P < 0.001); per SD increase in PHR was associated with depression (β= -0.0575, P = 0.02); and mediation analysis revealed that PHR mediated 5.02% of the association between LE8 and depression.

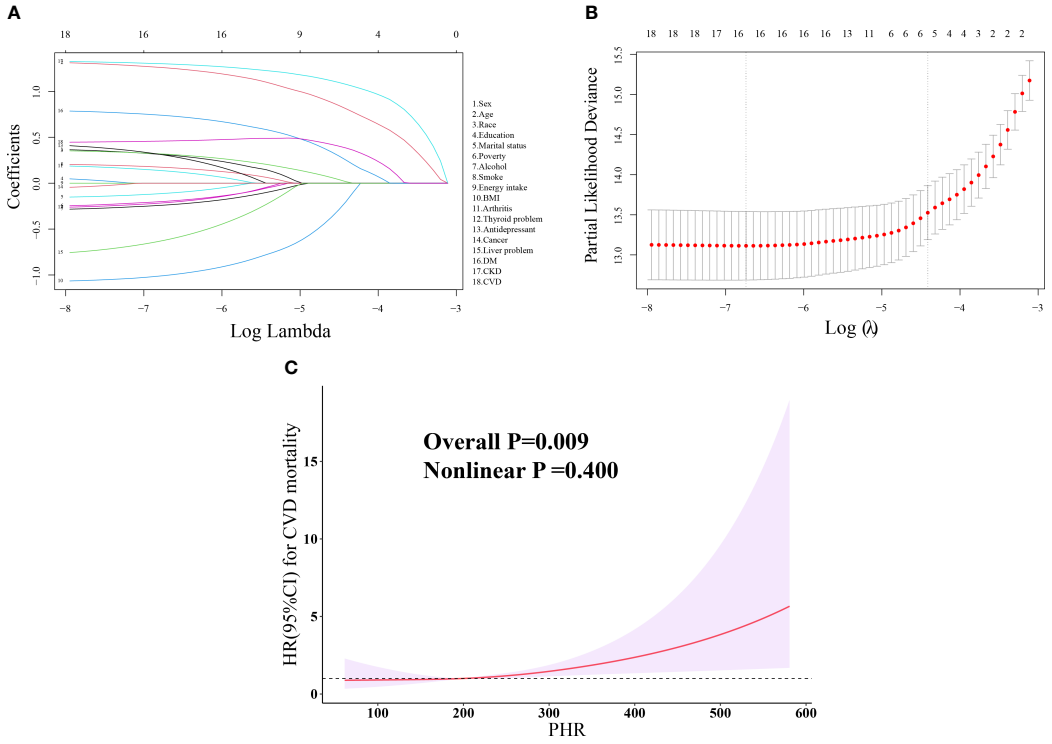


FIGURE 2
LASSO regression of the 18 covariates associated with CVD mortality and Dose-response relationship between PHR and CVD mortality among depression patients. **(A)** The screening path corresponds to 18 covariates that contribute to CVD mortality among depression patients. **(B)** The association between the log-transformed λ and Partial Likelihood Deviance for CVD mortality among depression patients. The red dashed line and its error bars represent the average Partial Likelihood Deviance value and the corresponding 95% CI. The left black dashed line represents the optimal λ value obtained when calculating the minimum Partial Likelihood Deviance, while the right black dashed line represents the λ value of the simplest model obtained at one standard error from the minimum Partial Likelihood Deviance. **(C)** Dose-response relationship between PHR and CVD mortality among depression patients. Three knots (10th, 50th, 90th percentiles) were selected for fitting the restricted cubic spline model, and the median value of PHR (198.54) was served as the reference point, and models were adjusted for age, race, BMI, DM, CVD CKD, statins, and antiplatelet. HDL-C, high-density lipoprotein cholesterol; PHR, platelet-to-high-density lipoprotein cholesterol ratio; BMI, body mass index; DM, diabetes; CVD, cardiovascular disease; CKD, chronic kidney disease; LASSO, least absolute shrinkage and selection operator; HR, hazard ratio; CI, confidence interval.

4 Discussion

In this large-scale nationally representative observational study, our findings provide strong evidence supporting a linear increase in depression risk with PHR. Current alcohol consumption significantly enhances the association between PHR and depression. Furthermore, compared to HDL-C, PHR has a better predictive ability for depression. Additionally, there is a positive linear dose-response relationship between PHR and CVD mortality in patients with depression. Sensitivity analyses, including without considering weights, multiple imputations of missing covariates, additional adjustment for metabolic syndrome, and excluding individuals who died within two years of follow-up in survival analysis, all support our findings. Finally, mediation analysis shows that maintaining good cardiovascular health significantly prevents the occurrence of depression by reducing PHR. In conclusion, our results suggest that PHR is an intermediate factor between cardiovascular disease and depression. On one hand, higher PHR in patients with depression is associated with a higher risk of cardiovascular death. On the other hand, maintaining good cardiovascular health can reduce the risk of depression through PHR reduction.

Previously, a large-scale retrospective study from Beijing, China found no difference in Platelet-to-HDL ratio (PHR) between Unipolar depression (UD), bipolar depression (BD), and healthy controls (HC). However, in a multivariate logistic regression model adjusting for confounding factors, a positive correlation between

TABLE 3 Weighted multivariate Cox regression analysis for the risk of PHR with CVD mortality among depression patients.

	Model 0		Model 1	
	HR (95% CI)	P	HR (95% CI)	P
PHR (per SD^{+})	1.15(0.85,1.54)	0.36	1.38(1.05, 1.81)	0.02
PHR (per 100^{+})	1.17(0.84,1.63)	0.36	1.44(1.06, 1.95)	0.02

Model 0: Not adjusted;
Model 1: Adjusted for age, race, BMI, DM, CVD, CKD, statins, and antiplatelet.
PHR, platelet-to-high-density lipoprotein cholesterol ratio; CVD, cardiovascular disease; BMI, body mass index; DM, diabetes; CKD, chronic kidney disease; SD, standard deviation; HR, hazard ratio; CI, confidence interval.

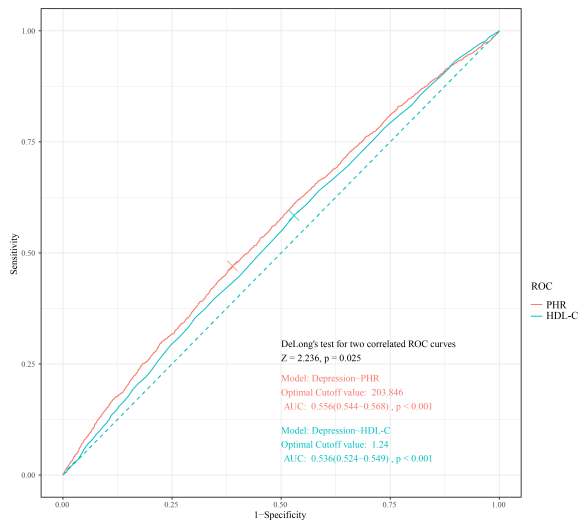


FIGURE 3
Unadjusted ROC curves to predict depression for PHR and HDL-C. ROC, receiver operating characteristic; HDL-C, high-density lipoprotein cholesterol; PHR, platelet-to-high-density lipoprotein cholesterol ratio.

PHR and Unipolar depression (UD) was observed (36). In our study, although the association between PHR and depression gradually decreased after considering confounding factors, PHR remained significantly associated with depression in all models. PHR is defined as the ratio of platelet count (PC) to HDL-C concentration (22, 37). Platelets play a crucial role in inflammation, as they produce numerous cytokines and chemokines when activated, leading to various immune effects (17). Platelets are considered a potential intermediate factor linking mental illness and inflammatory responses (38–40). Studies indicate that patients with depression exhibit platelet over-activation and inflammatory responses (18). However, there are also findings suggesting a low level of platelet activation in depression (19). Thus, further evidence is needed to clarify the role of platelets in depression. The relationship between HDL-C and depression is complex. Lower serum HDL-C levels are associated with a higher risk of depression or more severe depressive symptoms (41–45). Conversely, some studies suggest that higher HDL-C levels are correlated with more severe depressive symptoms (46, 47). These contradictory findings imply a more intricate dynamic regulatory mechanism, emphasizing the value of this study. Furthermore, cardiovascular disease patients are more prone to depression compared to the general population. Individuals with depression are more likely to develop cardiovascular disease, with higher mortality rates, possibly linked to increased inflammatory markers (48–51). Our results support PHR as a novel inflammation marker that significantly predicts CVD mortality in patients with depression. Additionally, through mediation analysis, we established that better cardiovascular health prevents depression by reducing PHR, further supporting the interplay of PHR in depression and CVD. However, the specific mechanistic relationship of PHR requires further elucidation.

Subgroup analysis showed a significant interaction between current alcohol consumption and PHR. Alcohol is a central nervous system depressant that slows down the activity of the central nervous system. Long-term alcohol consumption can lead to structural abnormalities in the brain and increase the risk of depression (52, 53). In a large multi-ethnic cohort, increasing levels of alcohol consumption were associated with higher levels of all HDL markers. Mild drinkers had lower levels of HDL markers compared to moderate drinkers (54). Huang et al. (55) also found that moderate alcohol consumption may be associated with a slower decrease in HDL-C, but excessive alcohol consumption may have a negative impact on HDL-C. On the other hand, a relationship has been established between alcohol consumption frequency and quantity and platelet count reduction (56). Wine, particularly its phenolic compounds, seems to have a more pronounced inhibitory effect on platelet aggregation (57).

5 Advantages and limitations

To our knowledge, this is the first observational study to explore the relationship between PHR and depression in the entire adult population of the United States; representing a weighted sample of 160 million adult individuals. Furthermore, our study

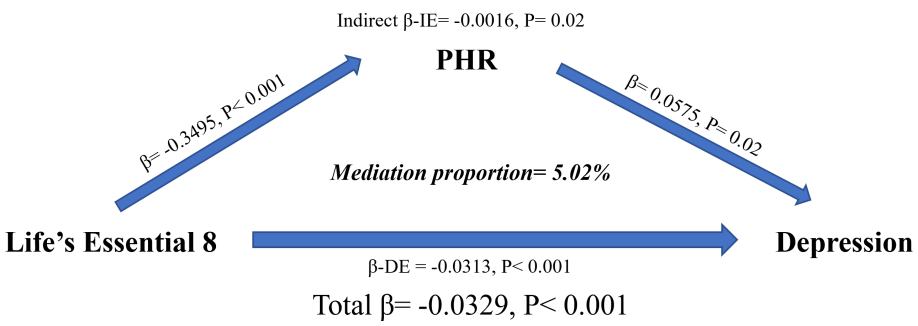


FIGURE 4
Estimated proportion of the association between LE8 and depression mediated by PHR. All models were adjusted for age, sex, ethnicity, education, marital status, poverty-income ratio, total energy intake, BMI, smoking, drinking, arthritis, thyroid problems, cancer, diabetes, liver diseases, CVD, CKD, statins, and antiplatelet. Mediation proportion = β-IE/Total β. IE, indirect effect; DE, direct effect; PHR, platelet-to-high-density lipoprotein cholesterol ratio; LE8, life's essential 8; BMI, body mass index; CVD, cardiovascular disease; CKD, chronic kidney disease.

comprehensively considered risk factors for depression, therefore, the conclusions are relatively reliable. Lastly, considering that HDL-C and platelet count are routine indicators that are easily accessible, they may have significant potential in the diagnosis of depression and cardiovascular prognosis assessment in depressed populations. However, there are some limitations to consider. First, regarding the relationship between PHR, HDL-C, and depression, further validation is needed in prospective studies involving larger cohorts due to the cross-sectional design. Second, HDL-C comprises multiple subgroups with different biological characteristics, but in this study, we did not have data on the quantity and size of HDL subgroups or particles. Third, potential confounding factors were inevitably overlooked. Fourth, depression diagnosis was based on self-reporting, which could be prone to bias. Fifth, depression is related to platelet function, yet this study did not obtain data on platelet functional parameters (58, 59). Sixth, this study assessed HDL-C concentration and platelet count based on a single measurement, which could introduce potential bias. Seventh, some treatments may affect the association between PHR and depression; however, in the current study, more detailed exploration cannot be carried out. Finally, it must be admitted that although the use of antidepressant drugs has been adjusted, some patients may have received effective treatment and therefore will not be diagnosed with depression during the investigation. To reduce this potential bias, we redefine depression. In additional analysis 1, using antidepressants or PHQ-9 ≥ 10 was defined as depression to define some potential depressive populations as depression. In additional analysis 2: Not using antidepressants and having PHQ-9 ≥ 10 was defined as depression to explore the association between PHR and untreated depression. In additional analysis 3, after excluding the participants with PHQ-9 ≥ 10 score, the individuals who use antidepressants and have PHQ-9 < 10 was defined as depression to explore the association between PHQ-9 and the effectively treated depression. In additional analysis 4: we exclude the people who use antidepressants to rule out the interference of antidepressants. Reassuringly, all four additional analyses above further confirmed the robust association of PHR with depression (Supplementary Table 7).

6 Conclusion

In conclusion, our study identified the bridging role of PHR in depression and CVD among U.S. adults. PHR appears to be a potential mechanism for the co-occurrence of these two conditions, and further validation of our findings is needed in more clinical trials and mechanistic studies in the future.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: https://www.cdc.gov/nchs/nhanes/index.htm.

Ethics statement

The studies involving humans were approved by The National Center for Health Statistics institutional review board. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

HZ: Conceptualization, Formal analysis, Methodology, Software, Writing – original draft, Writing – review & editing. YX: Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – review & editing. YYX: Conceptualization, Investigation, Methodology, Supervision, Validation, Writing – review & editing.

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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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1402336/full#supplementary-material

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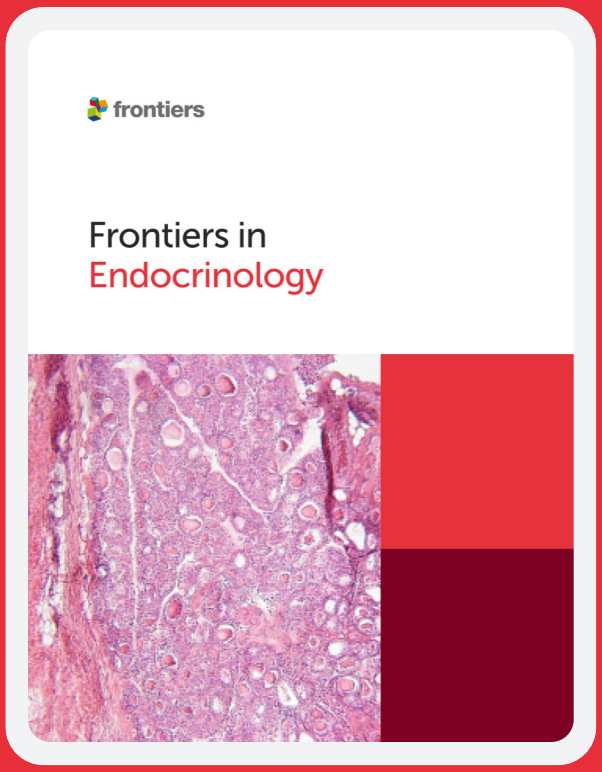
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