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Sex differences and cardiovascular therapeutics

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Sex differences and cardiovascular therapeutics

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Table of contents

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Editorial: Sex differences and cardiovascular therapeutics

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Editorial on the Research Topic
Sex differences and cardiovascular therapeutics

Introduction

Cardiovascular diseases (CVDs) remain a leading cause of morbidity and mortality worldwide (1). Sex and gender as a biological variable, are crucial factors that impact every aspect of clinical and public health practice and research (2–4). In recent times, research investigating sex differences has gained more attention, partly benefit from federal agencies emphasizing the importance of considering sex as a significant biological factor (5). Within cardiovascular medicine, sex and gender affect disease presentation, pathophysiology, diagnostic assessment, responses to treatments, and overall health outcomes (6, 7). Historically, CVD has been perceived as primarily affecting men; however, it is increasingly recognized as a leading cause of morbidity and mortality in women as well (8). While men tend to develop CVD earlier in life, CVD prevalence increases significantly in postmenopausal women, narrowing the gap between the sexes (9). Men have traditionally experienced higher rates of CVD-related events, but women are more likely to die following an acute cardiovascular event (10). The existence of these gender disparities has prompted significant attention, highlighting the crucial significance of considering gender variations in the prevention, diagnosis, treatment, and overall management of CVD (11).

Traditional risk factors such as hypertension, diabetes, dyslipidemia, and smoking affect both sexes, but their impacts can vary between men and women (12–14). Additionally, women may experience unique risk factors including pregnancy-related complications such as gestational diabetes and preeclampsia, as well as endocrine disorders in reproductive age such as polycystic ovary syndrome (PCOS) and early menopause, which are associated with accelerated development of CVD and impaired CVD-free survival (15–17). Biological differences include genetic differences, variation in sex hormonal status, vascular anatomy, endothelial function, and plaque composition, which contribute to differences in the pathophysiology of CVD between men and women (18, 19). Women showed less plaque inflammatory infiltration compared to plaques from age-matched men (20–22). In addition, women often undergo fewer diagnostic tests and experience delays in diagnosis compared to men,

leading to disparities in timely intervention and treatment (23). Despite accumulating evidence, the precise roles of biological sex and the sociocultural aspect of gender in the development and consequences of CVDs have not been fully explained. The interplay between sex-specific disparities in genetic and hormonal mechanisms and the intricate nature of gender, including its various components and influencing factors, which give rise to different disease patterns in men and women, requires further investigation.

The extents to which biological factors, such as genes and hormones, contribute to cardiovascular traits and outcomes are still not fully grasped. Heightened recognition of gender's impact has prompted endeavors to assess gender in both retrospective and prospective clinical studies, leading to the creation of gender scores. Yet, the combined or conflicting influences of sex and gender on cardiovascular characteristics, as well as on the mechanisms underlying CVDs, have not been systematically elucidated. The majority of medication are withdrawn after FDA approval due to unexpected adverse effects in women (24). Additionally, there are differences in the effectiveness and side effects of cardiovascular medications between men and women (25). Current guidelines do not provide sex-specific recommendations on the use of antithrombotic drugs in patients with coronary artery disease. Nevertheless, the effectiveness of antithrombotic medications might be impacted by genetic and biological factors associated to sex (26). Women generally exhibit greater platelet reactivity at baseline and in response to low-dose aspirin treatment in comparison to men (27). Despite receiving high-dose statin therapy following acute coronary syndrome, women showed a smaller absolute reduction in low-density lipoprotein cholesterol (LDL) cholesterol levels compared to men (28). Understanding these sex differences is crucial for providing personalized and effective cardiovascular care. It requires including more women in clinical trials, analyzing data by sex, and considering sex-specific factors in treatment decisions. By doing so, healthcare providers can optimize outcomes and reduce disparities in cardiovascular care between men and women.

Sex hormones in CVD

Sex hormones, including estrogen, progesterone, and testosterone, play significant roles in cardiovascular health and disease (29, 30). Estrogen, primarily found in premenopausal women, exerts cardioprotective effects (8, 29, 31). It helps maintain healthy blood vessel function by promoting vasodilation, reducing inflammation, and inhibiting the formation of atherosclerotic plaques. Estrogen also influences lipid metabolism, favoring higher levels of high-density lipoprotein (HDL) cholesterol and lower levels of LDL cholesterol, contributing to a reduced risk of CVDs such as heart attacks and strokes (32, 33).

The differences in estrogen levels between men and women, as well as the changes that occur during menopause, contribute to the variation in CVD occurrence between the sexes. Before menopause, women generally have higher levels of estrogen, potentially explaining their lower risk of CVD compared to men of the same age. However, after menopause, when estrogen levels decline, women's risk of CVD increases and may approach that of men (34). Progesterone, another female sex hormone, also plays a role in cardiovascular health, though its effects are less well understood compared to estrogen. Some research suggests that progesterone may have protective effects on the cardiovascular system, such as promoting vasodilation and inhibiting smooth muscle cell proliferation in blood vessels (35, 36).

Androgens, including testosterone and other male sex hormones, can influence cardiovascular health in both men and women. Low levels of testosterone in men have been associated with an increased risk of CVD, including coronary artery disease and heart failure (18). Testosterone influences factors such as blood pressure regulation, lipid metabolism, and the development of atherosclerosis. However, the relationship between testosterone levels and cardiovascular risk is complex, and both low and high levels of testosterone have been implicated in various cardiovascular conditions (33). In addition to testosterone, other androgens such as dehydroepiandrosterone (DHEA) and its sulfate (DHEAS) may also impact cardiovascular risk factors (18).

Overall, sex hormones play intricate roles in cardiovascular physiology and pathology. Understanding the interplay between sex hormones and cardiovascular health is essential for developing personalized approaches to preventing and managing CVDs.

Genetic factors that are specific to each sex and influence cardiovascular characteristics

Genetic factors play a significant role in cardiovascular characteristics, with some of these factors being specific to each sex (37). The presence of sex chromosomes (XX in females and XY in males) not only determines primary sexual characteristics but also influences cardiovascular health (38, 39). For instance, genes on the Y chromosome may impact cardiac function (40). Moreover, genetic variations in lipoprotein metabolism can influence the metabolism of lipoproteins differently in men and women (41, 42). For example, certain genetic variants may have a more pronounced effect on the levels of HDL cholesterol in women compared to men, or vice versa (43). Additionally, genes involved in blood pressure regulation may exhibit sex-specific effects. For instance, variations in genes related to the renin-angiotensin-aldosterone system (RAAS) may influence blood pressure in varying ways between men and women (44). Understanding these sex-specific genetic factors is crucial for developing personalized approaches to cardiovascular disease prevention, diagnosis, and treatment. It underscores the importance of considering sex as a biological variable in cardiovascular research and clinical practice.

Our Research Topic: "*Sex Differences and Cardiovascular Therapeutics*," featured twelve papers comprising original research papers and reviews (Table 1). These highlights offer a comprehensive perspective on gender-related differences in various CVDs, potential factors contributing to these distinctions,

TABLE 1 Twelve highly viewed research papers, published in our special topic entitled "*Sex differences and cardiovascular therapeutics*", are summarized.

Paper title	Summary	References
Sex differences in the renin-angiotensin aldosterone system and its roles in hypertension, cardiovascular, and kidney diseases	- Hypertension is less common in premenopausal women than in men. 1. Animal studies have demonstrated that females have greater nitric oxide (NO) bioavailability than males due to a higher capacity for generating NO in women, while increased oxidative stress in men leads to endothelial dysfunction and activation of the renin-angiotensin-aldosterone system (RAAS). 2. The RAAS is regulated by estrogen, which binds to estrogen receptor-α (ER-α) expressed in the vascular endothelium, promoting endothelial repair, vasodilation, and NO production. 3. Estrogen can modify RAAS activity by controlling the expression of key substrates, enzymes, receptors, and protein synthesis. 4. Estrogen has antihypertensive effects by upregulating substrates and enzymes in counter-regulatory RAAS pathways, such as increased expression of ACE2 and activation of the MAS receptor and Ang III/AT2 receptor. - Compared to men, women with high blood pressure had a higher risk for adverse cardiac events. - Women had worse blood pressure control rates compared to men (SPRINT trial). - There is an increased prevalence of adverse drug reactions in females due to increased drug bioavailability.	Nwia et al.
Sex differences in patterns of referral and resource utilization in the cardiology clinic: an outpatient analysis	There were higher referral rates of women compared to men from primary care due to palpitations in women (n = 676; 19.2%) and ECG abnormalities in men (n = 570; 23.2%). Additionally, compared to men, women were older. Women also had fewer cardiology hospitalizations and a lower mortality rate. Moreover, women under 65 years old had more admission to the emergency rooms.	Vicent et al.
Association of sex with post-arrest care and outcomes after out-of-hospital cardiac arrest of initial shockable rhythm: a nationwide cohort study	Compared to men, women were older. There were no significant differences observed in survival outcomes between males and females. Additionally, there was no significant difference noted between male and female patients who received in-hospital interventions such as extracorporeal cardiopulmonary resuscitation or targeted temperature management.	Hosomi et al.
Sex-specific association of low-renin hypertension with metabolic and musculoskeletal health in Korean older adults	In postmenopausal women, low-renin hypertension was associated to a lower femur neck T-score and a deteriorated trabecular bone score, indicating an increased risk of osteoporosis and subsequent fracture.	Lee et al.
Oxidative stress and inflammation distinctly drive molecular mechanisms of diastolic dysfunction and remodeling in female and male heart failure with preserved ejection fraction rats	Ren-2 male transgenic (TG) rats exhibited cardiac enlargement, left ventricular hypertrophy, left ventricle diastolic dysfunction, and hypertension compared to female TG rats. Both males and females displayed high levels of proinflammatory cytokines, along with significant alterations in apoptotic and autophagy pathways.	Zhazykbayeva et al.
Sex differences in long QT syndrome	There is a higher incidence of Long QT Syndrome (LQTS), with malignant arrhythmias being associated with female sex during postpartum, menopausal, and perimenopausal due to sex hormone differences. Women with LQTS show a higher risk of ventricular tachycardia and sudden cardiac death in comparison to the relatively low risk during pregnancy.	Díez-Escuté et al.
Diabetes and heart failure associations in women and men Results from the MORGAM consortium	Men have a greater absolute risk of heart failure than women regardless of diabetes status. Additionally, there are no sex-specific differences in the relative risk of heart failure between diabetic men and women.	Chadalavada et al.
The role of the pregnancy heart team in clinical practice	There are increased maternal mortality and morbidity rates in pregnant women with cardiovascular diseases. Peripartum cardiomyopathy and pre-existing cardiovascular diseases are the leading causes of heart failure during pregnancy. The prevalence of acute myocardial infarction associated with pregnancy also increases during pregnancy. Furthermore, there is an increased risk of corrected congenital heart disease, valvular heart diseases, and cardiomyopathies during pregnancy.	Lucà et al.
The emerging role of estrogen's non-nuclear signaling in the cardiovascular disease	Estrogen protects against vascular injury, suppress neointima hyperplasia, and reduces the development of atherosclerosis. Additionally, estrogen suppresses metabolic disorders and reduces pressure overload-induced cardiac hypertrophy.	Tokiwa et al.
Sex differences in coronary artery bypass grafting-related morbidity and mortality	CABG in women exhibited a stronger correlation with elevated risks of diseases such as hypertension, type 1 diabetes, diabetic retinopathy, Alzheimer's disease, aortic aneurysms, gout, and chronic kidney disease compared to the risk increases noted in men. Additionally, there was an increased risk of cardiac death after CABG in women compared to men.	Nurkkala et al.
Sex difference and outcome trends following transcatheter aortic valve replacement	Men exhibited markedly higher prevalence rates of hyperlipidemia, diabetes mellitus, chronic renal disease, peripheral artery disease, and coronary artery disease. Men showed a higher prevalence of previous cardiac interventions, including prior percutaneous coronary intervention, and a greater frequency of prior device implantation. Women demonstrated elevated in-hospital mortality rates. Additionally, women exhibited significantly higher rates of pericardial, cardiac, pulmonary, hemorrhagic, vascular, and neurological complications. Conversely, men had higher rates of acute renal failure, device-related mechanical complications, and pacemaker implantation.	Elbaz-Greener et al.
Cardiovascular sex-differences: insights via physiology-based modeling and potential for noninvasive sensing via ballistocardiography	On average, the size of the female heart is approximately one-fourth smaller than that of the male heart. In women, the Left Ventricle (LV) is typically smaller than in men, resulting in lower end-diastolic volume (EDV) and end-systolic volume (ESV), as well as smaller stroke volume (SV). LV ejection fraction (EF) is higher in women than in men. Additionally, women exhibit a smaller size and higher contractility of the Right Ventricle (RV), leading to lower EDV, SV, ESV, and cardiac output (CO) compared to men. Vessel diameters and lengths tend to be smaller in females when compared to males.	Zaid et al.

and management strategies. Collectively, these papers contribute to our understanding of sex differences in cardiovascular therapeutics and emphasize the importance of tailored approaches to prevention, diagnosis, and treatment based on gender-specific considerations.

Conclusion

Sex differences play a significant role in the epidemiology, pathophysiology, clinical presentation, diagnosis, management, and outcomes of cardiovascular diseases. Recognizing these differences and implementing sex-specific approaches in research, clinical practice, and public health initiatives are essential for reducing disparities and improving cardiovascular outcomes for both men and women. While the differences between sexes in the occurrence and complications of CVDs are widely acknowledged, there are relatively limited data in both clinical and pre-clinical studies that adequately explore the underlying mechanisms regarding sex as a biological variable in CVDs.

Author contributions

FS: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Writing – original draft. KX: Conceptualization, Writing – review & editing. YL: Conceptualization, Writing – review & editing. YS: Conceptualization, Writing – review & editing. XJ: Conceptualization, Writing – review & editing. HW: Conceptualization, Writing – review & editing. XY: Conceptualization, Funding acquisition, Supervision, Formal Analysis, Visualization, Writing – review & editing.

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Sex difference and outcome trends following transcatheter aortic valve replacement

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Background: Based on worldwide registries, approximately 50% of patients who underwent transcatheter aortic valve replacement (TAVR) are female patients. Although TAVR procedures have improved tremendously in recent years, differences in outcome including mortality between sexes remain. We aimed to investigate the trends in TAVR in the early and new eras of utilization and to assess TAVR outcomes in female patients vs. male patients.

Methods: Using the 2011–2017 National Inpatient Sample (NIS) database, we identified hospitalizations for patients with the diagnosis of aortic stenosis during which a TAVR was performed. Patients' sociodemographic and clinical characteristics, procedure complications, and mortality were analyzed. Piecewise regression analyses were performed to assess temporal trends in TAVR utilization in female patients and in male patients. Multivariable analysis was performed to identify predictors of in-hospital mortality.

Results: A total of 150,647 hospitalizations for TAVR across the United States were analyzed during 2011–2017. During the study period, a steady upward trend was observed for TAVR procedures in both sexes. From 2011 to 2017, there were significantly more TAVR procedures performed in men [80,477 (53.4%)] than in women [70,170 (46.6%)]. Male patients had significantly higher Deyo-CCI score and comorbidities. Differences in mortality rates among sexes were observed, presenting with higher in-hospital mortality in women than in men, OR 1.26 [95% CI 1.18–1.35], $p < 0.001$.

Conclusion: Utilization of TAVR demonstrated a steady upward trend during 2011–2017, and a similar trend was presented for both sexes. Higher in-hospital mortality was recorded in female patients compared

to male patients. Complication rates decreased over the years but without effect on mortality differences between the sex groups.

KEYWORDS

TAVR, aortic valve replacement, transcatheter aortic valve replacement, gender, interventional cardiology

Introduction

Aortic stenosis (AS) is characterized by left ventricular outflow obstruction. This results in decreased cardiac output, which leads to major morbidity and mortality. AS is a progressive disease. The prevalence in octogenarians is 9.8 vs. 0.2% in adults aged 50–59 years, which suggests degenerative etiology as the main cause of the disease (1). Aortic valve replacements (AVR) *via* surgical aortic valve replacements (SAVR) and transcatheter aortic valve replacements (TAVR) are well-known treatment options for patients with severe symptomatic aortic stenosis. Based on worldwide registries, approximately 50% of patients who underwent AVR are female patients (2–5).

Although AVR procedures have improved tremendously in recent years, differences in outcomes, including mortality between sexes, remain. Physiological, anatomical, and comorbidity differences between male patients and female patients contribute to the heterogeneity in AVR procedural and long-term outcomes (5–14).

Specifically, female patients tend to be older with a higher frailty score and a lower body mass index (6–13, 15–17). Women have greater periprocedural complications (18–23), such as more frequent bleeding, and more vascular complications than men following the same procedure (8, 14, 18–25). Thus, after AVR, women suffer significantly more than men from in-hospital and 30-day morbidity and mortality (3–10, 13, 21–23). However, although the short-term outcomes are worse, female patients have shown better long-term outcomes with higher survival rates (6, 9, 11, 12, 14, 16, 26–32) known as the sex paradox.

The revolutionary shift from SAVR to a less invasive procedure as TAVR has improved outcomes in women (12, 19, 20, 22–28) as seen in the last decade. Data suggest that short-term outcomes in female patients also improved (21), and the sex-related differences diminished over the years (4, 24, 25, 30–32).

A paucity of literature from the latest TAVR era suggests that the so-called sex paradox may not exist with the new, improved technology and a better patient selection process (4, 9, 30).

This study aimed to investigate temporal trends in sex-related differences in a large cohort of the US database from the National Inpatient Sample (NIS) registry, specifically the in-hospital outcomes in male patients vs. female patients who underwent TAVR procedures during 2011–2017.

Methods

Data collection

The data were obtained from the NIS database, the Healthcare Cost and Utilization Project (HCUP), and the Agency for Healthcare Research and Quality (AHRQ) (33). Data from the NIS datasets were de-identified, and therefore, this study was deemed exempt from institutional review by the Human Research Committee.

As described previously (34), the NIS is the largest collection of all-payer data on inpatient hospitalizations in the United States. The dataset represents an approximate 20% stratified sample of all inpatient discharges from US hospitals (35, 36). This information includes patient-level and hospital-level factors such as patient demographic characteristics, primary and secondary diagnoses, procedures, AHRQ comorbidities, length of stay (LOS), hospital region, hospital teaching status, hospital bed size, and cost of hospitalization. National estimates were calculated using the patient-level and hospital-level sampling weights provided by NIS.

For this study, we obtained data for the years 2011 to 2017. The International Classification of Diseases, ICD-9-CM, and ICD-10-CM Revisions were used. Clinical modifications were used for reporting diagnoses and procedures in the NIS database. For each index hospitalization, the database provided a principal discharge diagnosis and a maximum of 14 or 24 additional diagnoses (depending on the year), in addition to a maximum of 15 procedures.

We identified patients aged 18 years or older with a primary diagnosis of aortic stenosis based on ICD-9-CM codes 395.0, 395.2, 396, 396.2, 746.3, 424.1 and based on ICD-10-CM codes I35.0, I35.2, Q23.0, I06.0, I06.2, and I08.0, who underwent in-hospital TAVR procedure codes for PR1-PR15. ICD-9-CM codes 35.05 (trans-femoral) and 35.06 (trans-apical) and ICD-10-CM codes 02RF37Z, 02RF38Z, 02RF3JZ, 02RF3KZ, and X2RF332 (trans-femoral) and 02RF37H, 02RF38H, 02RF3JH, and 02RF3KH (trans-apical) were used.

We used the Deyo-Charlson Comorbidity Index (Deyo-CCI), which predicts the risk of death within 1 year of hospitalization for patients with specific comorbid conditions. Higher Deyo-CCI scores indicated a greater burden of comorbid diseases and were associated with mortality 1 year after admission (37). The Deyo-CCI index has been used extensively in studies from administrative databases, with proven validity in predicting short- and long-term outcomes (38–40). Deyo-CCI uses the ICD-9-CM and ICD-10-CM diagnosis and procedure codes, the administrative data for 17 comorbidities with differential weights of 1 to 6, to calculate the final score index, ranging from 0 to 33. The following patient demographics were collected from the database: age, sex, and race. Prior comorbidities were identified from the AHRQ. Detailed information on Deyo-CCI is provided in Appendix Table 1.

Outcomes

The primary outcome in this study was all-cause in-hospital mortality. The secondary outcome of interest included in-hospital complications. In-hospital TAVR-related complications were defined (36, 40) as follows: (1) pericardial complications, defined as tamponade, hemopericardium, pericarditis, and pericardiocentesis; (2) cardiac complications (during or resulting from procedure), defined as cardiac block, myocardial infarction, cardiac arrest, congestive heart failure, cardiogenic shock, and others; (3) pulmonary complications, defined as pneumothorax/hemothorax, diaphragm paralysis, postoperative respiratory failure, and other iatrogenic respiratory complications; (4) hemorrhage/hematoma complications, defined as hemorrhage/hematoma complicating a procedure, acute post-hemorrhagic anemia, and hemorrhage requiring transfusion; (5) vascular complications, defined as accidental puncture or laceration during a procedure, injury to blood vessels, arteriovenous fistula, injury to retroperitoneum, vascular complication requiring surgical repair, reopen, and other vascular complications; (6) infection, defined as fever, septicemia, and post-procedural aspiration pneumonia; (7) neurological, defined as nervous system complication, unspecified, central nervous system complication, iatrogenic cerebrovascular infarction or hemorrhage cerebrovascular effect, and transient ischemic attack; (8) diaphragmatic paralysis; (9) acute renal failure; (10) reopen and conversion to open surgery; (11) device-related mechanical complication; (12) paravalvular leak (PVL); and (13) permanent pacemaker implantation (PPM). Detailed information on all ICD-9-CM and ICD-10-CM codes used to identify in-hospital complications is summarized in Appendix Table 2. Length of stay (LOS) was defined as the time interval in days from hospital admission to hospital discharge.

Statistical analysis

The chi-square (χ^2) test and Wilcoxon rank sum test were used to compare categorical variables and continuous variables, respectively. Rao-Scott *F*-adjusted chi-square test was used to represent differences in baseline characteristic frequencies of TAVR patients and between-gender differences. LOS (continuous) was compared based on non-parametric confidence intervals according to Zhou and Dinh (41).

Trends

Piecewise regression analyses were performed to assess temporal trends in TAVR utilization in male patients and in female patients in response to an empirical inflection point corresponding to the early vs. late TAVR eras, before 2014 vs. after 2014. *P*-values were computed using Rao-Scott *F*-adjusted chi-square test to represent differences between year groups in complication frequencies before vs. after 2014.

Predictors of mortality/complications

We generated a weighted logistic regression model using "TRENDWT" to identify independent predictors of in-hospital complications and in-hospital mortality (further details are found in Appendix Table 3). Congruent with the HCUP NIS design, the hospital identification number was used as a random effect with patient-level factors clustered within hospital-level factors. We retained all predictor variables that were associated with our primary outcome of mortality and secondary outcome of at least one complication with $p < 0.1$ in our final multivariable regression model. For LOS analysis, we generated a logistic regression model. For all analyses, we used SAS® version 9.4 software (SAS Institute Inc., Cary, NC). A *p*-value < 0.05 was considered statistically significant.

Results

We analyzed data out of 30,153 unweighted hospitalizations in the NIS database from 2011 to 2017. After implementing the weighting method, these represented a total of 150,647 hospitalizations for aortic stenosis in patients who underwent in-hospital TAVR during the index hospitalization.

Baseline characteristics

In this study, from 2011 to 2017, there were significantly more TAVR procedures performed in men [80,477 (53.4%)] than in women [70,170 (46.6%)]. However, in patients

TABLE 1 Baseline and TAVR procedural characteristics categorized by sex.

	Total	Male	Female	*P*-Value
Population, *n* (%)				
Unweighted	30,153	16,108 (53.4)	14,045 (46.6)	<0.001
Weighted	150,647	80,477 (53.4)	70,170 (46.6)	
Age, years %				<0.001
18–49	0.4	0.5	0.3	
50–59	1.6	1.9	1.3	
60–69	8.3	8.7	7.7	
70–79	26.9	28.6	25.0	
≥80	62.8	60.3	67.7	
Race, %				<0.001
White	82.7	83.8	81.6	
Comorbidities, %				
Hypertension	52.3	50.3	54.5	<0.001
Hyperlipidemia	65.6	67.5	63.5	<0.001
Cerebrovascular disease	5.1	5.0	5.2	0.44
Congestive heart failure	28.8	29.3	28.3	0.07
Diabetes mellitus	35.1	37.1	32.8	<0.001
Renal failure	35.9	40.3	30.9	<0.001
Chronic pulmonary disease	30.9	31.0	30.7	0.60
Peripheral vascular disorders	26.8	29.4	23.9	<0.001
Prior CAD/IHD	27.6	30.4	24.3	<0.001
Prior PCI	9.4	10.6	8.1	<0.001
Prior Pacemaker/ICD/CRTD	13.0	15.2	10.5	<0.001
Prior sternotomy, %	32.0	41.3	21.4	<0.001
Deyo-CCI, %				<0.001
0	6.3	5.5	7.2	
1	11.2	9.8	12.8	
2 or higher	82.5	84.7	80.0	
Length of stay (days)	5.7 ± 0.1	5.5 ± 0.1	6.0 ± 0.1	0.001
Mortality	2.3	2.1	2.7	0.001

CAD, cardiovascular disease; CRTD, cardiac resynchronization therapy devices; Deyo-CCI, Deyo-Charlson Comorbidity Index; ICD, implantable cardioverter-defibrillator; IHD, ischemic heart disease; TAVR, transcatheter aortic valve replacement.

older than 80 years, there was a female predominance (67.7%). Most patients were Caucasian (82.7%), and the mean age was 80.6 ± 8.2 years. Baseline and procedural characteristics categorized by sex are presented in Table 1.

Regardless of sex, most patients also presented with hypertension and hyperlipidemia (>50%). Male patients had significantly higher Deyo-CCI score and higher proportions of hyperlipidemia, diabetes mellitus, chronic renal disease, peripheral artery disease, and coronary artery disease and tended to be smokers. Furthermore, male patients had a higher rate of previous cardiac intervention as prior percutaneous coronary intervention and a higher rate of prior device implantation (Table 1).

AVR utilization trends

Our data show that the annual number of TAVR procedures has increased from 1,215 in 2011 to 48,480 in 2017 (Figure 1). The same trend characterized both female patients and male patients. A significant and steady upward trend was observed for TAVR procedures in both sexes, rising from 670 in 2011 to 26,450 in 2017 for male patients and from 545 in 2011 to 22,030 in 2017 for female patients (Figure 1).

Using a piecewise regression analysis, a significant steady upward trend was observed for TAVR procedures from 2011 to 2017, with an additionally pointed elevation after 2014 in both sexes ($p = 0.001$ for male patients and $p < 0.005$ for female patients) (Figure 1).

Clinical outcomes

All-cause in-hospital mortality during the study period was 2.3% (Table 1). Differences in mortality among sexes were observed, with higher in-hospital mortality in women (2.7%) than in men (2.1%) (Figure 2A). Over time, there was improved mortality, with similar trends observed in both men and women, peaking in 2013 and dropping down to a minimum in 2017, the last observation year (Figure 2A).

Women had a longer LOS than men (6.0 ± 0.1 vs. 5.5 ± 0.1, $p = 0.0001$, respectively). TAVR LOS decreased significantly in men and women from 2011 to 2017 but was still higher in women (Figure 2B).

Procedural complications

Procedural complications categorized by sex are presented in Table 2. Following TAVR, female patients had significantly higher rates of pericardial, cardiac, pulmonary, hemorrhagic, vascular, and neurological complications (Table 2). Acute renal failure, device-related mechanical complications, and pacemaker implantation were significantly higher in male patients, although similar downward trends were observed for both sexes (Table 2).

Trends in complication rates during 2011–2017 are presented in Figures 3A–F. A significant downward trend was observed in a majority of the complications rate in the early vs. late TAVR era in both male and female patients (Figures 3A–F).

Multivariable analysis

The multivariable regression model analysis adjusted for potential confounders is presented in Table 3. Women had a higher mortality risk with an odds ratio of 1.26 (95%CI 1.18–1.35), $p < 0.001$ (Table 3).

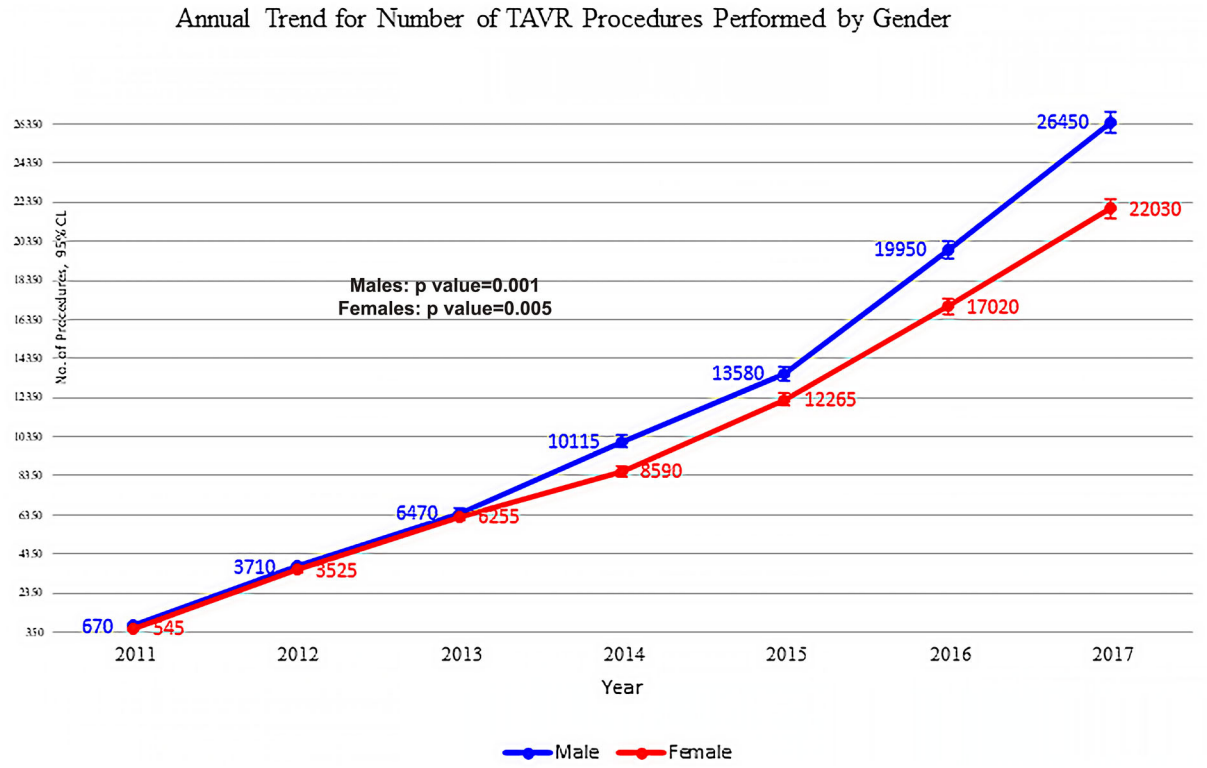

FIGURE 1
Annual trend for the number of procedures performed by sex and procedure. Piecewise linear regression between years before and after 2014; *p*-value is 0.001 for male patient and 0.005 for female patient.

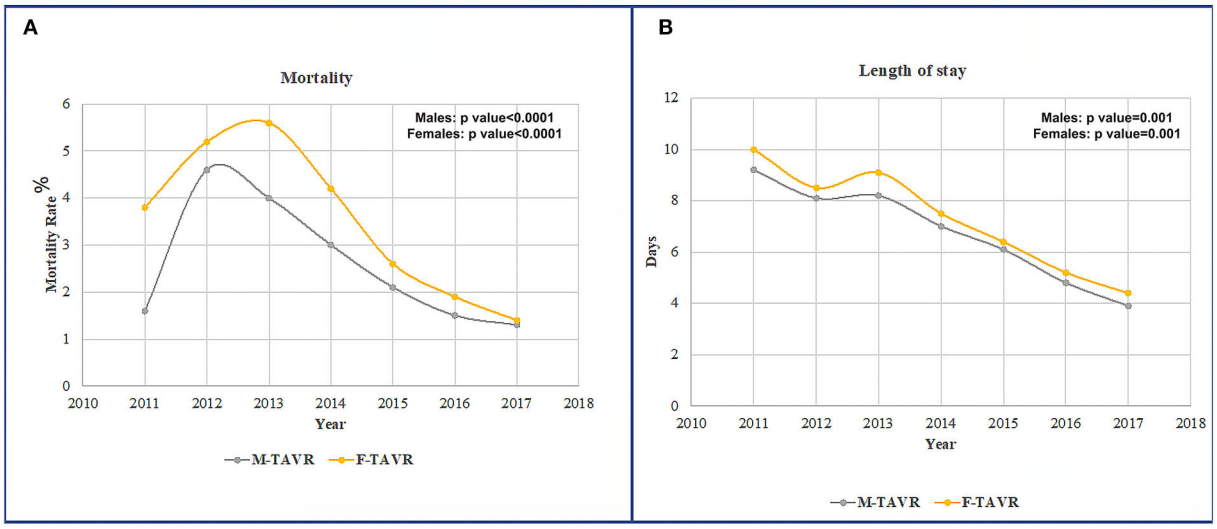

FIGURE 2
(A) Mortality rates in male patients vs. female patients after TAVR during 2011–2017. **(B)** LOS rates in male patients vs. female patients after TAVR during 2011–2017.

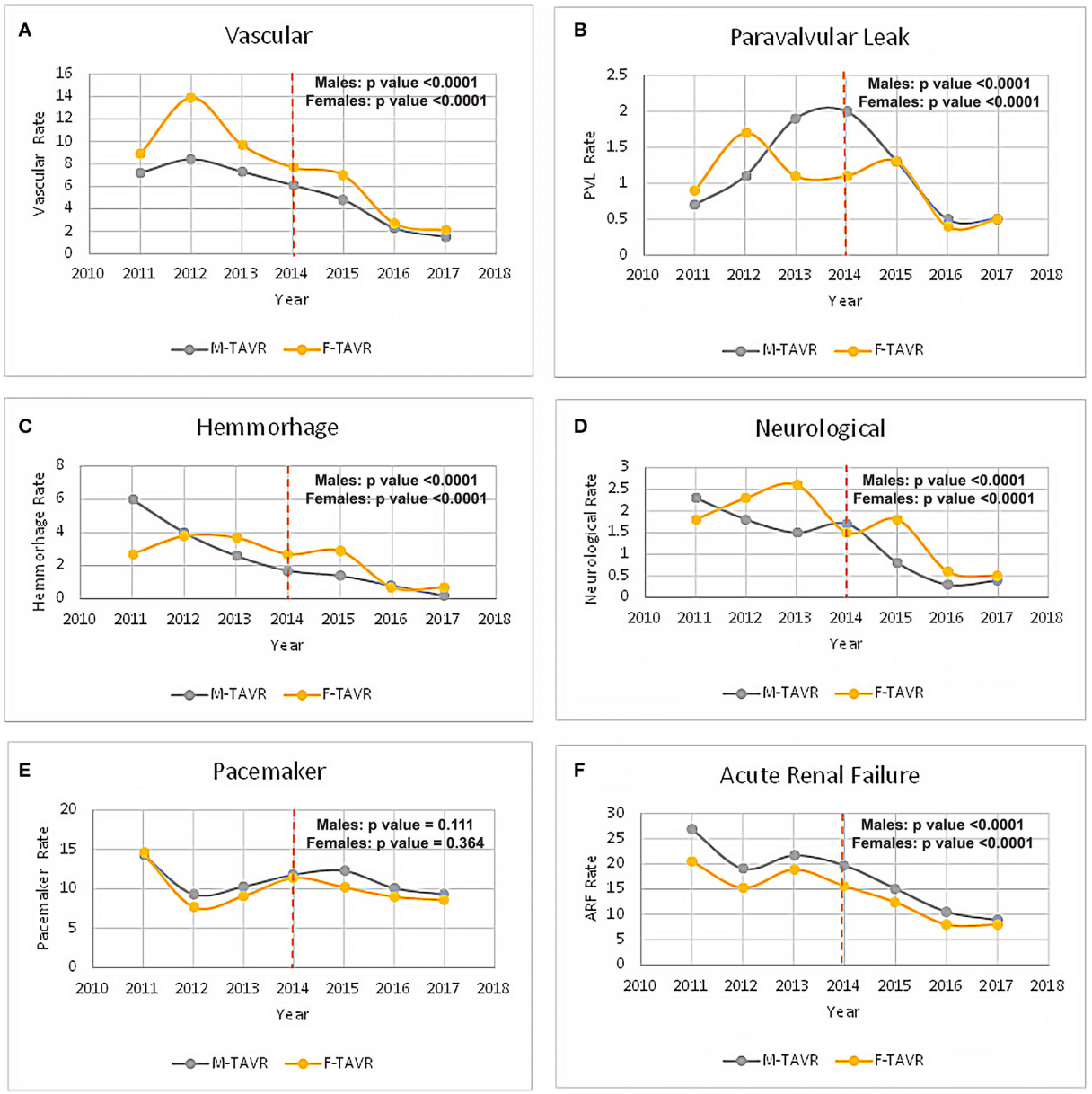

FIGURE 3
Complications following TAVR in male patients vs. female patients in the early vs. late TAVR era. **(A)** Vascular complication. **(B)** Paravalvular leak. **(C)** Hemorrhage complication. **(D)** Neurological complication. **(E)** New pacemaker implantation. **(F)** Acute renal failure.

Table 4 presents separate analyses for each gender. Women had fewer comorbidities independently associated with mortality than men. Renal failure, peripheral vascular disease, and higher Deyo-CCI score were independent risk factors for both sexes, while female patients with peripheral vascular disease had a higher probability of mortality (Table 4).

Discussion

This retrospective study found significant differences in male patients vs. female patients undergoing TAVR procedures between 2011 and 2017. Differences in in-hospital mortality rates among sexes were observed for TAVR, with higher in-hospital mortality in women than in men.

TABLE 2 TAVR procedural complications categorized by sex.

	Total	Male	Female	*P*-Value
Pericardial	2.7	1.8	3.7	<0.001
Cardiac	9.0	8.0	10.1	<0.001
Pulmonary	5.1	4.7	5.6	0.001
Hemorrhage/Hematoma	1.4	1.1	1.8	<0.001
Vascular	4.3	3.7	5.1	<0.001
Infection	2.1	2.1	2.2	0.64
Neurological	0.9	0.8	1.1	0.001
Acute renal failure	12.3	13.3	11.1	<0.001
Cardiogenic shock	2.3	2.4	2.2	0.11
Diaphragmatic paralysis	0.1	0.2	0.1	0.27
Re-open	0.2	0.2	0.3	0.11
Mechanical complication device related	2.3	2.5	1.9	<0.001
Pacemaker	9.9	10.5	9.4	0.001
Paravalvular leak	0.9	1.0	0.8	0.09

TABLE 3 Multivariate analysis for predictors of mortality from 2011 to 2017 in the TAVR cohort.

Predictor	Odds ratio (95% CI)	*P*-Value
Gender		<0.001
Male	1.00 (reference)	N/A
Female	1.26 (1.18, 1.35)	<0.001
Age group		<0.001
18–49 yrs	1.00 (reference)	N/A
50–59 yrs	1.90 (0.97, 3.72)	0.060
60–69 yrs	1.27 (0.67, 2.42)	0.460
70–79 yrs	1.34 (0.71, 2.52)	0.362
80–89 yrs	1.51 (0.80, 2.82)	0.202
90 yrs or older	2.11 (1.12, 3.97)	0.020
Race		<0.001
White	1.00 (reference)	N/A
Asian or Pacific Islander	0.94 (0.67, 1.32)	0.718
Black	0.59 (0.47, 0.74)	<0.001
Hispanic	1.32 (1.13, 1.54)	<0.001
Native American	2.11 (1.25, 3.57)	0.005
Comorbidities		
Hypertension		<0.001
No	1.00 (reference)	N/A
Yes	0.49 (0.46, 0.53)	<0.001
Hyperlipidemia		<0.001
No	1.00 (reference)	N/A
Yes	0.48 (0.45, 0.51)	<0.001
Cerebrovascular disease		0.058
No	1.00 (reference)	N/A
Yes	1.13 (1.00, 1.28)	0.058
Congestive heart failure		0.025
No	1.00 (reference)	N/A
Yes	1.10 (1.01, 1.20)	0.025
Diabetes mellitus		<0.001
No	1.00 (reference)	N/A
Yes	0.72 (0.66, 0.77)	<0.001
Renal failure		<0.001
No	1.00 (reference)	N/A
Yes	1.47 (1.37, 1.57)	<0.001
Chronic pulmonary disease		<0.001
No	1.00 (reference)	N/A
Yes	1.17 (1.09, 1.26)	<0.001
Peripheral vascular disorders		<0.001
No	1.00 (reference)	N/A
Yes	1.30 (1.21, 1.39)	<0.001
Prior CAD/IHD		<0.001
No	1.00 (reference)	N/A
Yes	0.81 (0.74, 0.89)	<0.001
Prior PCI		<0.001
No	1.00 (reference)	N/A
Yes	0.59 (0.52, 0.67)	<0.001
Prior cardiac surgery		<0.001
No	1.00 (reference)	N/A
Yes	0.61 (0.56, 0.67)	<0.001
Deyo-CCI		
0	1.00 (reference)	N/A
1	1.09 (0.91, 1.32)	0.354
2 or higher	1.49 (1.28, 1.74)	<0.001

AVR, aortic valve replacement; SAVR, surgical aortic valve replacement; TAVR, transcatheter aortic valve replacement; IHD, ischemic heart disease; PCI, percutaneous coronary intervention; NA, no available.

After TAVR procedures, this study observed that women had significantly higher in-hospital mortality rates than men over the years. LOS was also significantly higher in women compared to men. There was a peak in mortality and LOS around 2014 and then a steady and significant decrease in these clinical outcomes over the years in both groups. Still, women have poor short-term outcomes compared to men.

The vastly increasing number of procedures performed led to better outcomes and fewer complications over the years due to more experienced operators, better patient selection, and better technology (42, 43). Hence the notion that women will benefit from these advances and have better or equal outcomes than men, unlike their worse outcomes compared to men with SAVR (30, 44).

The "sex paradox" describes the discordance between the higher rates of short-term mortality and complications in women compared to better long-term survival (13, 22, 45–48). Fewer baseline comorbidities could explain this paradox in women who appear to start the process in better general health (13, 23), which might be the explanation for findings in previous papers suggesting lower long-term mortality in women. They are healthier, and thus, if they do not suffer short-term complications, they live longer (4, 29). As reproduced in this study, for various reasons, women suffer more periprocedural complications (23), primarily vascular and bleeding (49). Perhaps this is not a paradox at all but a manifestation of women's higher rates of periprocedural complications. Female patients were found to have smaller anatomy of the atrioventricular area and smaller annular diameters (50). Women also have significantly smaller vascular anatomy, which could be associated with higher rates of vascular complications

TABLE 4 Multivariable analysis for predictors of TAVR mortality from 2011 to 2017 by sex.

	Male		Female	
Predictor	**Odds ratio (95% CI)**	***P*-Value**	**Odds ratio (95% CI)**	***P*-Value**
Comorbidities				
Hypertension		<0.001		<0.001
No	1.00 (reference)	N/A	1.00 (reference)	N/A
Yes	0.53 (0.47, 0.59)	<0.001	0.46 (0.42, 0.52)	<0.001
Hyperlipidemia		<0.001		<0.001
No	1.00 (reference)	N/A	1.00 (reference)	N/A
Yes	0.46 (0.42, 0.51)	<0.001	0.50 (0.45, 0.55)	<0.001
Cerebrovascular disease		<0.001		0.311
No	1.00 (reference)	N/A	1.00 (reference)	N/A
Yes	1.40 (1.18, 1.66)	<0.001	0.91 (0.76, 1.09)	0.311
Congestive heart failure		<0.001		0.651
No	1.00 (reference)	N/A	1.00 (reference)	N/A
Yes	1.25 (1.11, 1.41)	<0.001	0.97 (0.86, 1.10)	0.651
Diabetes mellitus		<0.001		<0.001
No	1.00 (reference)	N/A	1.00 (reference)	N/A
Yes	0.77 (0.69, 0.86)	<0.001	0.66 (0.59, 0.74)	<0.001
Renal failure		<0.001		<0.001
No	1.00 (reference)	N/A	1.00 (reference)	N/A
Yes	1.55 (1.40, 1.71)	<0.001	1.40 (1.27, 1.54)	<0.001
Chronic pulmonary disease		<0.001		0.224
No	1.00 (reference)	N/A	1.00 (reference)	N/A
Yes	1.30 (1.18, 1.44)	<0.001	1.06 (0.96, 1.18)	0.224
Peripheral vascular disorders		<0.001		<0.001
No	1.00 (reference)	N/A	1.00 (reference)	N/A
Yes	1.27 (1.14, 1.40)	<0.001	1.33 (1.20, 1.47)	<0.001
Prior CAD/IHD		0.004		0.001
No	1.00 (reference)	N/A	1.00 (reference)	N/A
Yes	0.81 (0.71, 0.94)	0.004	0.82 (0.73, 0.93)	0.001
Prior sternotomy		<0.001		<0.001
No	1.00 (reference)	N/A	1.00 (reference)	N/A
Yes	0.61 (0.55, 0.69)	<0.001	0.60 (0.52, 0.69)	<0.001
Deyo-CCI		<0.001		0.001
0	1.00 (reference)	N/A	1.00 (reference)	N/A
1	0.97 (0.71, 1.33)	0.852	1.16 (0.92, 1.47)	0.198
2 or higher	1.69 (1.31, 2.17)	<0.001	1.37 (1.13, 1.67)	0.002

Deyo-CCI, Deyo-Charlson Comorbidity Index; IHD, ischemic heart disease; TAVR, transcatheter aortic valve replacement; NA, no available.

(51). Data from the TVT registry showed a significantly higher rate of TAVR performed *via* alternative access, 45% in women compared to 35% in men, possibly explaining their higher complication rate (16). Our data show that >93% trans-femoral approach was used in the study cohort, which included the late TAVR era. This could be the explanation for vascular complications reduction in both women and men. Further investigation into this phenomenon is warranted. The increase in the trans-femoral approach in the late TAVR era could be explained by better patient selection and devices and delivery systems that improved significantly and by the learning curve of new technology that entered the market.

The periprocedural complication rate decreased significantly between the early and late periods of this study with 2014 being the cutoff point. This is supported in other studies as well (23, 24, 27, 29). We tried to understand whether this reduction affects the mortality differences between the sex groups. The observation that women are more susceptible to early complications and thus have higher in-hospital mortality persisted throughout the study periods. Despite the decline in mortality in the late study period, the difference remained, with higher mortality in women participants compared to men. More extensive studies focusing on female early mortality in these procedures are crucial for understanding how to improve outcomes in this population.

Our study should be interpreted in the context of several limitations. First, the NIS database is a retrospective administrative database containing discharge-level records and is susceptible to coding errors, and reporting may not be consistent across different institutions. Second, the NIS does not include detailed clinical information and therefore cannot rule out residual confounding of the associations we observed. Additionally, the NIS precluded using follow-up beyond the same index hospitalization. These limitations are counterbalanced by the real-world, nationwide nature of the data, as well as the mitigation of reporting bias introduced by selective publication of results from specialized centers. We used a logistic model for the complications.

In conclusion, while a significant downward trend in complication rates was observed, in-hospital mortality remains higher in female patients. This should be further investigated to understand the mechanism behind this phenomenon to reduce early mortality in this group.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: The data were obtained from the NIS database, the Healthcare Cost and Utilization Project (HCUP), and the Agency for Healthcare Research and Quality (AHRQ). Data from the NIS datasets were de-identified and therefore this study was deemed exempt from institutional review by the Human Research Committee. Requests to access these datasets should be directed to hcup@ahrq.gov.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

GE-G and GR conceived the idea and design of the study and drafted the manuscript. ER and MY drafted the manuscript. SC contributed to the data analysis and interpretation and provided revisions to the manuscript. HW and SR contributed to the data interpretation and provided major revisions to the manuscript. DP provided major revisions to the manuscript. OA is the principal investigator, conceived the idea and design of the study, and provided revisions to the manuscript. GE-G had access to all the study data, takes responsibility for the accuracy of the analysis, has the authority over manuscript preparation, and the decision to submit the manuscript for publication. All authors read and approved the manuscript.

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/fcvm.2022.1013739/full#supplementary-material

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Sex differences in coronary artery bypass grafting-related morbidity and mortality

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Background: Coronary artery bypass grafting (CABG) is associated with both cardiovascular disease (CVD) and non-CVD traits. In addition, women's prognosis after coronary events and revascularizations is worse than in men. As the course of CVD in women differs from that of men, we performed a phenome-wide analysis on the sex differences in CABG -related morbidity and mortality.

Materials and methods: We performed an untargeted analysis on the sex differences in predictors and outcomes of CABG. We studied a sample of 176,680 FinnGen participants, including 5,950 individuals who underwent CABG (4,988 men and 962 women) and were followed between 1998 and 2019. Over 1,100 different traits were analyzed for both sexes and the results were adjusted with age, smoking status and BMI. Cox proportional hazards models with sex-trait interactions were used to estimate the associations between (1) traits and incident CABG; and (2) CABG and incident traits.

Results: In women, CABG was more strongly related to greater increases in risk of diseases such as hypertension, Alzheimer's, aortic aneurysms, gout, and chronic kidney disease compared to risk increases observed in men (all interaction p-values < 0.03). After CABG, men had 2.5-fold (p = 3.1E−15) and women 6.3-fold (p = 9.4E−08) greater risk of cardiac death compared to same-sex individuals who did not undergo CABG (p for interaction 8.2E−4). Moreover, the risk of death in women remained higher even 12 years after CABG, whereas the long-term risk of death in men was not increased, compared to same-sex individuals who did not undergo CABG.

Conclusion: The adverse outcomes after CABG, both quantity and quality, also appear to differ between men and women. In women, CABG is related to greater long-term increases in risk of cardiac death and several other disease

states than in men. Consideration should therefore be given to whether women receive adequate long-term post-operative therapy and follow-up as CABG is not associated with equally improved cardiovascular disease prognosis in women than in men.

KEYWORDS

sex-difference, women, survival, morbidity, coronary artery bypass grafting

Introduction

Outcomes of coronary heart disease (CHD) and coronary artery bypass crafting (CABG) have constantly improved over the past decades (1–3). However, women's prognosis after coronary events and revascularizations, including CABG, still remain markedly impaired compared to that of men (4, 5). The mechanisms explaining this observed sex difference are undoubtedly multifactorial and partially related to older age at the time of surgery and greater comorbidity (6). Especially diabetes and hypertension are more common in women with CHD and are related to increased risk in women compared to men (6–10). Also, women with CHD and acute coronary syndrome often present themselves with atypical symptoms which may lead to a delayed diagnosis and treatment, resulting in worse outcomes (11, 12).

The obvious benefits of CABG are significantly improved quality of life (13) and decreased mortality (4, 14, 15), but conversely, the procedure exposes the patient to several other late post-operative comorbidities and even unexpected conditions such as depression (16). Most previous studies have assessed the impact of CABG on cardiovascular disease (CVD) morbidity (17–19), but CABG is also found to associate with several non-CVD traits such as anemia, gastrointestinal traits, septicemia, lung cancer, Alzheimer's disease and chronic obstructive pulmonary disease (20), which may be reflective of the shared comorbidities between these diseases and CHD. Despite the known sex-differences in the course of CHD, the definite reason for the poorer survival after coronary procedures in women remains unclear, highlighting the urgent need for further studies.

Some sex differences in the CABG-related morbidity are known to exist (5). To our knowledge, however, no phenome-wide untargeted analysis of the sex differences in traits that are associated with future and prior CABG, including non-CVD traits, has been performed. Thus, we investigated the sex-dependent differences in CABG correlates, which could further explain the observed sex differences in CABG morbidity and mortality. To address the question, we considered health data of $>300{,}000$ participants of the FinnGen study and then performed a systematic analysis of $>1{,}100$ CABG predictors and outcomes in men and women.

Materials and methods

Study sample

The original study sample comprised 309,154 individuals from the FinnGen Data Freeze 7 which included Finnish participants from national hospital biobanks, prospective epidemiologic studies, and disease-based cohorts. All participants in the FinnGen study were linked to data from the nationwide National Hospital Discharge, Cause of Death, Cancer, and Medication Reimbursement registers by using personal identification codes.

From the original study sample 132,474 individuals with missing BMI and smoking data were excluded from the study and thus the final study sample consisted of 176,680 individuals (82,794 men and 91,568 women). Of these participants, 5,950 (4,988 men and 962 women) individuals underwent CABG surgery during follow-up. All study participants provided an informed written consent before their participation in the FinnGen study. This study protocol was approved by The Coordinating Ethical Committee of the Hospital District of Helsinki and Uusimaa, as described in the **Supplementary material**.

Register-based traits and follow-up

The analysis period extended from January 1, 1998, to December 31, 2019. The predictor- and outcome-traits were defined by ICD and ATC codes as described in the **Supplementary Table 1**. In total, 4,182 different traits with incidence $n \geq 1$ were defined in the FinnGen study and thus formed 8,364 pairs of events (traits preceding CABG and CABG preceding traits). However, event pairs with any subgroup size of less than 10 individuals were excluded from the study to comply with the privacy protocol of the FinnGen study.

Statistical analysis

We used a case-cohort design with Cox's regression models to study the associations between different traits and CABG

in men and in women separately (21). The cohort size was 10,000. Cox's regression models with sex-trait interaction term were performed separately for (1) each predictor trait and CABG and (2) for each CABG and outcome trait. We adjusted *p*-values for multiple testing and false discovery rate (FDR) using Benjamini–Hochberg correction (22). Event pairs that had a significant interaction term at FDR-corrected $P < 0.05$ were selected for sex-stratified analyses. All Cox regression models were adjusted for those known CHD-risk factors that were available in FinnGen: sex, birth year, smoking status, and body-mass-index (BMI). BMI was defined as weight (kg)/height^2 (m^2). Height and weight for BMI calculation were measured by nurses and smoking status was determined by self-report. We excluded from the analyses individuals that had outcome before baseline or had outcome event before the predictor event. We ignored the time for predictor events before the start of the study (if the predictor preceded the study start).

From sex-stratified Cox regression models a ratio of hazard ratios (rHR = HR women/HR men) was calculated for each predictor-CABG pairs and CABG-outcome pairs. For the main results reported in **Tables 1**, **2**, we applied a clinical significance limit of rHR > 1.5 or rHR < 0.66 with $n > 200$ and associations outside these thresholds limits were only reported in the **Supplementary Table 1**. From the remaining traits, we further excluded similar and overlapping traits using most significant interaction *p*-value as the rule-in criteria.

To further investigate the impact of sex on all-cause of mortality, we used a case-control approach for men and women who underwent CABG. Each case (individuals who underwent CABG) was then matched with a control (individuals who did not undergo CABG) 1:1 for sex, birth year, BMI and smoking status using nearest neighbor matching with propensity score as distance. This resulted 999 cases and 999 controls for females and 5,222 cases and 5,222 controls for males. For cases, age of the first CABG was used as index age, while for controls index age was either age at year 1998 or 0, if birth year was after year 1998. An individual was included only if the end of follow up was after the index age. Using this dataset, we created Kaplan Meier curves (**Figure 1**) and logistic regression models (**Figure 2**) for cases and controls. Logistic regression models were performed separately for both sexes and for different follow up lengths.

Python 3.7.3. (lifelines library, Python Software Foundation, Beaverton, OR) and R 4.0.4 (The R Foundation, Vienna, Austria) was used for the statistical analyses.

Results

Patient characteristics

We studied 176,680 individuals (82,794 men and 91,568 women) from FinnGen database Data Freeze 7. The mean birth year of the sample was 1957.6 ± 18.4, 52.5% were women, 25.6% were active smokers, and the mean BMI was 27.4 ± 5.3 kg/m^2. Mean BMI was 27.3 ± 5.8 kg/m^2 in women, and 27.5 ± 4.6 kg/m in men, respectively. In total, 27,196 (32.8%) of men and 17,454 (19.1%) of women were past or current smokers. A total of 5,950 (4,988 men and 962 women) individuals underwent CABG surgery during follow-up period. The mean age at the time of the procedure was 64.7 ± 9.4 years and median follow-up time for CABG was 9.3 ± 6.1 years. From all potential 8,364 event pairs, we performed 1,192 analyses where trait preceded CABG procedure and 1,423 analyses where CABG procedure preceded trait (**Supplementary Table 1**). The ICD-codes for the observed predictor- and outcome traits are illustrated in more detail in **Supplementary Table 2**.

Sex-differences in coronary artery bypass grafting-related morbidity

We observed five traits that were more strongly associated with future CABG in women than in men (**Table 1**). These traits were type 1 diabetes, thoracic aortic aneurysms, other heart diseases, other chronic obstructive pulmonary disease, diseases of the ear and mastoid process and disorders of choroid and retina.

We observed several sex differences in the associations between CABG and incident traits (**Table 2**). In general, the associations between CABG and incident traits (hypertension, cardiac death, Alzheimer's, retinal disorders, thoracic aortic aneurysm, gout, chronic kidney disease, and fatigue) were greater in women than in men. CABG was only related to lower risk of knee arthrosis in women than in men.

Sex differences in risk of death

The risk of cardiac death after CABG was 2.5- and 6.3-fold in men and in women as compared with controls of the same sex, respectively (**Table 2**). In the Kaplan–Meier survival analysis, the risk of short- and long-term death for women after CABG was higher than in the control group of matched women without CABG. In men, the Kaplan–Meier analysis demonstrated an increased cumulative risk for death during the first year after CABG but this difference in mortality was similar to the matched male control group during the rest of the follow up (**Figure 1**).

In the logistic regression analysis, an increased odds for all-cause death were observed in women with CABG compared to matched women during follow-up (**Figure 2**). The odds ratios for death were highest during the first 3 years after surgery and remained higher than in the control group for 12 years (**Figure 1**). In contrast, men after CABG had only modestly increased odds of death during the first year of follow-up, after

TABLE 1 Predictors with sex related risk prior to coronary artery bypass grafting (CABG).

Predictor	Men (n = 4988)			Women (n = 962)			HR-ratio	Interaction q-value
	n	HR	q-value	n	HR	q-value		
Type 1 diabetes	145	3.2	7.0E−09	63	12.8	6.1E−17	4.0	5.1E−07
Thoracic aortic aneurysm	179	2.7	5.0E−07	23	15.6	3.9E−07	5.7	0.002
Other heart diseases	2186	4.8	3.3E−150	539	7.1	2.1E−83	1.5	0.003
Other chronic obstructive pulmonary disease	177	0.9	0.660	29	1.4	0.308	1.5	0.005
Diseases of the ear and mastoid process	442	0.8	0.006	138	1.4	0.023	1.8	0.027
Disorders of choroid and retina	259	1.2	0.102	101	2.4	1.1E−05	1.9	0.049

False discovery rate adjusted interaction q-value < 0.05 was considered statistically significant. 1.5-fold increase or decrease in HR and over 200 incidents in each predictor category was considered clinically significant. CABG, coronary artery bypass crafting; n = number of CABG patients with the predictor, HR ratio = women HR/men HR.

TABLE 2 Association of CABG and incident outcomes by sex.

Outcome	Men (n = 4988)			Women (n = 962)			HR-ratio	Interaction q-value
	n	HR	q-value	n	HR	q-value		
Hypertensive diseases (excluding secondary)	918	1.0	0.87	176	1.6	0.15	1.5	2.8E−05
Death due to cardiac causes	1471	2.5	3.1E−15	252	6.3	9.4E−08	2.5	8.2E−04
Alzheimer's disease	347	1.6	0.006	82	4.2	7.3E−06	2.7	0.006
Other retinal disorders	381	1.3	0.022	112	4.1	3.7E−07	3.0	0.010
Thoracic aortic aneurysm	358	2.1	2.7E−09	34	6.1	3.8E−09	2.9	0.011
Metabolic disorders	1207	3.0	2.8E−16	274	7.5	9.6E−12	2.6	0.020
Gout	296	2.5	9.6E−11	35	5.8	1.3E−05	2.3	0.024
Gonarthrosis, primary, with knee surgery	182	1.1	0.47	40	0.5	0.04	0.5	0.027
Chronic kidney disease	405	2.7	3.3E−13	68	5.5	2.3E−06	2.0	0.032
Malaise and fatigue	424	2.3	9.7E−10	130	9.4	3.8E−17	4.0	0.039

False discovery rate adjusted interaction p-value < 0.05 was considered statistically significant. 1.5-fold increase or decrease in HR and over 200 incidents in each outcome category was considered clinically significant. CABG, coronary artery bypass crafting; n = number of CABG patients with the outcome, HR-ratio = women HR/men HR.

which the odds of death were lower than in the control group of matched men.

Discussion

In a systematic analysis of 5,950 CABG patients and over 1,100 different traits from the FinnGen study, we evaluated the sex-dependent differences in mortality and morbidity before and after CABG. In women, CABG was more strongly related to greater increases in risk of diseases such as hypertension, Alzheimer's, aortic aneurysms, gout, and chronic kidney disease compared to risk increases observed in men. We observed that the risk for cardiac death was significantly higher in women after CABG compared to women who did not undergo CABG. The risk of cardiac death for men who underwent CABG was also higher than in the matched control population without CABG. However, the combined acute and long-term risk of death was 2.5-fold greater in women than in men, when compared to non-operated counterparts of the same sex.

Female sex has previously been associated with increased cardiovascular mortality and morbidity after CABG (4, 5, 23). Recent meta-analysis on sex differences in CABG outcomes from 2021 reported female sex to associate with higher risk of operative (odds ratio 1.77) and late mortality (incidence rate ratio, IRR 1.16) (5). Also, same study reported higher risk for major adverse cardiac event (IRR 1.40), myocardial infarction (IRR 1.28) and stroke (IRR 1.31) for women after CABG compared to men (5). Indeed, several previous studies have assessed the post-operative risk in women in relation to men. In our study, we assessed the association of CABG with mortality and morbidity separately for both sexes, with age-, BMI- and smoking status -matched controls. We observed that after CABG, men have a moderately elevated risk of death as compared to other men, whereas in women this risk is markedly higher (**Figures 1**, **2**).

Sex differences in mortality after CABG are thought to be explained by a greater comorbidity burden in women. Both type 1 and 2 diabetes are associated with excess CVD risk in women as compared to men (10). In this study, we observed a statistically significant sex interaction with type 1 diabetes for future CABG (HR for men 3.2; HR for women 12.8; **Table 1**). Disorders of choroid and retina, which include diabetic retinopathy, were also more strongly associated with future

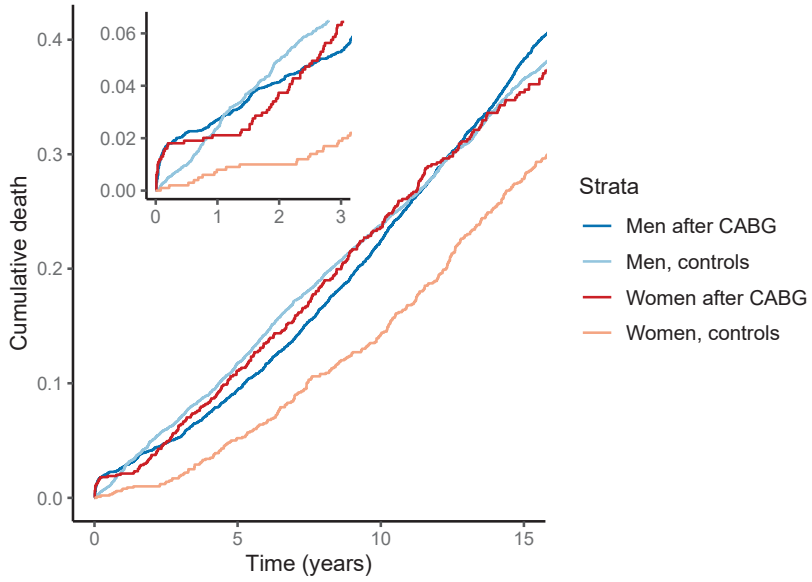

FIGURE 1
Cumulative death by sex after coronary artery bypass grafting (CABG). The survival curves are from Kaplan-Meier estimator. We matched the models for age, BMI and smoking status. The risk for death is elevated in women after CABG compared to matched women. In contrast, after 1 year men with CABG had similar risk of death than the matched men in the control group.

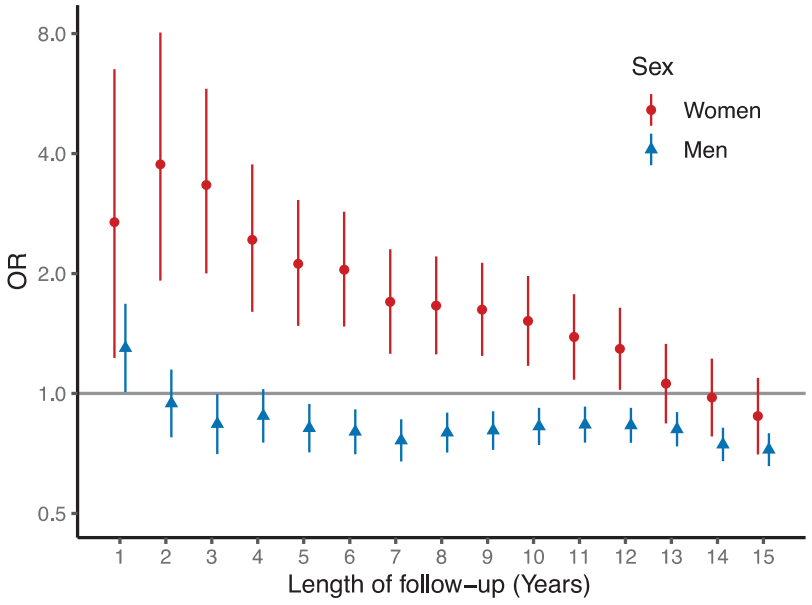

FIGURE 2
Odds ratios for death by sex after coronary artery bypass grafting (CABG). Logistic regression models are matched and adjusted for age, BMI and smoking status. Women had increased OR for any death for 12 years after CABG compared to matched and risk adjusted women. In contrast, men had similar or moderately reduced risk for any death than the men in the matched and risk adjusted control group.

CABG in women than in men. Furthermore, hypertension was observed to have sex-dependent interaction after CABG with 1.5-fold risk in women compared to men. The other traditional CVD risk factors, apart from hypertension, carried a similar risk for future CABG in both sexes. These findings therefore highlight the need for adequate control of diabetes and hypertension particularly in women to prevent future CABG.

Thoracic aortic aneurysms were observed to associate strongly with CABG in women both before and after CABG (**Table 2**). Male sex, old age, aortic atherosclerosis,

hypercholesterolemia, genetic predisposition, smoking, and hypertension are risk factors that are known to associate with aortic aneurysm formation (24). However, CABG-operated women had a higher aortic aneurysm risk before and after CABG than in women without CABG. In men, however, these risks were much more modest. These findings suggest that aneurysm risk factors seem to potentiate more strongly in CABG-operated women than in men.

The similar results observed for COPD further emphasize the importance of smoking as a strong CABG risk factor in women (10). In this study, COPD was more strongly associated with future CABG in women than in men. It its known that COPD is a CVD risk factor due to common risk factors, such as smoking. Women with COPD have less cardiovascular comorbidities than men but conversely a more rapid COPD progression (25). Smoking is also a stronger risk factor for MI in women than in men (26). Thus, the observed sex differences in the COPD-CABG associations may be a result of their shared risk factors, thus highlighting the importance of smoking cessation in women undergoing revascularization (27).

Disorders of the choroid and retina were more strongly related to risk of CABG in women than in men. This relation was similar when CABG was the predictor variable. This miscellaneous category included phenotypes, such as macular degeneration, retinal breaks, diabetic retinopathy, maculopathy, and preretinal fibrosis, which all share risk factors with CVD. Smoking and age are risk factors for macular degeneration in both women and men, but obesity, hypertension, and low physical activity are associated with increased risk of macular degeneration only in women (28). Diabetic retinopathy is the most common microvascular complication of diabetes and is more common in men (29). However, as diabetes is a more potent risk factor for death and cardiovascular disease in women (30), the observed association between retinal disorders and future CABG is likely to represent the effects of diabetes and smoking.

We also observed that diseases of ear and mastoid process were associated with future CABG particularly in women. This phenotype consists of several miscellaneous diseases such as sensorineural hearing loss, benign paroxysmal vertigo, and infections of the outer and middle ear. Hearing loss is known to associate with atherosclerosis and cardiovascular disease with a possible underlying mechanism of microvascular impairment (31, 32). This association is stronger in women than in men (31). Therefore, the increased risk for CABG in women with sensorineural hearing loss may be considered a surrogate of CVD, particularly with concomitant retinopathy.

CABG was also associated with several other incident traits after the operation. For instance, CABG was a stronger risk factor for Alzheimer's disease in women than in men. This is could be explained by the common risk factor profiles associated with CVD and Alzheimer's, including hypertension, diabetes and obesity (27). Another possibility could be that women are more susceptible to cognitive impairment caused by the operation and the anesthesia themselves.

Coronary artery bypass grafting was found to associate with incident gout more strongly in women than in men (**Table 2**). Hyperuricemia, an increase of uric acid in circulation, is required for gout, but hyperuricemia also independently associates with coronary artery disease and other CVD, especially in elderly women (33). However, results have been conflicting as other studies consider hyperuricemia as a risk factor while other studies have not found any association with these two conditions (33). Our finding could be explained by sex differences in medications, such as diuretics, and comorbidities.

Coronary artery bypass grafting in women was associated with incident CKD and the risk was 2.0-fold compared to men. Acute kidney injury is common immediately after CABG and also a risk factor for progression to later CKD and increases morbidity and CVD mortality (34). This sex-dependent observation may be one factor in explaining the worse outcomes of women after CABG. The reasons underlying this finding remain unclear and warrant further research.

History of CABG in women was more strongly associated with incident metabolic disorders, comprised of hypercholesterolemia, hyponatremia or hypokalemia, in women than in men. As expected, CABG was associated with increased risk of hypercholesterolemia after CABG. However, this risk was greater in women than in men. This association most likely reflects that women are more susceptible of post-operative electrolyte disorders and less aggressive lipid-lowering therapy.

Further, CABG was related to a reduced risk of knee replacement surgery in women compared with men. The research on the relation of osteoarthrosis and CVD has provided conflicting results. Some publications have suggested that individuals with osteoarthrosis, and particularly women, have an increased CVD risk (35–37), whereas others studies have not found an association between CVD and knee osteoarthritis (38). The possible mechanisms behind the link between osteoarthritis and CVD could be related to shared causal factors, such as vascular inflammation and microvascular changes (39). However, the causes underlying the observed sex differences warrant further study, although is possible that women with a history of CABG are less often referred to surgical interventions than men.

Finally, the phenotype other heart diseases was observed to associate more robustly with future CABG in women than in men. This phenotype includes various disease entities such as heart and valve infections, cardiomyopathies, conduction disorders, arrythmias, and various types of heart failure. This heterogeneity of the diagnoses renders further interpretation of the observed association challenging. Similarly, the phenotype malaise and fatigue, with an HR of 9.4 in women after CABG, is a very unspecific diagnosis, but this finding may represent poorer control of symptoms in women.

A limitation and strength of this untargeted epidemiological study is the large number of different traits available for statistical analysis. Despite adjustments for age, BMI and smoking status, the unavailability of exact measurements of lipid-, glucose- and blood pressure levels prevents a more detailed assessment of individual risk profiles. Also, detailed information on ejection fraction, number of coronary bypasses, and the acuity of surgery were not available in this register-based study. Furthermore, as in other studies, the number of women who underwent CABG was low compared to that of men. To address these potential biases, we adjusted for multiple testing and used clinically significant thresholds of HR $>$ 1.5 or HR $<$ 0.66 for the main results. Moreover, as statistical associations do not indicate causality, more research is needed in order to discover the mechanisms of the observed connections to improve the outcomes in women with CHD.

Conclusion

Women are at higher risk for diseases after CABG, which further increase the risk of death. After CABG, the relative risk of long-term death in women is significantly higher than in men. Consideration should be given to whether women receive adequate treatment post-CABG, as CABG does not improve life expectancy in women as much as in men.

Data availability statement

The FinnGen data may be accessed through Finnish Biobank's Finngenious portal at www.fingenious.fi and at www.FinnGen.fi/en.

Ethics statement

Patients and control subjects in FinnGen provided informed consent for biobank research, based on the Finnish Biobank Act. Alternatively, separate research cohorts, collected prior the Finnish Biobank Act came into effect (in September 2013) and start of FinnGen (August 2017), were collected based on study-specific consents and later transferred to the Finnish biobanks after approval by Fimea (Finnish Medicines Agency), the National Supervisory Authority for Welfare and Health. Recruitment protocols followed the biobank protocols approved by Fimea. The Coordinating Ethics Committee of the Hospital District of Helsinki and Uusimaa (HUS) statement number for the FinnGen study is Nr HUS/990/2017. The FinnGen study was approved by Finnish Institute for Health and Welfare (permit numbers: THL/2031/6.02.00/2017, THL/1101/5.05.00/2017, THL/341/6.02.00/2018, THL/2222/6.02.00/2018, THL/283/6.02.00/2019, THL/1721/5.05.00/2019, and THL/1524/5.05.00/2020), Digital and population data service agency (permit numbers: VRK43431/2017-3, VRK/6909/2018-3, and VRK/4415/2019-3), The Social Insurance Institution (permit numbers: KELA 58/522/2017, KELA 131/522/2018, KELA 70/522/2019, KELA 98/522/2019, KELA 134/522/2019, KELA 138/522/2019, KELA 2/522/2020, and KELA 16/522/2020), Findata (permit numbers: THL/2364/14.02/2020, THL4432/14.06/2020, THL6619/14.06.00/2020, THL/1284/1406.00/2021, THL/4055/14.06.00/2020, THL/3433/14.06.00/2020, THL/5189/14.06/2020, THL/5894/14.06.00/2020, THL/209/14.06.00/2021, THL/688/14.06. 00/2021, THL/1965/14.06.00/2021, and THL/5546/14.02.00/2020), Statistics Finland [permit numbers: TK-53-1041-17 and TK/143/07.03.00/2020 (earlier TK-53-90-20)]. The Biobank Access Decisions for FinnGen samples and data utilized in FinnGen Data Freeze 7 include: THL Biobank BB2017_55, BB2017_111, BB2018_19, BB_2018_34, BB_2018_67, BB2018_71, BB2019_7, BB2019_8, BB2019_26, BB2020_1, Finnish Red Cross Blood Service Biobank 7.12.2017, Helsinki Biobank HUS/359/2017, Auria Biobank AB17-5154 and amendment #1 (August 17 2020), Biobank Borealis of Northern Finland_2017_1013, Biobank of Eastern Finland 1186/2018 and amendment 22 §/2020, Finnish Clinical Biobank Tampere MH0004 and amendments (21.02.2020 & 06.10.2020), Central Finland Biobank 1-2017, and Terveystalo Biobank STB 2018001. The patients/participants provided their written informed consent to participate in this study.

Author contributions

JN wrote the original draft and revisions. AK and JP analyzed and interpreted the patient data. JA and TN planned, reviewed, and edited the manuscript. 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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.1021363/full#supplementary-material

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Sex differences in long QT syndrome

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Long QT Syndrome (LQTS) is a rare, inherited channelopathy characterized by cardiac repolarization dysfunction, leading to a prolonged rate-corrected QT interval in patients who are at risk for malignant ventricular tachyarrhythmias, syncope, and even sudden cardiac death. A complex genetic origin, variable expressivity as well as incomplete penetrance make the diagnosis a clinical challenge. In the last 10 years, there has been a continuous improvement in diagnostic and personalized treatment options. Therefore, several factors such as sex, age diagnosis, QTc interval, and genetic background may contribute to risk stratification of patients, but it still currently remains as a main challenge in LQTS. It is widely accepted that sex is a risk factor itself for some arrhythmias. Female sex has been suggested as a risk factor in the development of malignant arrhythmias associated with LQTS. The existing differences between the sexes are only manifested after puberty, being the hormones the main inducers of arrhythmias. Despite the increased risk in females, no more than 10% of the available publications on LQTS include sex-related data concerning the risk of malignant arrhythmias in females. Therein, the relevance of our review data update concerning women and LQTS.

KEYWORDS
long QT syndrome, gender, arrhythmias, sudden cardiac death, woman

1. Introduction

Congenital long QT syndrome (LQTS) is a cardiac channelopathy, characterized by ventricular repolarization and polymorphic ventricular tachycardia (*torsades de pointes*, TdP), leading to malignant arrhythmias, syncope and sudden cardiac death (SCD) at a young age. It is clinically recognized by a prolonged QT interval in the surface electrocardiogram (ECG). LQTS is one of the most common inherited arrhythmia conditions (1:2000/1:2500). LQTS is caused by rare genetic alterations in cardiac ion channels or accessory ion channel subunits, mainly following an autosomal-dominant pattern of inheritance. To date, there are 17 genes potentially associated with LQTS, but definite deleterious alterations have been identified in three genes (*KCNQ1*, *KCNH2* and *SCN5A*) that account for about 90% of all LQTS cases (1). Due to the potential risk of malignant arrhythmias, it is crucial to accurately identify and manage patients. Continuous advances in diagnosis and personalized treatment, as well as prevention, have been achieved in the last few years. However, risk stratification remains the main,

challenge in clinical practice at present. Variable expressivity and incomplete penetrance are hallmarks of LQTS, impeding a conclusive risk stratification. Currently, the existence of differences in sex in LQTS is widely accepted, showing females with an increased risk of developing polymorphic ventricular arrhythmia or SCD than men after the onset of adolescence (2). In addition, women experience a decreased risk during pregnancy, but increased in postpartum period and perimenopause. These differences exist due to hormone levels, which vary depending on the menstrual cycle, gestation, and the postnatal period. Despite being widely accepted as definite risk factor, no more than 10% of studies focused on the pathophysiological mechanism occurring in females suffering from LQTS have been published to date (**Figure 1**). Herein, we will review evidence from basic and clinical studies involving female-susceptibility to LQTS. A better understanding of the role of sex-related differences in LQTS will lead to improvement in risk stratification.

2. Clinical findings

Congenital LQTS is a primary cardiac electric disease characterized by the prolongation of the QT interval, usually associated with T-wave abnormalities. To make a definite diagnosis, it is essential to exclude secondary causes of the prolonged QT interval, such as QT-prolonging drugs or electrolyte imbalances (3). High risk of life-threatening arrhythmias includes T wave alternans and functional 2:1 atrioventricular block in the ECG. In addition to the baseline ECG, QTc behavior can also be assessed during stress testing and 24 h Holter recording, preferably 12-lead. Therefore, the "*Schwartz score*" was developed as a diagnostic criterion to support the diagnosis of the disease if a score of >=3,5 is attained. Adrenaline testing, supine-to-standing ECG or mental stress tests are of lower diagnosis yield (4). It is widely accepted that QT intervals are generally longer in healthy women; therefore, sex-specific cut-off values for prolonged QTc should be applied in order to accurately diagnose LQTS −470 ms in men and 480 ms in women- (5). In addition, women are more susceptible of developing a QT prolongation at slower heart rates, making QTc duration at rest and during sleep critical markers of arrhythmic risk (6). Therefore, adult women with LQTS are at higher risk of malignant arrhythmias due to the influence of a hormone, requiring pharmacological therapy (7). Taking into account all these points, a novel risk score for patients with long QT syndrome has been developed, offering accurate prognostic information to guide clinicians in identifying the patients at the highest risk of life-threatening arrhythmias (8).

The use of β-blockers (preferably nadolol) is the most effective therapy in both sexes (especially in LQTS type 1 and 2), despite the fact that response varies by sex and underlying genotype. Patients with LQTS type3 may benefit from mexiletine or even flecainide; left cardiac sympathetic denervation may be also offered despite rarely and only for special cases. Finally, a reduced number of LQTS patients are suitable for ICD, such as primarily survivors of cardiac arrest and patients at a high-risk for SCD, with recurrent syncope despite adequate pharmacological therapy.

3. Genetic and cellular basis

The normal QT interval in the ECG represents the time from the beginning to the end of ventricular depolarization. LQTS is characterized by a prolonged QT interval due to cardiac repolarization dysfunction leading to risk for ventricular tachyarrhythmias, syncope, and even SCD (3). Indeed, QT prolongation is related to a combination of modifiable and

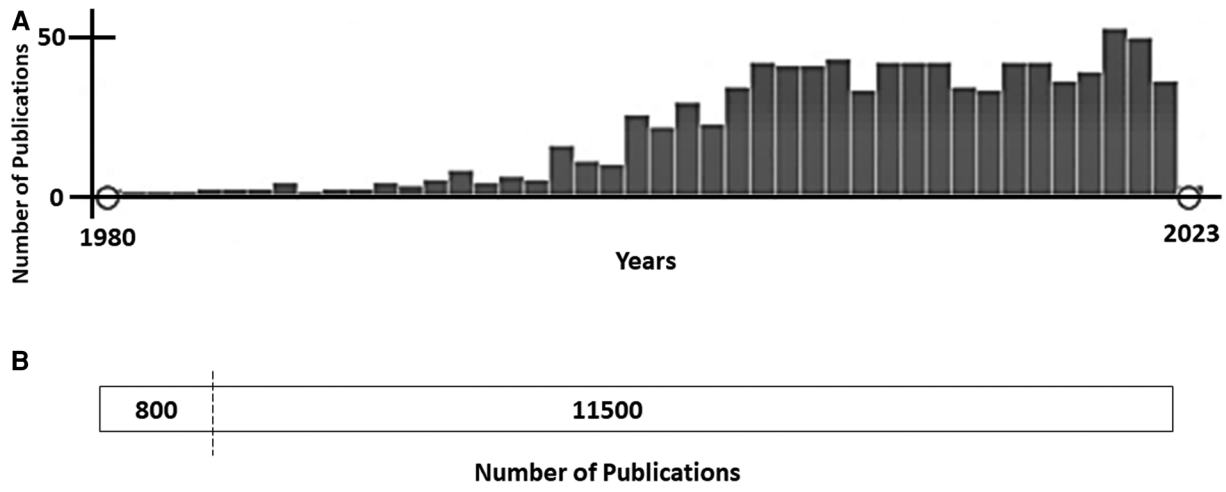

FIGURE 1
(A)- publications focused on long QT syndrome (pubMed, January 2023). From approximately 11,500 publications about Long QT Syndrome, less than 10% (800 publications) included any data concerning females/women or gender/sex differences. (B)- Time-line of publications focused on Long QT Syndrome and sex differences (PubMed, January 2023). Since 2005, the number of publications including any data concerning gender/sex differences has been maintained at nearly 45–50 studies per year.

unmodifiable risk factors. Electrolyte anomalies are the most common risk factors associated with prolonged QT. Among those, hypokalemia has the main arrhythmogenic effect, as well as hypocalcemia and hypomagnesemia. They may be responsible for prolonging the QT interval, but may also be a major risk factor for drug-induced LQTS, one of the most frequent reasons for QT prolongation (9). Genetic background, as well as older age and female sex, are the most important unmodifiable risk factors. Focused on genetics, rare deleterious alterations located in genes encoding ion channels or associated proteins have been reported as a cause of LQTS. Diagnosed families follow an autosomal dominant pattern of inheritance, with characteristic incomplete penetrance and variable expressivity. Nowadays hundreds of rare alterations have been reported in more than 15 genes (10), despite only a limited number of variants that are definitively classified as deleterious following current ACMG recommendations (11). These causative variants are located mainly in three genes (*KCNQ1, KCNH2,* and *SCN5A*), and are responsible up to 75% of all patients with LQTS (3, 10). Loss-of-function deleterious variants in the potassium channels *KCNQ1* and *KCNH2* are responsible for LQT1 and LQTS2, respectively. They cause decreasing activity of the slow delayed rectifier current (IKs) and rapid delayed rectifier current (IKr) (phase 3 of the action potential), respectively. In contrast, gain-of-function deleterious variants in the *SCN5A* gene (sodium channel, phase 0 of an action potential) are responsible for LQTS3. They cause persistent sodium influx that extends through the plateau phase. A loss of IKs or IKr function, or gain of INa function predisposes ventricular myocytes to early afterdepolarizations, then triggering malignant arrhythmias. Other rare alterations have been reported in minor genes, accounting for 5% of LQTS (*CACNA1C, CALM1, CALM2, CALM3,* and *TRDN*), whereas about 20% of all diagnosed patients do not have an identifiable deleterious variant in any of the current known genes related to LQTS (3). We must also remark that co-inheritance of a second rare variant that affects ventricular repolarization is described as a "second hit". These variants are classified as deleterious in the same gene (compound heterozygosity) or in a different gene (digenic heterozygosity). Compound deleterious variants are present in 5%–10% of LQTS patients and it is well-accepted as cause of a more severe phenotype (12). Concerning genetic risk after the onset of adolescence, women with pathogenic variants in *KCNQ1* (LQTS1) and *KCNH2* (LQTS2) are at increased risk of malignant arrhythmias, especially LQT2 due to a pore loop pathogenic variant (13) (**Figure 2**).

4. Pregnancy, post-partum and perimenopausal periods

It is widely accepted that there exists a slight female phenotypic predominance in LQTS, with the female sex being a risk factor for malignant arrhythmias. This increased risk occurs after adolescence due to sex hormone differences, with testosterone being a main cause of QT-interval duration in men (2). Low risk of arrhythmias is widely accepted during pregnancy but this risk increases during postpartum, menopausal, and perimenopausal periods (**Figure 2**). The complex interaction of sex hormones and cardiac ion currents/action potential is widely accepted but it still remains to be clarified (14). What is clear is that sex differences in the electrical substrate are not the result of a simple change in the expression of a single or even a few ionic

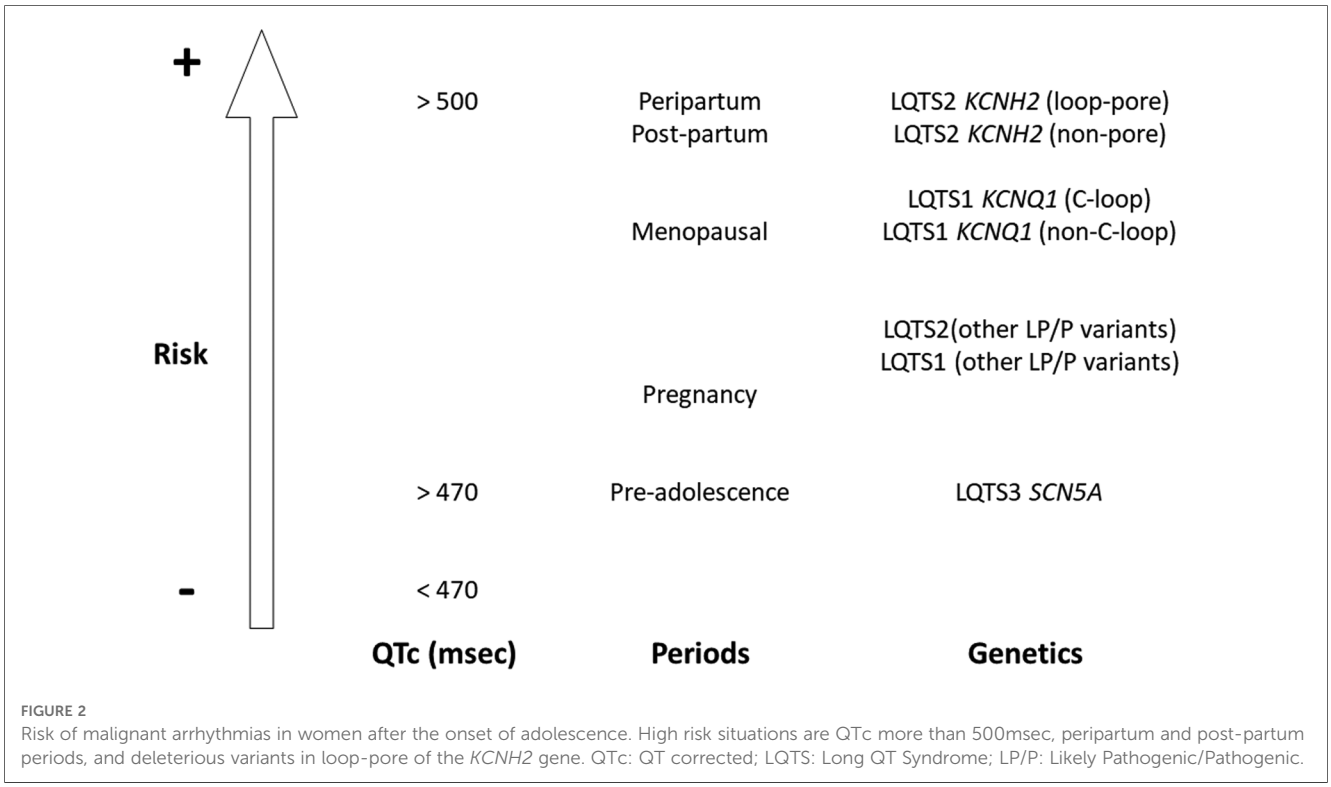

FIGURE 2
Risk of malignant arrhythmias in women after the onset of adolescence. High risk situations are QTc more than 500msec, peripartum and post-partum periods, and deleterious variants in loop-pore of the *KCNH2* gene. QTc: QT corrected; LQTS: Long QT Syndrome; LP/P: Likely Pathogenic/Pathogenic.

currents (7). As a preventive measure, a multidisciplinary approach to women with LQTS ensures comprehensive risk assessment and optimal patient care.

4.1. Pregnancy

Fluctuations in sex hormone levels during pregnancy could potentially provoke cardiac events. In addition to sex hormone levels, other internal or external factors may alter cardiac electrophysiology during pregnancy and postpartum (alterations in adrenergic activity, disrupted sleep pattern), that can contribute to changes in arrhythmic risk (14). It is widely reported that pregnancy decreases risk of malignant arrhythmias in females diagnosed with LQTS, especially in LQTS type 1 (15). The choice of b-blockers has not been established due to the limited evidence available nowadays. One thing that is for certain is that the use of b-blockers such as propranolol is effective in reducing the risk of arrhythmic events (15, 16). However, lower fetal birth weight has been reported, leading to non-selective b-blocker use, mainly metoprolol (17). Because of the risk in mothers with LQTS for stillbirth and the higher risk of miscarriages, a more stringent follow-up of these patients during pregnancy might be necessary. Finally, concerning contraceptives, no conclusive studies have been published to date, with the risk of arrhythmias remaining unknown (18).

4.2. Postpartum

Concerning post-partum follow-up, women with LQTS, especially LQTS type 2, show a higher risk of VT and SCD in comparison to the relatively low risk during pregnancy. After postpartum, the risk of cardiac events returns to basal levels before pregnancy. However, there are no general recommendations and approved schemes on how women with LQTS should be supervised after delivery. Limited data suggest increased risk in the early post-partum period, particularly in patients with LQTS2 (15). In addition, the use of b-blockers is mostly well tolerated during the postpartum period to prevent life-threatening manifestations. It is also important to note that b-blockers are secreted in breast milk, but hypoglycemia and bradycardia may occur in breastfed infants, albeit rarely (19). Therefore, dose adjustment of beta-blockers may be needed and postpartum care remains the same as in routine cases (20).

4.3. Menopausal and perimenopausal periods

Nowadays, only one study has been published focusing on females after the onset of menopause who have been diagnosed with LQTS. Available data suggest a higher risk of malignant arrhythmias in LQTS-type2 women (21). In contrast, there are studies in post-menopausal period showing that estrogen replacement therapy (ERT) prolongs the QTc interval more, compared to those postmenopausal women taking no hormones or taking combined estrogen-progestin replacement therapies (22, 23). It suggests that progesterone has a similar protective effect to testosterone (24).

5. Role of hormones in arrhythmogenic risk

The underlying molecular mechanisms that cause patients with LQTS to have sex-dependent variability in arrhythmogenic risk remain to be elucidated. However, several studies suggest that it results from the effect of certain hormones on cardiac ion channels. Animal experiments have shown that estradiol could act as a pro-arrhythmic agent in LQTS2, whereas progesterone would have a protective role (25). This effect of estradiol is attributed to its interaction with certain potassium ion currents (26), and due to the effect on the transcription of some genes like *KCNE2* and *RyR2*, being mediated by an estrogen receptor-dependent process (27, 28). However, such effects have not been supported in human studies, in which a more complex interaction between estrogen and progesterone appears to exist (29). For example, a recent study in women with LQTS found an inverse relationship of RR interval with estradiol levels during the menstrual cycle (30). In addition, progesterone was found to show an inverse association with the corrected QT interval and with the ratio of progesterone to estradiol in women with LQTS2. This shortening of the QT interval was observed during the luteal phase, and was mainly attributed to increased progesterone levels in this phase (30). Such associations were not maintained in women with LQTS1, supporting that the observed differences in LQTS subtypes are due to different effects of hormones on the channels, with different sensitivity between genotypes. Likewise, it has been shown that progesterone may have anti-arrhythmic effects, which may help to reduce the risk of arrhythmias (31). These effects of progesterone on the QT interval are related to an increase in intracellular calcium reuptake from the sarcoplasmic reticulum mediated by SERCA2a (32). Similar to progesterone, testosterone decreases I_{CaL} current and increases potassium channel currents (I_{Kr}, I_{Ks}, I_{K1}), reducing the QTc interval in animals and humans (33–35).

During pregnancy, the combination of hormones is complex. Apart from the interaction of estrogen and progesterone, oxytocin released towards the end of pregnancy, during labor and lactation, would have direct effects on sodium channels and cause a direct acute inhibition on the potassium I_{Ks} channel (36), which could explain the increased risk in post-partum women with LQTS2. Data on the potential impact of prolactin on cardiac electrophysiology are limited, but some animal studies suggest that it would act similarly to oxytocin (36).

6. Conclusions

LQTS is a rare heterogenous group of arrhythmogenic entities, characterized by a prolonged QT interval. In recent years, a

continuous improvement in diagnosis as well as genetic/pathophysiological mechanism has been performed, however, risk stratification remains a current challenge. It is widely accepted that sex is an independent risk factor due to females having a high risk of malignant arrhythmias associated with LQTS. Hormones seem to be the main reason for reported sex differences, despite the fact that the link between the sex hormones and susceptibility to malignant arrhythmias is still a matter of debate. Additional studies will help to unravel the pathophysiological mechanism involved in sex differences, helping to define a proper risk stratification in LQTS patients.

Author contributions

GS-B, OC, and EA developed the concept. ND-E, EM-B, EA, PC, SC, JC, FC, VF, CH, and GS-B acquired, pre-processed, and analyzed the data. ND-E, EA, OC, and GS-B prepared the manuscript. EA, OC, and GS-B supervised the study. 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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The emerging role of estrogen's non-nuclear signaling in the cardiovascular disease

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Sexual dimorphism exists in the epidemiology of cardiovascular disease (CVD), which indicates the involvement of sexual hormones in the pathophysiology of CVD. In particular, ample evidence has demonstrated estrogen's protective effect on the cardiovascular system. While estrogen receptors, bound to estrogen, act as a transcription factor which regulates gene expressions by binding to the specific DNA sequence, a subpopulation of estrogen receptors localized at the plasma membrane induces activation of intracellular signaling, called "non-nuclear signaling" or "membrane-initiated steroid signaling of estrogen". Although the precise molecular mechanism of non-nuclear signaling as well as its physiological impact was unclear for a long time, recent development of genetically modified animal models and pathway-selective estrogen receptor stimulant bring new insights into this pathway. We review the published experimental studies on non-nuclear signaling of estrogen, and summarize its role in cardiovascular system, especially focusing on: (1) the molecular mechanism of non-nuclear signaling; (2) the design of genetically modified animals and pathway-selective stimulant of estrogen receptor.

KEYWORDS

estrogen, non-nuclear signaling, cardiovascular disease, genetically modified animal, membrane-initiated steroid signaling

1. Introduction

Cardiovascular disease (CVD) is the leading cause of death in many countries, and its total burden is increasing dramatically (1–3). Sexual dimorphism has been observed in various CVDs. Women are less susceptible to coronary artery disease than men; however, their morbidity increases after menopause, reaching male levels (4, 5). Furthermore, menopause is associated with an increased prevalence of metabolic syndrome, a CVD risk factor (6, 7). These findings suggest a cardiovascular protective role of female sex hormones, especially estrogen, which have been consistently reported in basic research. Although hormone replacement therapy (HRT) was expected to decrease CVDs in postmenopausal women, a randomized controlled trial (RCT) by the Women's Health Initiative failed to demonstrate an improvement in CVD morbidity and was terminated early owing to adverse events including breast cancer (8). However, subanalysis of the RCT revealed conjugated equine estrogens had a tendency to lower CVD morbidity in relatively early postmenopausal women (9). Additionally, oral estradiol administration suppressed carotid artery atherosclerosis when treatment was initiated within six years, but not ten or more years after menopause (10). These reports indicate that HRT can induce cardiovascular benefits with careful application and encourage further research on the molecular mechanisms of estrogen signaling.

Estrogen receptors (ERs) regulate gene expression as transcription factors in the nucleus, known as nuclear signaling. However, a subpopulation of ERs is present at the plasma membrane and initiates intracellular signaling, referred to as "non-nuclear signaling" or "membrane-initiated steroid signaling". Despite their relatively small numbers compared to nuclear ERs (11, 12), an increasing body of evidence suggests the essential role of non-nuclear signaling in various physiological functions, including cardiovascular effects (13, 14). Additionally, G-protein-coupled estrogen receptor (GPER), a distinct subtype of ER, has been identified as another mediator of non-nuclear signaling.

In this article, we first describe the characteristics of ERs and the molecular mechanism of non-nuclear signaling. Next, the role of non-nuclear signaling in cardiovascular systems is discussed through studies using genetically modified animals and pathway-selective stimulators. A concise review of GPER is also provided.

2. Structure and ligand of estrogen receptors

Endogenous estrogens exert physiological effects by binding to their receptors (ERs). Two subtypes of ERs, ERα and ERβ, belong to the nuclear hormone receptor superfamily and share common structural characteristics (15–17). ER consists of six distinct domains (A to F domains) (18). The N-terminal A/B domains contain a transcriptional activation domain (AF1), which facilitates the transcriptional function of ER. The C domain is a DNA-binding domain (DBD) that interacts with a specific DNA sequence called estrogen response elements (EREs) located in the transcriptional regulatory region of estrogen-responsive genes. The D domain is a flexible hinge region between domains C and E, and contains a nuclear localization signal (NLS) and a nuclear export signal (NES). The E domain corresponds to the ligand-binding domain (LBD), which harbors another transcriptional activation domain (AF2). The C-terminal of ER is the F domain. Although ERα and ERβ are encoded by two genes, their C and E domains are highly homologous (19), while the other domains are relatively divergent (20). In addition, splicing variants of ERα and ERβ have distinct physiological functions (18).

Upon binding to their ligands, ERs undergo a conformational change and form a stable dimer (21–23), which then enter the nucleus guided by NLS (24–26). ERs regulate gene expression with associated coregulators (27–30), and phosphorylation of ER also enhances their transcriptional activity in a ligand-independent manner (31–33).

Estradiol (E2) is the most potent endogenous estrogen in pre-menopause women, whereas estrone (E1) plays a larger role after menopause, and estriol (E3) shows a greater importance during pregnancy (34). Estetrol (E4) is synthesized during pregnancy by fetal liver enzymes (35). Additionally, various natural and synthetic exogenous compounds act as ER ligands (36). A group of synthesized estrogenic compounds, known as selective estrogen receptor modulators (SERMs), exhibit dual functionality as both agonist and antagonist of ER in different organs due to tissue- or cell-specific difference in the recruitment of cofactors (36, 37). It has been reported that E4 exhibits the activity of an natural SERM (38).

Some oxysterols, which are oxygenated derivatives of cholesterol, function as ER ligands. 27-hydroxycholesterol (27HC) inhibits E2-induced nitric oxide synthase expression and re-endothelialization of murine carotid artery (39). In contrast, 27HC promotes breast cancer progression in an ER-dependent manner (40, 41), suggesting its characteristic as an endogenous SERM (42). 27HC also regulates bone homeostasis, partially mediated by ERs (43, 44). Similarly, 25-hydroxycholesterol exhibits ERα-mediated breast and ovarian cancer cell proliferation and prevents hypoxia-induced cardiomyocyte apoptosis (45).

3. Non-nuclear signaling of ERs

3.1. Mechanism of plasma membrane localization

In addition to their role in nuclear signaling, ERs also mediate rapid intracellular signaling. In 1967, Szego and Davis showed that estrogen increased cyclic adenosine monophosphate (cAMP) concentration in the rat uterus within minutes (46). Following studies documented rapid calcium uptake of endometrial cells after E2 administration and E2 binding to the cell membrane (47, 48). Further research has identified the existence of membrane-bound ERα and ERβ (11, 49, 50), which are responsible for rapid signaling, such as the activation of extracellular signal-regulated kinase (ERK), protein kinase B (PKB, also known as Akt), and endothelial nitric oxide synthase (eNOS) (51–54). This signaling is referred to as "non-nuclear signaling" or "membrane-initiated steroid signaling". However, it should be noted that this signaling can also induces the transcriptional response subsequently (55, 56).

Palmitoylation, a posttranslational modification of ER plays an essential role in trafficking to the plasma membrane (57–60). A conserved amino acid motif in the E domain of ERα and ERβ is responsible for palmitoylation (61) by DHHC-7 and −21 (62). Caveolin-1, the main component of caveolae (63), is colocalized with ERα (64) and the amino acid substitution of S522A in ERα impairs the interaction with caveolin-1 and plasma membrane localization (65). Striatin, a scaffold protein, is another component of the signaling complex of membrane-bound ERα (66).

3.2. Signaling complex of membrane-localized ER

On the plasma membrane, ERs form functional modules with associated proteins (**Figure 1**), including G-protein. In human umbilical vein endothelial cells, membrane ERα interacts with Gα_{13}, which activates the RhoA/Rho Kinase/Moesin pathway and induces cell migration (67). Furthermore, E2-bound membrane

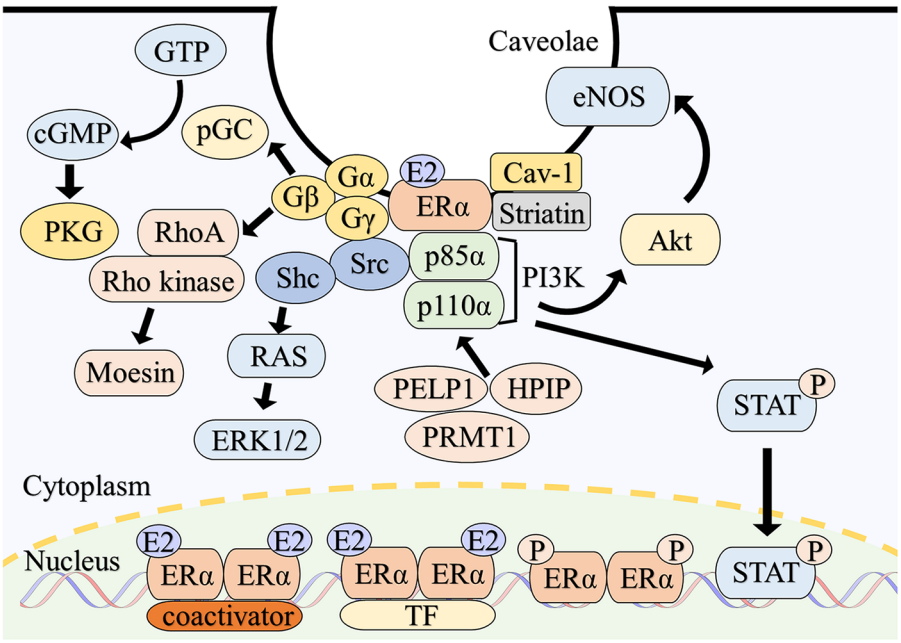

FIGURE 1
Overview of intracellular signaling of estrogen receptor *α*. Classically, E2-bound ER*α* dimerizes and translocate to the nucleus. ER*α* directly binds to estrogen response elements of the target genes with coactivators and modulates gene expressions. ER*α* also binds to DNA indirectly in association with other transcription factors. Phosphorylation of ER*α* also enhances transcriptional activity. A subpopulation of ER*α* is localized to the caveolae of the plasma membrane through the interaction with caveolin-1 and striatin. ER*α* on the plasma membrane assembles a functional complex with associated proteins such as G proteins, Src and PI3K, resulting in the rapid activation of multiple intracellular signaling. Abbreviations; Akt, protein kinase B; Cav-1, caveolin-1; cGMP, cyclic guanosine monophosphate; eNOS, endothelial nitric oxide synthase; E2, estradiol; ERK, extracellular signal-regulated kinase; ER*α*, estrogen receptor *α*; GTP, guanosine triphosphate; HPIP, hematopoietic PBX-interacting protein; PELP1, proline-, glutamic acid- and leucine-rich protein 1; pGC, particulate guanylate cyclase; PI3K, phosphatidylinositol-3 kinase; PKG, cGMP-dependent protein kinase; PRMT1, protein arginine methyltransferase 1; P, phosphorylation; Shc, src homology and collagen; STAT, signal transducer and activator of transcription; TF, transcription factor.

ER*α* links to G*α*i-2/3 and stimulate particulate guanylate cyclase-A, which causes the generation of cyclic guanosine monophosphate (cGMP). In consequence, activated cGMP-dependent protein kinase (PKG)-I stimulates cystathionine *γ*-lyase in endothelial cells, resulting the rapid release of hydrogen sulfide, which acts as a vasodilator (68). Human ER*α* binds to G*α*i and G*βγ* at amino acids of 251–260 and 271–595 of ER*α* respectively. Disruption of the interaction of ER*α* with G*α*i or G*βγ* inhibits E2-induced Src and ERK phosphorylation (69). Point mutations in the G*α*i-binding domain of ER*α* diminish E2-stimulated activation of ERK and eNOS (70).

Src, a proto-oncogene, plays a critical role in Ras/ERK activation by E2-bound ER*α* (71). Src phosphorylates human ER*α* at Tyrosine 537 (72), and the SH2 domain of Src subsequently binds to ER*α*, modulating Src activity (73). A similar mechanism is observed with ER*β* (73). Disruption of the ER*α*/Src association inhibits E2-induced proliferation of MCF-7 cell, which is an estrogen-responsive tumor cell (74). Src homology and collagen (Shc) is also contribute to ERK1/2 activation, with Src acting as an upstream regulator of Shc (75). Additionally, Proline-, glutamic acid- and leucine-rich protein 1 and hematopoietic PBX-interacting protein assist in the complex formation of ER*α*, Src and p85*α* subunit of phosphatidylinositol-3-OH kinase (PI3K) (76, 77). Protein arginine methyltransferase 1 is also involved in this process, mediating the methylation of ER*α* arginine 260 (78).

Nitric oxide production by eNOS is a vital function of endothelial cells (79). E2-bound ER*α* on the plasma membrane binds to the p85*α* subunit of PI3K and rapidly stimulates eNOS *via* the PI3K-Akt pathway (52, 80), primarily in caveolae (81). G*α*i and heat shock protein 90 are also involved in E2-induced eNOS activation (82, 83).

While studies on non-nuclear ER*β* signaling are limited, it has been reported that ER*β* activates eNOS in endothelial cell caveolae (84). Additionally, both ER*α* and ER*β* activate ERK1/2 and Akt in a subtype-specific manner (85).

4. Genetically modified animal models for ER non-nuclear signaling

Several genetically modified mouse models have been generated to investigate the role of non-nuclear signaling of ER*α* function (**Table 1**). Although the specific method of inhibiting non-nuclear signaling differ in each mouse model, these mice consistently exhibit a lack of rapid activation of eNOS by E2, which is known to play a pleiotropic role in maintaining cardiovascular homeostasis (107).

The C451A-ER*α* mouse model, which is characterized by the inhibition of palmitoylation and translocation of ER*α* to the plasma membrane by the substitution of cysteine 451 with

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TABLE 1 Comparison of the phenotypes of genetically modified mouse models.

	C451A-ERα	R264A-ERα	DPM	KRRKI	$ERα^{KI/KI}Tie2^{Cre}$	$ERαAF1^{0}$	$ERαAF2^{0}$	H2NESKI	GPER knockout
Female fertility	Infertile (86)	Fertile (87)	Fertile (88)	Infertile (89)	Fertile (90)	Infertile (91)	Infertile (92)	Infertile (93)	Fertile (94)
Uterine morphology	Atrophic (86)/Normal (95)	Normal (87)	ND	Normal (89)	Normal (90)	Atrophic (96)	Atrophic (96)	Atrophic (93)	Normal (94)
Uterine hypertrophy by E2	Impaired (86)/Preserved (95)	Preserved (87)	ND	Preserved (89)	Preserved (90)	Impaired (91)	Impaired (92)	Impaired (93)	Preserved (94)
Vascular effect of E2									
Acceleration of reendothelialization	Abrogated (95)	Abrogated (87)	ND	ND	Abrogated (90)	Preserved (91)	Preserved (92, 95)	Augmented without E2 (93)	ND
Inhibition of neointimal hyperplasia (mechanical wire injury model)	ND	ND	Abrogated (88)	ND	Abrogated (90)	Abrogated (97)	ND	ND	ND
Prevention of atherosclerosis	Preserved (98)	Preserved (87)	ND	ND	ND	Preserved (91)	Abrogated (92, 98)	ND (no statistically significant change) (93)	Abrogated (99)
Cardiac phenotype	ND	ND	ND	Impairment of E2-dependent cardioprotection by PDE5 inhibitor (89)	ND	ND	ND	ND	Impairment of E2 protection against ischemia/reperfusion injury (100) Systolic and diastolic dysfunction (cs-GPER KO) (101, 102)
Metabolic disorders									
Body weight	No effect (103)	ND	ND	Increased (104)	ND	No effect (96)	Increased (96)	Increased (93)	Increased (105) /Decreased (106)
Visceral fat accumulation	No effect (103)	ND	ND	Increased (104)	ND	No effect (96)	Increased (96)	Increased (93)	Increased (105)
Glucose intolerance	Mildly impaired (103)	ND	ND	Impaired (104)	ND	Mildly impaired (96)	Impaired (96)	Impaired (93)	Impaired (106)

ND, not determined; cs-GPER KO, cardiomyocyte-specific GPER knockout.

alanine, exhibits a complete absence of membrane-localized ERα (86, 95), leading to the abrogation of non-nuclear signaling.

Based on the importance of human ERα amino acids 251–260 in non-nuclear signaling (69, 70), the R264A-ERα mouse model was generated by replacing arginine 264 of murine ERα with alanine, which corresponds to arginine 260 of human ERα (87). While the C451A-ERα female mouse exhibits infertility and impaired reproductive organ development (86), the R264A-ERα female mice remain fertile with intact reproductive organs, which suggests a difference in the degree of non-nuclear signaling inhibition between the two models.

Another mouse model of non-nuclear signaling inhibition was produced by disrupting the interaction between ERα and striatin, which is facilitated by amino acids 183–253 of human ERα and is crucial for the membrane localization of ERα (66). The disrupting peptide mouse (DPM) was generated through transgenic overexpression of a peptide containing the amino acid sequence of ERα 176–253, which disrupts the interaction between ERα and striatin. DPM mice exhibit a lack of rapid E2-induced phosphorylation of Akt or ERK in endothelial cells, resulting in the failure to activate eNOS (88).

By developing the concept of DPM mice, it was discovered the substitution of lysine 231, arginine 233 and 234 into alanine (KRR to AAA) of human ERα inhibits its interaction with striatin (108). In a human endothelial cell line with modified ERα, the rapid activation of ERK, Akt, and eNOS by E2 administration is abrogated, while the direct genomic reaction is preserved. To investigate the effects of this modification *in vivo*, a mouse model was established in which the endogenous ERα was replaced with the modified ERα (KRR knock-in: KRRKI mice) (104).

Recently, a novel mouse model called ERα$^{KI/KI}$Tie2^{Cre} mice was established. In this mouse model, ERα non-nuclear signaling is inactivated by disrupting its binding to the p85α subunit of PI3K through an arginine 263 to alanine mutation in a tissue-specific manner under the Cre-loxP system (90).

In this section, we summarize the findings obtained from these models and compare them with the mouse model with inactivated nuclear signaling, specifically with regards to the cardiovascular and metabolic systems.

4.1. Protection against vascular injury

Estrogen accelerates re-endothelialization and suppresses neointimal hyperplasia after vascular injury, with ERα playing an essential role (109, 110). Neither the C451A-ERα, R264A-ERα nor ERα$^{KI/KI}$Tie2^{Cre} mice exhibits E2-induced acceleration of re-endothelialization after electric perivascular injury (87, 90, 95). Similarly, E2 administration does not improve neointimal hyperplasia after mechanical wire injury in DPM or ERα$^{KI/KI}$Tie2^{Cre} mice (88, 90). In contrast, the mouse models with inactivated nuclear signaling by ERα AF1 or AF2 domain deletion (ERαAF1^{0}, ERαAF2^{0}) have demonstrated preserved E2 acceleration of carotid artery re-endothelialization after electric injury (91, 92, 95).

These findings suggest that ERα non-nuclear signaling plays the predominant role in the vascular protection by estrogen. This idea is supported by previous research demonstrating that E2 suppresses the proliferation of vascular smooth muscle cells (VSMCs), an underlying mechanism of neointimal hyperplasia (111). This effect is mediated through the formation of a complex of membrane ERα and striatin and protein phosphatase 2A (PP2A), leading to subsequent kinase inactivation (112). In VSMCs derived from DPM mice, the estrogen-induced complex formation and anti-proliferative effect is abrogated.

However, it is worth noting that E2-induced suppression of neointimal hyperplasia after mechanical wire injury is abolished in ERαAF1^{0} mice (97). This result suggests that ERα nuclear signaling may also contribute to vascular protection or that there may be differences in the underlying biological mechanism of each vascular injury model.

The mouse models with genetic modification within D-domain provide valuable insight into the function of non-nuclear signaling in vascular protection. Amino acid substitution in the hinge region and NES of ERα D-domain alter the pattern of intracellular distribution of ERα. While wildtype ERα predominantly is localized in the nucleus, ERα with modifications in the putative NLS of the hinge region and NES (H2 + NES ERα) is exclusively localized in the cytoplasm. This altered localization is assumed to be caused by enhanced NES function, which is partially restored by leptomycin B, a nuclear export inhibitor. H2 + NES ERα exhibits impaired transcriptional activity but maintains a non-nuclear response of ERK1/2 phosphorylation after E2 administration (113, 114). The mouse model with mutated ERα (H2NESKI) exhibits an interesting cardiovascular phenotype where the degree of carotid artery re-endothelialization after electric injury is similar to that of E2-treated wild-type female mice, even in the absence of estrogen by ovariectomy. This observation suggests an intrinsically enhanced non-nuclear ERα signaling in H2NESKI mice (93).

4.2. Atherosclerosis prevention

Estrogen reduces the development of atherosclerotic lesion through ERα in mouse models of atherosclerosis with apolipoprotein E-deficient (*Apoe*$^{-/-}$) or low-density lipoprotein receptor-deficient (*Ldlr*$^{-/-}$) (115–118). While ERα non-nuclear signaling appears to play a critical role in protecting against vascular injury, ERα nuclear signaling is essential for preventing atherosclerosis by estrogen. This effect is preserved in C451A-ERα (98) and R264A-ERα mice (87), but abolished in ERαAF2^{0} mice (92) that are crossed with *Ldlr*$^{-/-}$ mice. Notably, the AF1 domain of ERα appears to be dispensable for atherosclerosis prevention (91), suggesting the individual function of each domain.

4.3. Effect on metabolic homeostasis

Estrogen has been shown to suppress metabolic disorders, which are a common risk factor of CVDs. Female mice with

whole-body ERα knockout exhibit several metabolic disorders, including glucose intolerance, body weight gain and visceral fat accumulation (96). Both nuclear and non-nuclear signaling of ERα appear to play a substantial role in these metabolic effects.

KRRKI mice also exhibits these dysfunctions, which are due to the disruption of the signal complex of membrane ERα, striatin, and PP2A in the hypothalamus, resulting in lower levels of physical activity and energy expenditure (104). In contrast, C451A-ERα mice only exhibit partial abnormalities (103), indicating that the mechanism of non-nuclear signaling inhibition may be relevant to the metabolic phenotype.

Regarding nuclear signaling, deletion of ERαAF2 induces similar abnormalities, with the disappearance of estrogen-regulated metabolic gene expression response to estrogen in the liver and adipose tissue. In contrast, deletion of ERαAF1 causes only mild hyperglycemia in the glucose tolerance test, suggesting a minor contribution of the AF1 domain (96).

4.4. Cardiac phenotype

E2 administration attenuates pressure overload-induced cardiac hypertrophy in female mice (119), as well as inhibits angiotensin II or endothelin-1-induced hypertrophy of neonatal rat cardiomyocytes (120). While ERβ seems to play the predominant role in mediating estrogen's protective effect against hypertrophy (121–124), ERα also contributes to this effect (125).

The KRRKI mouse model has shed light on the role of ERα non-nuclear signaling in pressure overload-induced heart failure (89, 126). Phosphodiesterase 5 (PDE5) inhibitors prevent cardiac remodeling in mice by myocardial PKG activation (127). Interestingly, the efficacy of PDE5 inhibitors in female is dependent on estrogen, which stimulates cGMP synthesis *via* the eNOS/soluble guanylate cyclase pathway (128). Notably, in female KRRKI mice, PDE5 inhibitors failed to activate PKG and provide cardiac protection against pressure overload-induced heart failure, even in the presence of estrogen (89).

Other mouse models with genetic modification within the DBD (129, 130), LBD (131), and a transgenic mouse expressing only a functional E domain of ERα at the plasma membrane (132) also provide meaningful insights into intracellular estrogen signaling. However, the effect of these genetic modifications on the cardiovascular system have not been determined.

5. Selective stimulator of non-nuclear ER signaling

Estrogenic compounds and estrogen derivatives that selectively stimulate a subpopulation of ERs also offer important insights into non-nuclear signaling. Estradiol-bovine serum albumin conjugate (E2-BSA), estrogen-dendrimer conjugate (EDC), and pathway-preferential estrogens (PaPEs) have been developed as selective stimulator of non-nuclear signaling and widely used in various studies, including cardiovascular research.

5.1. Estradiol-bovine serum albumin conjugate

17β-estradiol conjugated to bovine serum albumin (E2-BSA) is membrane impermeable and therefore selectively stimulates cell-surface ERs. The exposure to E2-BSA leads to an increase in intracellular calcium and NO release in human artery endothelial cells, which is presumed to be mediated by non-nuclear signaling (133).

However, it should be noted that some criticisms have been raised regarding the suitability of E2-BSA as a tool to evaluate membrane-bound ER function: (1) E2-BSA solution contains some free E2 by cleaving from BSA, which may activate nuclear signaling; (2) the pattern of E2-BSA binding to ERs is influenced by the BSA linking site, which leads to different biological responses (134, 135).

5.2. Estrogen-dendrimer conjugate

A novel conjugate of estrogen and a polyamidoamine dendrimer was developed, in which the estrogens were linked to the dendrimer through a hydrolytically stable bond (136). This conjugate, known as estrogen-dendrimer conjugate (EDC), selectively stimulates ERs localized to the plasma membrane and cytoplasm since its positive charge and large size prevent it from entering the nucleus. EDC rapidly induces phosphorylation of ERK, Shc, and Src in MCF-7 cells, with limited impact on the expression of estrogen-responsive genes.

In bovine artery endothelial cells, EDC activates eNOS and promotes cell proliferation and migration. Moreover, EDC accelerates re-endothelialization of carotid artery after electric injury (137), which is consistent with the results of studies conducted on genetically modified mice.

In an ischemia/reperfusion model, E2 decreases cardiomyocyte apoptosis and infarct size (138, 139). Pretreatment with EDC similarly reduces infarct size and mitigates the decline in left ventricular function (140). This effect is accompanied by S-nitrosylation,of myocardial proteins, which plays an important role in cardioprotection (141).

It is noteworthy that continuous administration of EDC did not activate ER-mediated gene transcription *in vivo*, as evidenced by the bioluminescence assay using ERE-luciferase reporter mouse and real-time PCR (137). This finding not only confirms the high selectivity of EDC in stimulating non-nuclear signaling but also demonstrates the chemical stability of E2-dendrimer bound in EDC, which prevents the release of free E2.

5.3. Pathway-preferential estrogens

A novel estrogen compound that preferentially activates a subset of ERs has been developed through a distinct mechanism

from EDC (142). Typically, an initial signal triggered by transient ER-ligand binding is sufficient to activate non-nuclear signaling, in which subsequent kinase cascades play a predominant role. In contrast, activation of nuclear signaling often requires sustained ER-ligand binding to induce a series of subcellular processes. Therefore, it is anticipated that the modified estrogen, which possesses appropriately reduced affinity to ERs, will activate non-nuclear signaling effectively while avoiding the stimulation of nuclear signaling.

Pathway-preferential estrogens (PaPEs) are synthesized from estradiol by altering its steroid structure and adding modifications. PaPE-1 binds to ERα and ERβ 50,000 times less effectively than E2, while still retaining essential chemical features. PaPE-1 rapidly stimulates kinase phosphorylation in MCF-7 cells without directly activating genomic target genes. Additionally, PaPE-1 accelerated re-endothelialization of murine carotid arteries after electric injury (142).

In contrast to E2, neither PaPE-1 nor EDC prevents plaque formation in $Ldlr^{-/-}$ female mice fed a hypercholesterolemic diet (98). This result confirms the pivotal role of ER nuclear signaling in the atheroprotective effect of E2, as demonstrated by ERαAF2^{0} mice (98).

6. Function of G-protein-coupled estrogen receptor (GPER)

GPER, called as GPR30 previously, constitutes a significant part of non-nuclear signaling of estrogen. GPER is a seven transmembrane G protein-coupled receptor (GPCR) of estrogen, identified in 1997 (143). GPER induces rapid activation of protein kinase A (PKA) as well as multiple signaling pathways that are also downstream of ERα (144–150).

Interestingly, GPER is not localized only at plasma membrane but also in intracellular compartments including endoplasmic reticulum and Golgi apparatus (151–99). The cellular distribution of GPER varies depending on the types of tissue and cells (151–154), and dynamically changes through intracellular trafficking (155, 156).

GPER mediates multiple physiological effects of estrogen on various organs, including the cardiovascular system. In contrast, it has been reported that GPER is dispensable for estrogenic effects in the reproductive system (94). Here, we outline the role of GPER in the cardiovascular system. For the detailed function and regulation of GPER, please refer to excellent comprehensive reviews elsewhere (157–159).

6.1. Vascular phenotype

G-1, a selective agonist of GPER (160), induces acute eNOS activation in rat aorta and cultured aortic endothelial cells (161). G-1 also activates protein kinase A in vascular smooth muscle cells, phosphorylating myosin light chain kinase (MLCK), while increasing intracellular calcium concentration, resulting in the relaxation of smooth muscle cells and thus vasodilatation (162–164). GPER-deletion in mice abrogates vasodilatory response to G-1 (105) and results in greater arterial constriction after vasoconstrictor exposure (165, 166) and development of high blood pressure with age (106).

GPER also mediates vasculo-protective effects of estrogen against atherosclerosis. In ovary-intact female mice fed on atherogenic diet, GPER deletion reduces vascular NO bioavailability and aggravates aortic inflammation and atherosclerosis (99), which contrasts with the dispensable role of ERα non-nuclear signaling in atherosclerosis (87, 98). G-1 induces differentiation of smooth muscle cell and suppresses proliferation (167), which could lead to the amelioration of atherosclerosis given the major pathogenic role of dedifferentiated smooth muscle cells (168).

6.2. Cardiac phenotype

GPER stimulation also confers cardiac protection in a PI3K-dependent mechanism. The pretreatment with G-1 attenuates contractile dysfunction and reduces infract size following ischemia/reperfusion injury (169), which is also accompanied by the suppression of proinflammatory cytokines in myocardium (170) and the inhibition of calcium-induced mitochondria permeability transition pore opening (171). Consistently, GPER-deficient male mice lose the protective effect of E2 against ischemia/reperfusion injury (100).

G-1 inhibits angiotensin II-induced cardiomyocyte hypertrophy (172). Studies using genetic models support the role of GPER in the heart. Over-expression of GPER using adeno-associated virus with G-1 stimulation ameliorates cardiac remodeling from chronic pressure-overload (173). Consistently, cardiomyocyte-specific GPER knockout induces cardiac dysfunction with increased cardiac oxidative stress and collagen deposition in female mice (101, 102).

6.3. Metabolic phenotype

GPER-deficient female mice lack estrogenic response to insulin, exhibiting hyperglycemia and glucose intolerance (106). Another line of GPER-null animals shows obese phenotype with visceral fat accumulation (105).

7. Conclusion

The series of studies focusing on non-nuclear signaling brought a new perspective on intracellular signaling and expanded our understanding of estrogen function. Further research advance would lead to a therapeutic approach that effectively distinguish cardiovascular protective effects from unfavorable ones such as cancer-progression and thrombosis.

Author contributions

HT wrote the manuscript. KU and ET critically revised the manuscript and contributed to the conception and design of this review paper. All authors contributed to the article and approved the submitted version.

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The role of the pregnancy heart team in clinical practice

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Significant maternal and fetal morbidity and mortality risk has been shown to be associated with cardiovascular disease in pregnancy. Several determinants, such as the increasing number of females with corrected congenital heart disease in reproductive age, a more advanced maternal age associated with cardiovascular risk factors, and a greater prevalence of preexisting comorbidities related to cardiac disorders such as cancer and COVID-19), lead to a higher incidence of cardiac complications in pregnancy in the last few decades. However, adopting a multidisciplinary strategy may influence maternal and neonatal outcomes. This review aims at assessing the role of the Pregnancy Heart Team, which should ensure careful pre-pregnancy counseling, pregnancy monitoring, and delivery planning for both congenital and other cardiac or metabolic disorders, addressing several emerging aspects in the multidisciplinary team-based approach.

KEYWORDS

acquired heart disease, corrected congenital heart disease, pregnancy heart team, cardio obstetric team, pre-conception counseling, multidisciplinary team-Based approach, postpartum followup

Introduction

Maternal mortality (MM) has increased in the last twenty years (1). A substantial role of cardiovascular diseases (CVD) in this rising trend has been well-established (2), and more than 33% of pregnancy-related deaths have been attributed to CVD (3–6). Corrected congenital heart disease (cCHD), valvular heart diseases (VHD), and cardiomyopathies are the most frequent CVD in pregnancy (7). Moreover, because of the improvements in cCHD surgery, it has become more and more frequent that females survivors with cCHD embark on pregnancy (2, 8); in addition, a more advanced maternal age (9) and, consequently, a greater prevalence of cardiovascular (CV) risk factors have been shown to contribute to CV deaths and morbidity (2). Indeed it has been recognized that women over 30 years have a higher MM rate (9). Conversely, the development of complications such as intrauterine growth restriction (IUGR), preterm birth, preeclampsia, and other

hypertensive disorder of pregnancy are more likely to be related to future CVD after delivery (10).

Remarkably, it has been estimated that more than 68% of CV-related MM could have been avoided (5). Therefore, in order to minimize MM, a multidisciplinary approach to pregnancy-associated conditions has been advocated (11, 12). In the latest decades, it has been proposed to create a cardio-obstetric or pregnancy heart team (PHT) involving cardiologists, gynecologists, obstetrics, anesthesiologists, nurses, and other specialists according to the specific clinical competencies required. Women with CVD or CV risk factors should be referred to this multidisciplinary team to improve care and outcomes for those at higher risk. The role of PHT is not only limited to the pregnancy period, but it is also crucial before pregnancy and in the post-delivery period (11–13). Indeed, women referred to PHT should receive appropriate counseling on maternal and fetal risk and the potential teratogenic effects of several drugs. Contraception should also be provided if required. An accurate clinical examination and close follow-up during pregnancy and, importantly, planning delivery should be provided. Finally, in the post-partum period, women should be carefully monitored for managing CV complications.

However, although the positive impact of team-based multidisciplinary strategies on pregnancy outcomes has been assessed, the role of the PHT has not been definitively recognized in clinical practice, and significant gaps exist in implementing a multiplanar approach for reducing pregnancy-associated comorbidity CVD burden. This paper aims to comprehensively discuss the efficacy and appropriateness of multidisciplinary evaluation, which enables an improvement in quality care.

Epidemiology

Maternal death is defined as a non-accidental, pregnancy-related fatal event occurring during pregnancy or within 42 days of its termination, irrespective of its duration and location (14). Moreover, all life-threatening events occurring during pregnancy and delivery are defined as severe maternal morbidities.

It has been estimated that the global maternal mortality ratio (MMR) is 216/100,000 live births (1), with a wide variability between developing and more developed countries (1). Indeed, social and geographic differences have also been considered to influence the pregnancy outcome, and a significant geographical heterogeneity has been shown. Black and Hispanic ethnicities belonging have been considered pregnancy-related mortality risk factors (15). Notably, a particularly high MMR (12/100.000) has been reported in the United States (1). Conversely, a lower mortality rate was recorded in European countries (1, 16), ranging from 2.7/100.000 to 10.9/100.000 live births in Norway and Slovakia, respectively (16). It has been reported that MM occurs mostly in preterm women or at delivery. However, a prevalence of MM of 20% until six weeks postpartum and later has been reported. CVD has been shown to cause more than ¼ of MM so that they are considered one of the major causes of MM (17).

Physiopathology

Blood volume expansion, higher cardiac output, lower systemic vascular resistance, obstruction of the vena cava, anemia, and systemic blood pressure (BP) fluctuations are hemodynamic changes that physiologically characterize each pregnancy and can result in the worsening of preexisting CVD (18). Therefore, underlying CV conditions (19) can be exacerbated by pregnancy.

Conversely, acquired CVD, can develop during pregnancy (19).

Pregnancy heart team

Although the awareness of MMR has increased in the last decades, the management of these patients still needs to be better organized.

In order to improve the quality of care for complex pregnant women avoiding discrepancies among different hospitals, the development of a PHT including cardiologists, gynecologists, anesthesiologists, and other specialized figures such as geneticists, neonatologists, cardiac surgeons, endocrinologists, and oncologists has been proposed (13, 17, 20, 21). Teams has been proposed. PHT should be finalized not only to guarantee accurate monitoring during the pregnancy and delivery but also should be organized to last from pre-conception counseling (22) to the postpartum follow-up, including labor and delivery time (14, 17–19).

Pre-conceptional counseling

Pre-conceptional counseling before pregnancy is crucial for identifying high-risk patients for maternal and fetal complications (22, 23). Contraception in young patients with CVD should also be encouraged by the PHT in order to avoid unplanned pregnancies (22). Moreover, women should be supported by the PHT and provided with the opportunity to choose the best timing for the pregnancy and undergo planned treatment. Remarkably, the inheritance of several conditions should also be faced. Heredity is expected to manifest in 50% of patients with genetic disorders associated with cCHD, such as DiGeorge (22q11 deletion) (24, 25), Marfan (26), Heart-hand syndromes (26, 27) Holt-Oram (28), Nooman (29), Alagille (30), CHARGE (31), Williams-Beuren (32), Cutis laxa (33), Vascular Ehlers–Danlos (vED) (34), and Silver-Russel syndromes (35). Several scores have been proposed to assess risk in pregnant women or those planning pregnancy. CARPREG II (Cardiac Disease in PregnancyStudy) (36), ZAHARA (Zwangerschap Bij Aangeboren Hartafwijking) (37), and modified WHO (World Health Organization) (38–40) have been validated to clinically evaluate the CVD burden, in order to favor not only preconception counseling, but also pregnancy and delivery

management, and eventually termination of pregnancy in particularly high-risk conditions.

The modified World Health Organization (mWHO) risk score identifies five risk classes (WHO I, II, II-III, III, and IV) (38–40), investigating not only the risk assessment of CV events but also obstetric complications, such as miscarriage, postpartum hemorrhage, hypertensive disorders, prematurity, intrauterine growth restriction (IUGR), low birth weight (ELBW), and perinatal mortality (38).

Preconception counseling, frequency of controls during pregnancy, delivery time and modality, and postpartum care should be based on the risk assessment (8, 41–47).

Postpartum follow-up

Postpartum follow-up should be adequately monitored in women with known CVD, considering the fact that women with CVD remain at high risk for late CV complications (48). Females with an increased risk for adverse long-term CV outcomes can be identified using pregnancy risk prediction tools (48). The intrauterine device or progesterone-only subdermal implants can be used in the immediate postpartum period, taking into account the risk of thrombosis or bleeding.

Patients at great risk for developing CV complications should be monitored for the following 72 h (49). Several CV events, such as peripartum cardiomyopathy (PPCM), pulmonary embolism (PE), spontaneous coronary artery dissection (SCAD), and aortic dissection (AD), can occur postpartum. The so-called "red flag" symptoms are thought to be relevant in the early detection of CV complications. A self-monitoring of the patients is also beneficial. After discharge, the first visit should be performed for high-risk patients within three days (50). A postpartum evaluation within the first three weeks after delivery with interim follow-up has been recommended, including a comprehensive medical examination within 12 weeks (50). Nevertheless, heart failure (HF), arrhythmias, hypertensive disorders, and hemorrhagic and infective pregnancy-related events complications have been considered the most frequent causes of rehospitalizations in the first 42 days postpartum (51).

Discussion

An increment in MM has been reported in the last two decades. CVD is considered the leading cause of MM and morbidity (3–6). Nowadays, advanced maternal age is commonly observed, being related to a more prevalence of comorbidities and CV risk factors such as hypertension, diabetes, and obesity (15).

In addition, the widespread use of assisted reproductive technology has been correlated with greater CV risk. On the other hand, the improved management of cCHD resulted in a more prevalence of adult females with cCHD. Due to the difficult management of CVD in pregnant women, referring these patients to highly specialized centers would be advisable to ensure a high quality of care and a multidisciplinary approach during pregnancy and in the first few months of postpartum (**Table 1**).

Heart failure

Pregnancy-related HF is a dangerous condition that requires an appropriate multidisciplinary approach. PPCM (53, 54) and pre-existing CVD (36, 55) have been reported to be the leading causes of HF development during pregnancy. However, diastolic dysfunction may also evolve in overt HF (56, 57). Therefore, if clinical signs and/or symptoms occur, echocardiographic parameters and biomarkers should be strictly monitored in order to detect HF early (11).

Women may become symptomatic for HF in the second trimester or earlier due to increased plasma volume, especially if structural cardiac disorders coexist (58). Nevertheless, it has been reported that 60% of pregnancy-related HF occurs postpartum, particularly in the 30 days following delivery (59). However, the diagnosis is frequently delayed or under-recognized.

Beta-blockers (except atenolol), thiazides, and loop-diuretics should be recommended, whereas angiotensin-converting enzyme (ACE) inhibitors/angiotensin II receptor blockers (ARBs), mineralocorticoid receptor antagonists, and angiotensin receptor-neprilysin inhibitor (ARNI) should not be used due to their fetotoxicity. Moreover, diuretics use should be limited to those cases in which pulmonary congestion (60). Hydralazine and nitrates may be safely used during pregnancy (61, 62). The delivery option should be evaluated if an acute refractory HF is detected. Sodium restriction should be recommended for all patients.

Peripartum cardiomyopathy (PPCM)

PPCM may occur during pregnancy or after delivery, generally in the earlier phases with idiopathic etiology. Its incidence ranges from 1 to 100 and 1–60,000 live births (63–67). African-American (AA) ancestry, a more advanced maternal age, multiple pregnancies, genetic predisposition, and hypertensive disorders have correlated with PPCM (63, 64, 68–70).

PPCM has been reported to be a leading cause of MM (71, 72). The diagnosis may be challenging because signs and symptoms may be masked by normal late pregnancy and postpartum features. A delay in detecting the diagnosis significatively increases MM so that an early diagnosis is crucial. Remarkably, PHT should provide the most appropriate medical strategy, carefully evaluating the potential teratogenic drugs effect and balancing advantages and drawbacks for the mother and fetus. Beta-blockers, loop diuretics, hydralazine/isosorbide dinitrate, and digoxin use may be encouraged, whereas ACE/ARB/aldosterone receptors antagonists must not be used. Moreover, to avoid thromboembolic events, anticoagulation should be considered during pregnancy in patients with LVEF <40%, prolonging to the first eight weeks after delivery (73).

Other pharmacological approaches, such as intravenous immune globulin use (74), pentoxifylline (an anti–tumor necrosis factor-alpha) (75), and bromocriptine prolactin inhibitor (76, 77) have also been proposed. After delivery, enalapril and

TABLE 1 Role of PHT evaluation before, during, and after pregnancy in the most common CVD.

CVD	Before conception	During peripartum	Delivery	Long term follow-up
CAD (15, 18, 38, 52)	–History of CAD –Evaluation of ongoing medications	–Assessment for possible ACS: symptoms, ECG, echocardiography –Evaluation of CAG indication –Management for antiplatelet therapy: (aspirin, clopidogrel for shortest duration) –Medications (beta-blockers, nitrates)	–Prefer vaginal delivery –Correct anemia and volume depletion (may exacerbate underlying ischemia) –Avoid hypotension/hypertension –Avoid bleeding and arrhythmias	–Contraception –Counseling for the evaluation of future pregnancies
Cardiomyopathies (38, 40)	–History of cardiomyopathy –Baseline echocardiogram –BNP/NTproBNP –Functional class classification –Evaluation of medications safety	–Acute HF management –Echocardiographic FU –Evaluation of medications' safety during pregnancy and postpartum –Avoiding hypotension and excessive diuresis –Anticoagulation in women with PPCM	–Based on hemodynamic conditions and choice of team –Monitor for 72 h after delivery	–Considering FU within 7–10 days. –Considering anticoagulation for 6–8 weeks if LVEF < 35% in women with PPCM –Contraception
Hypertrophic cardiomyopathy (38)	–Echocardiographic evaluation –Avoiding pregnancy if severe LV dysfunction or severe symptomatic LVOTO occur	–Evaluation of medications safety: beta-blockers and calcium channel –Multidisciplinary clinical and echocardiographic approach (every 3 months or in case of hypotension)	–Vaginal delivery is preferred	–Close monitoring for volume depletion (blood loss may worsen LVOTO)
Arrhythmias (38, 40)	–History of arrhythmias –Devices –Drug evaluation: antiarrhythmics and anticoagulants	–Acute treatment of arrhythmias –Multidisciplinary approach –VA: amiodarone o synchronized ECV in case of hemodynamic instability –SVA: vagal maneuvers, if adenosine is not effective –Medical therapy: antiarrhythmics and anticoagulants –Zero x-ray CA may be considered in selected patients with frequent recurrences despite medical therapy	–Vaginal delivery is preferred	–Medical therapy: antiarrhythmics and anticoagulants –Consider CA
VHD (18, 38)	–Clinical and echocardiographic assessment –-Severe VHD should be treated before conception –-Consider valve repair or bioprosthetic valve replacement in order to minimize the need for anticoagulation	–Clinical and echocardiographic assessment	PHT for deciding mode and timing of delivery	–Clinical and echocardiographic assessment
Mitral stenosis (18, 38)	–Clinical and echocardiographic assessment –Valvuloplasty if the valve area is ≤1 cm^2	–Clinical and echocardiographic assessment –Valvuloplasty in patients with symptoms or pulmonary hypertension (sPAP) > 50 mmHg) under OMT –Treatment of HF	–Vaginal delivery is preferred –Caesarean section is generally considered in patients in NYHA class III/IV or with PHA	–Regular FU visits after delivery –Late prognosis depends mainly on stenosis progression. –Regular FU are required
Aortic stenosis (18, 38)	–Clinical and echocardiographic assessment –Consider reparative therapy –Pregnancy is generally well tolerated in mild to moderate AS	–Clinical and echocardiographic FU every two months –Acute HF management –Severe AS symptomatic despite medical therapy, percutaneous treatment shoul be considered	–Vaginal delivery is preferred in non-severe aortic stenosis –Caesarean delivery should be preferred in severe symptomatic AS	–Evaluation of AS degree –Disease progression is frequent after delivery –Close FU are required
Pulmonary artery hypertension (38)	–Consider echocardiography and right heart catheterization –It is confirmed PAH, pregnancy should be avoided –When pregnancy occurs, termination should be evaluated	–Treatment of pulmonary hypertensive crisis, thrombosis, and right HF	–PHT for the decision on the mode and timing of delivery	Counseling is necessary to discuss the need for ongoing therapies and to avoid future pregnancies
Atrial Septal Defect (38)	–Consider Surgical or Percutaneous ASD closure –Echocardiographic evaluation	–In unrepaired defect, treat thromboembolic complications and atrial arrhythmias	–Vaginal delivery is preferred	–Closure should be considered

(*continued*)

TABLE 1 Continued

CVD	Before conception	During peripartum	Delivery	Long term follow-up
Coarctation of the aorta (19, 38)	Consider repair	–Close BP monitoring for unrepaired CoA –Echocardiographic FU: aneurysms have an increased risk of complications, including dissection	–Vaginal delivery is preferred –Close BP monitoring	–Close BP monitoring
Fontan circulation (19, 38)	–Clinical and echocardiogram assessment –Pregnancy shoul be discouraged	–Frequent surveillance during pregnancy (monthly) –Consider anticoagulation for thromboembolic complications –Treat arrhythmias promptly	–The time and modality of the delivery should be programmed by PHT	–Surveillance in the first weeks after delivery
Tetralogy of Fallot (19, 38)	–Maternal screening for 22q11 deletion	–Clinical evaluation every three months –Treatment with diuretics and bed rest if right ventricular dysfunction develops	–PHT for deciding mode and timing of delivery	Close surveillance
Aortic Disease (38) **Marfan syndrome** **Vascular Ehlers–Danlos syndrome** **Turner syndrome**	–Genetic counseling –Counselling that evaluates the risks of aortic dissection (aortic dilation) –Imaging of the entire aorta (CT/MRI) –Pregnancy is not recommended in Vascular Ehlers-Danlos syndrome and Turner syndrome with severe dilatation of the aorta	Monitoring by echocardiography every month in high risk patients and every three months in low-risk patients –Strict BP control (prefer beta-blockers) –Aortic dissection occurring during pregnancy is a surgical emergency –Multidisciplinary team (cardiothoracic, cardiology, obstetric, and cardio-anesthetic physicians) must act rapidly to deliver the fetus by cesarean section in specialized cardiothoracic centers and promptly repair the dissection.If pregnancy is not viable, aortic surgery with the fetus in place should be performed	Vaginal delivery if the ascending aorta diameter is <45 mm Cesarean delivery should be considered when the aortic diameter exceeds 45 mm, and is recommended in patients with vascular Ehlers–Danlos syndrome type IV	FU of the dilated aorta
Hypertension (15, 38, 40, 52)	–History of chronic hypertension –Antihypertensive therapy –Prevention of eclampsia with low-dose aspirin –Chronic hypertension includes evaluation of target organ involvement and evaluation of secondary causes	–Modification of diet and lifestyle –Treatment of moderate to severe and acute hypertension (labetalol, alpha-methyldopa and calcium channel blockers as first-line therapy)	–Vaginal delivery with close BP control	–Adjustment of postpartum therapy –Monitoring BP –Postpartum BP monitoring is recommended within 72 h and no later than ten days after hospital discharge

CAD, coronary artery disease; ACS, acute coronary syndrome; ECG, electrocardiogram; CAG, coronary angiography; FU, follow-up; BNP, brain natriuretic peptide; NT-proBNP, N-terminal (NT)-pro hormone BNP; HF, heart failure; PPCM, peripartum cardiomyopathy; LVEF, left ventricular ejection fraction; LV, left ventricular; LVTO, left ventricular outflow tract obstruction; ECV, electrical cardioversion; VA, ventricular arrhythmias; CA, catheter ablation; sPAP, systolic pulmonary artery pressure; OMT, optical medical therapy; PAH, pulmonary arterial hypertension; MS, mitral stenosis; AS, aortic stenosis; ASD, atrial septal defect; BP, blood pressure; CoA, coarctation of the aorta.

spironolactone may be initiated, as well as beta-blockers and diuretics may be continued, preventing patients from fluid overload. Furthermore, if a severe LV dysfunction persists, PHT should consider wearable cardioverter/defibrillator options. Long-term follow-up also is recommended. Finally, contraception options should be guaranteed.

Coronary artery disease

Acute myocardial infarction associated with pregnancy (PAMI) has been shown to have a 3-fold increased prevalence in pregnancy compared to what has been expected in women of similar age and CV comorbidities (78), with a reported incidence of 1/16,000 deliveries (79). Pregnant women of all ages can be affected, particularly those aged more than 30 years (78). In addition to the traditional risk factors, other predisposing conditions, such as pre-eclampsia and eclampsia, have been described (79).

SCAD is the leading cause of PAMI, especially in the latest gestational period and in the early post-partum. The left anterior descending artery and left main segment are the most commonly involved vessels (80). Structural hormonally-mediated coronary alterations belonging to the hypercoagulable state of pregnancy have been proposed as PAMI-related mechanisms of coronary thrombosis in the absence of atherosclerosis. Transient spasms may also underline a SCAD if normal coronary artery anatomy is found (81).

ST-segment elevation myocardial infarction (STEMI) is the most common clinical manifestation of PAMI. LV function impairment and ventricular arrhythmias (VA) may occur (80).

Due to very high mortality (ranging from 5% and 7%) in both mother and fetus (80, 82), PHT evaluation is crucial. A percutaneous coronary intervention should be recommended regardless of pregnancy. However, radiation risks must be carefully taken into account, lowering fetal exposure in order not to exceed the cutoff (<1 rad during pregnancy) (83). Moreover, the increased risk of SCAD should be considered.

Conversely, a conservative approach should be evaluated in non-ST-elevation myocardial infarction (NSTEMI) (80). Remarkably, the PHT approach in this context is mandatory.

Congenital heart disease (CHD)

Due to the improvement in cardiac surgery that has raised congenital patients' survival (84), the percentage of pregnant women with cCHD requiring a PHT evaluation has increased in the last decades. Although MM has been dramatically lowered up to 0.5% (47), cCHD causes a significant morbidity burden, often resulting in arrhythmias and HF (55, 85–88), so that strict clinical follow-up should be performed. Moreover, CHD must be classified into subcategories, accurately assessing the pregnancy-related risk according to the mWHO risk score (38–40). PHT plays a crucial role in managing these patients, who must be provided with appropriate counseling to raise awareness of pregnancy-related risks (89).

Remarkably, also according to the European Society of Cardiology (ESC) (18, 19, 40), Fontan circulation, systemic right ventricle (RV), and uncorrected cyanotic CHD are considered high-risk congenital disorders which mostly need a PHT evaluation.

Metabolic disorders

Metabolic disorders such as gestational diabetes mellitus should be detected and treated the earliest as possible (23), due to potential complications and adverse long-term consequences for both mother and fetus (90). Therefore, identifying undiagnosed prediabetes or diabetes at the beginning of the pregnancy is essential to improve pregnancy outcomes (91).

Pulmonary arterial hypertension (PAH)

Pulmonary Arterial Hypertension (PAH) is likely to be due to a multifactorial etiology. Idiopathic or heritable etiology, as well as connective tissue disease, CHD (Eisenmenger syndrome), left heart disorders, pulmonary diseases, and thromboembolic diseases, have been reported as mechanisms for the development of PAH (92). Patients with PAH must be carefully evaluated in order to be provided with the most appropriate treatment. Moreover, delivery must be planned early delivery. Remarkably, counseling is crucial for women with known PAH to decide strategies, including targeted therapies, physical exercise, oxygen support, and whether to interrupt pregnancy.

Valvular heart disease (VHD)

Congenital and acquired VHD are important causes of MM and morbidity, despite the fact that rheumatic etiology has diminished in the last decades, remaining a leading cause in developing countries (40, 93, 94). Remarkably, mechanical prosthetic valve management in pregnancy is particularly complex, requiring an appropriate anticoagulation strategy, requiring a PHT-based approach before and during pregnancy (40, 93, 94).

Cancer

Notably, due to the rising prevalence of cancer at young ages, the number of survivors who reach reproductive age and desire a pregnancy is significantly increased (95). In these cases, it is crucial that the patient is aware of the influence of cancer-related treatments on fertility, the outcome of the pregnancy, and potential CV complications (96).

It has been recognized that LV dysfunction may develop in women survivors who have undergone cancer therapies at reproductive age (37). Moreover, pregnancy-related hemodynamic stress is likely to result in LV impairment and HF (97).

The main risk factors of CV events during pregnancy include a reduced LV systolic function prior to the pregnancy, history of chemotherapy with anthracyclines (cumulative dose of doxorubicin ≥ 250 mg/m^2) (98), history of radiotherapy (cumulative dose ≥ 35 Gy or direct radiation on the heart > 15 Gy), diagnosis and treatment of cancer at a young age (<10 years), a longer period of time from cancer treatment to first pregnancy (>15 years) (99, 100). The assessment of the basal BNP value during pregnancy allows early identification of systolic function impairment (101, 102). Moreover, women with a history of cardiomyopathy are at a higher risk of developing further LV failure during pregnancy (103).

Notably, late radiation-induced complications may occur after radiotherapy, manifesting as therapeutics-related cardiac dysfunction, premature CAD, valvular abnormalities, pericardial injury, HF, pericardial disease, and arrhythmias (104).

Therefore, it has been established that cancer survivors who are planning a pregnancy should undergo pre-conceptional counseling (100). Clinic surveillance, including echocardiographic evaluation, is advisable before pregnancy for patients previously treated with anthracyclines and chest radiation (100).

COVID-19

An increment of 33% in MMR during the COVID-19 pandemic has been reported (105).

It has been recognized that COVID-19 patients have associated injury of the heart and vessels involving microvascular and macrovascular damage. Arterial and venous thromboembolism, CAD, HF, and arrhythmias have been shown to increase in COVID-19. Pregnant women, compared to non-pregnant females

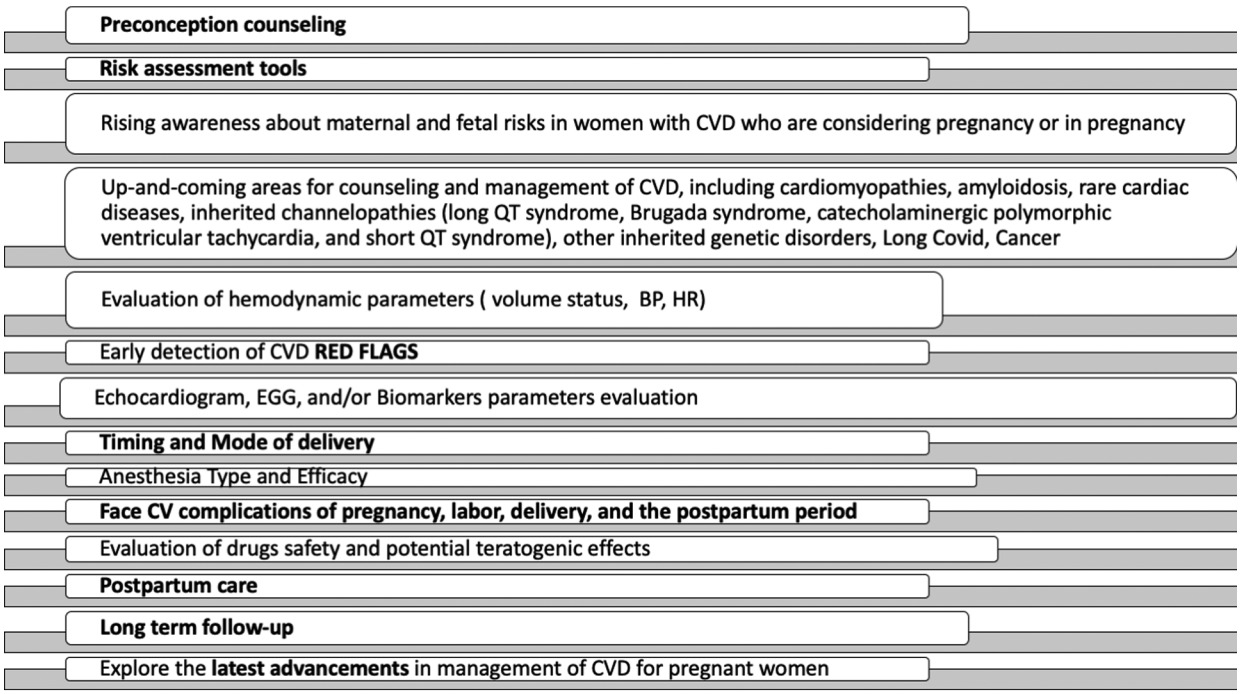

FIGURE 1
Pregnancy heart team: present and future directions. CVD, cardiovascular diseases; BP, blood pressure; HR, heart rate.

affected by COVID-19 disease, are more likely to have adverse outcomes. Moreover, severe infections (10%), intensive care unit (ICU) admission (4%), mechanical ventilation (3%), and extracorporeal membrane oxygenation (ECMO) needing (0.2%) have been reported to be more frequent in COVID-pregnant patients (106). Furthermore, COVID-19 complications may lead to preterm delivery, and the management of the pregnancy is substantially modified (107).

Assessing pregnant women with COVID-19 requires PHT to recognize COVID-19-related CV complications and to distinguish them from other pregnancy-related CV risk conditions (107). Notably, a more advanced maternal age, obesity, hypertension, and diabetes not only result in increasing CV risk in pregnancy but also the risk of severe COVID-19 disease. Accordingly, a higher neonatal ICU rate has been recorded in children of mothers affected by COVID-19. The increased risk of CV complications has been associated with a low vaccination rate in pregnant women. A more adverse outcome has been reported in unvaccinated women compared to vaccinated ones. Remarkably, vaccination during pregnancy should be strongly encouraged and should be included in the PHT program (108).

Conclusions

Progress in cardiovascular care and cardiac surgery has determined significant improvement in the conditions of women who choose to become pregnant. A close assessment before the pregnancy and monitoring during and after by a multidisciplinary group is able to reduce adverse events and improve maternal-fetal outcomes.

PHT management of comorbidities should be incorporated into pregnancy care in order to optimize appropriate and effective therapies (**Figure 1**).

Implementing PHT care will require a multidisciplinary team to address therapeutic optimization, active comorbid disease management, and evidence-based interventions. Therefore, optimizing care pathways in cardio-obstetric patients is a promising area of care innovation that should substitute the traditional care approaches.

Author contributions

All authors have seen and approved the manuscript being submitted, have contributed significantly, attest to the validity and legitimacy of the data and its interpretation, and agree to its submission. All authors agree with the content, and all give explicit consent to submit. All authors whose names appear on the submission: 1. Made substantial contributions to the conception, and design of the work and to acquisition, analysis, or interpretation of data. 2. Drafted the work or revised it critically for important intellectual content; 3. approved the version to be published; 4. agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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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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Diabetes and heart failure associations in women and men: Results from the MORGAM consortium

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Background: Diabetes and its cardiovascular complications are a growing concern worldwide. Recently, some studies have demonstrated that relative risk of heart failure (HF) is higher in women with type 1 diabetes (T1DM) than in men. This study aims to validate these findings in cohorts representing five countries across Europe.

Methods: This study includes 88,559 (51.8% women) participants, 3,281 (46.3% women) of whom had diabetes at baseline. Survival analysis was performed with the outcomes of interest being death and HF with a follow-up time of 12 years. Sub-group analysis according to sex and type of diabetes was also performed for the HF outcome.

Results: 6,460 deaths were recorded, of which 567 were amongst those with diabetes. Additionally, HF was diagnosed in 2,772 individuals (446 with diabetes). A multivariable Cox proportional hazard analysis showed that there was an increased risk of death and HF (hazard ratio (HR) of 1.73 [1.58–1.89] and 2.12 [1.91–2.36], respectively) when comparing those with diabetes and those without. The HR for HF was 6.72 [2.75–16.41] for women with T1DM vs. 5.80 [2.72–12.37] for men with T1DM, but the interaction term for sex differences was insignificant (p for interaction 0.45). There was no significant difference in the relative risk of HF between men and women when both types of diabetes were combined (HR 2.22 [1.93–2.54] vs. 1.99 [1.67–2.38] respectively, p for interaction 0.80).

Conclusion: Diabetes is associated with increased risks of death and heart failure, and there was no difference in relative risk according to sex.

KEYWORDS

diabetes, heart failure, sex differences, epidemiology, MORGAM

Introduction

The impact of diabetes is a global concern with an estimated 500 million people affected worldwide and its prevalence continues to rise (1). The cardiovascular complications of diabetes have the highest impact on mortality and morbidity in those with diabetes (2, 3). Heart failure is the most common cardiovascular complication, which can be asymptomatic initially and often in the absence of macrovascular ischemic disease (4–6).

Observational studies have noted sex differences in cardiovascular outcomes (2, 7, 8). A meta-analysis which included 12 million people demonstrated a relative increase in the risk of heart failure in women with diabetes compared to men (9). This study found a 47% higher relative risk in women with T1DM compared to men and 9% higher in women with T2DM. However, due to the lack of individual-level data, further investigation was not possible to better understand this observation. This was addressed in our recent study, in which a survival analysis was performed on the UK Biobank population consisting of approximately 500,000 participants (10). We also found that the increased relative risk of HF in women was more prominent in T1DM than T2DM (88% increased relative risk in women compared to men with T1DM, 17% in women with T2DM). Therefore, a hypothesis generated is that those with T1DM are more affected by the underlying pathological processes implicated in the increased risk of heart failure in those with diabetes. In addition, it was shown that this increased relative risk in women with diabetes was present even after adjusting for covariates such as age, body mass index, ethnicity, smoking, and alcohol use as well as confounders such as the presence of hypertension, hypercholesterolemia and coronary disease. Competing risk and mediation analysis also supported these findings, which was not possible to discern with the metanalysis.

It is not clear whether the increased relative risk seen for heart failure in women with diabetes, in particular T1DM, is generalizable to other populations. Therefore, we aim to validate the findings from the UK Biobank in external cohorts harmonized in the MORGAM (MOnica Risk, Genetics, Archiving and Monograph) study to better understand the effect of diabetes and sex on the risk of heart failure. This study provides a unique opportunity to assess whether the findings generated from standardized cohorts like the UK Biobank, can be replicated in cohorts representing populations spanning across Europe.

Materials and methods

Study cohorts

MORGAM is a multinational study aiming to explore associations of cardiovascular diseases with their classic and genetic risk factors and biomarkers using harmonized data from several population-based cohorts (11). Relevant data for this study were available from five countries: three cohorts from DAN-MONICA Study (Denmark, baseline measurements in 1982–1992), five cohorts from FINRISK Study (Finland, 1982–2002), one cohort from Moli-sani Study (Italy, 2005–2010), six cohorts from Northern Sweden MONICA Study (Sweden, 1986–2009) and four cohorts from Scottish Heart Health Extended Cohort (SHHEC) Study (United Kingdom, 1984–1995).

Figure 1 shows the numbers of participants from the MORGAM Centers after applying various exclusion criteria. After removing individuals with prevalent heart failure (HF) at baseline, incident diabetes after baseline and missing data for baseline diabetes, baseline HF or HF follow-up, the data from 88,559 subjects in total remained. At baseline 3,281 individuals were diabetic (including both type 1 and 2 diabetes) and 85,278 were non-diabetic.

Study design

As the aim of this study was to externally validate the UK Biobank's findings (10), our analytical approach including variable definitions was made as similar as possible to this previous work. The response variable was the first diagnosis of HF during follow-up. To improve comparability, the follow-up time was restricted to the maximum of 12 years. The number of incident HF cases was 2,772 within this restricted 12-year period.

Prevalent diabetes, including both type 1 and 2, was defined as self-reported or documented diabetes at baseline. Documented history of type 1 diabetes was available only for DAN-MONICA and FINRISK studies and documented history of type 2 diabetes was available for DAN-MONICA, FINRISK and Northern Sweden MONICA studies. Consequently, separate analyses by diabetes type were restricted to DAN-MONICA and FINRISK studies.

The diagnostic criteria for prevalent diseases and follow-up procedures of incident events vary by country and year. Baseline diseases were defined using data from hospital discharge registers, drug reimbursement registers and survey questionnaires. Follow-up data were obtained from causes-of-death registers, hospital discharge registers and death certificates. Further details of disease diagnostics, follow-up procedures and recruitment of each cohort are available online (12).

Baseline coronary disease was defined as documented or self-reported history of myocardial infarction or documented history of cardiac revascularization. Hypertension was defined as systolic blood pressure >140 mmHg, diastolic blood pressure >90 mmHg or use of antihypertensive medication. Hypercholesterolemia was defined as total serum cholesterol ≥6 mmol/L or taking drugs for lowering cholesterol levels.

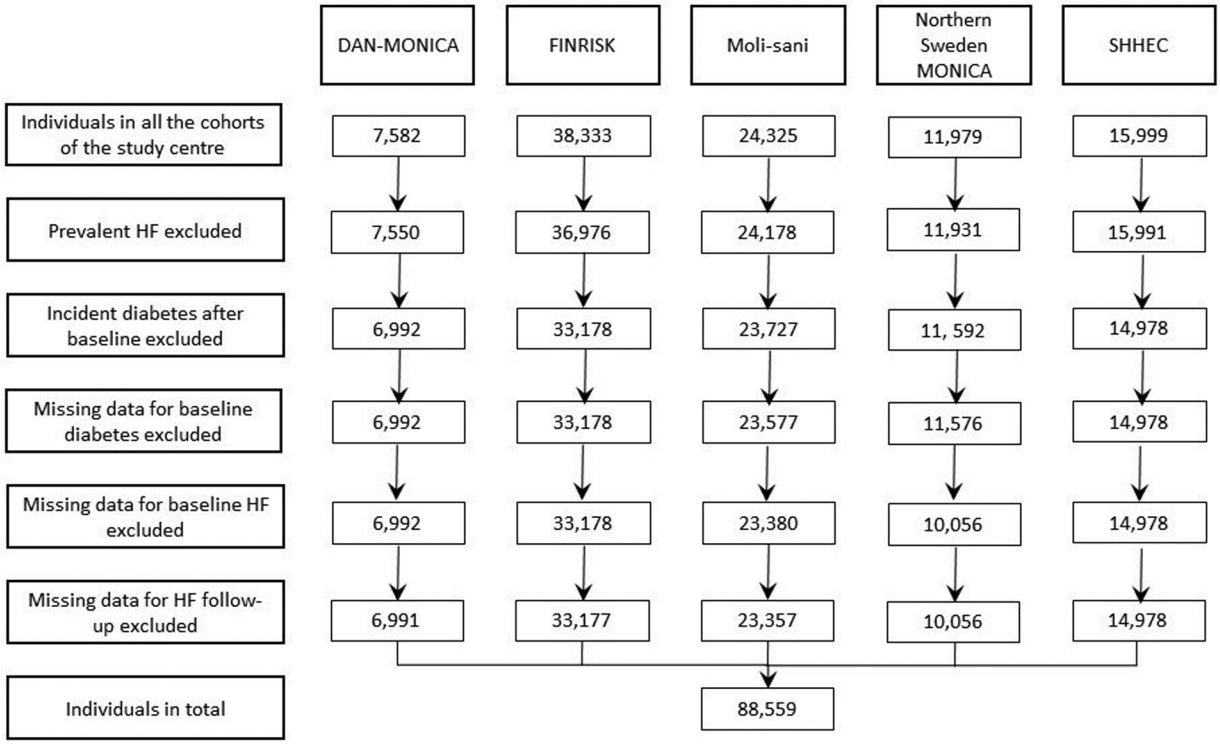

FIGURE 1
Flowchart of the numbers of participants from different MORGAM centers after data exclusion steps. SHHEC, Scottish Heart Health Extended Cohort; MONICA, Monitoring of Trends and Determinants in Cardiovascular Disease; HF, heart failure.

Smoking history had three categories: current smoker, previous smoker and never smoked. Body mass index (BMI) was derived from measured height and weight as kg/m^2. The history of alcohol consumption was not as comprehensively available as in survival analysis study performed in UK Biobank population (10), so we used the average daily consumption of alcohol (grams). Information on ethnicity was available only from DAN-MONICA and Moli-sani studies and limited to only two categories (European or other) in MORGAM data, so it was not possible to harmonize the variable to be comparable with the UK Biobank variable (four categories). Thus, we did not include ethnicity in Cox proportional hazard analysis.

Statistical analyses

The risk of HF against time in those with and without diabetes was visualized by plotting the cumulative probabilities of HF. Associations of diabetes status with heart failure in men and women were assessed by estimating hazard ratios (HR) with Cox proportional hazards models, which were also stratified by cohort. The models were fitted with an interaction effect of sex and diabetes as well as separately for data split by sex. Age, hypertension, smoking, BMI, hypercholesterolemia, alcohol consumption and coronary disease were used as covariates. Further details of the variable definitions and their use in the modelling are described in **Supplementary Table S1**.

The analyses were carried out both without adjustment for the competing risk of non-HF death and with adjustment using the Fine-Gray model (13). The timescale of the Cox models was the follow-up time which aligned with the UK Biobank analyses.

Missing data were handled using multiple imputation with random forest as the imputation method. The number of imputed datasets was ten. All analyses were carried out using R statistical software, version 4.2.1 (R Core Team) (14). R-package mice (15) was used for the imputation, survival-package (16) for the Cox models and crrSC-package (17) for the competing risks analyses.

Ethics declarations

The included studies have been approved by local ethic committees as follows: FINRISK Study: 1980s: no ethics approval required for observational studies, but there is a law which allows the use of these data for public health research, 1990s: Ethics committee of the National Public Health Institute (KTL), 2002: Ethics Committee of Epidemiology and Public Health in Hospital District of Helsinki and Uusimaa. DAN-MONICA Study: Ethics Committee of the Capital Region (formerly Copenhagen County), Denmark. Northern Sweden MONICA Study: Research Ethics Committee of Umeå University. Moli-sani Study: Università Cattolica del Sacro Cuore Facoltà di Medicina e Chirurgia "Agostino Gemelli", Rome. SHHEC Study: Ethical

approval was received from all relevant medical research ethics committees covering the individual populations involved.

Results

The participant characteristics are presented in **Table 1**. A total of 51.8% of participants were women, but only 46.3% of individuals with diabetes were women. People with diabetes were older, had higher BMI, were more likely to be hypertensive, less likely to be current smokers and had more coronary disease at baseline and more HF events during the follow-up. The amount of missing data was relatively low, except for ethnicity which was used only in a sensitivity analysis. Unadjusted absolute risk of heart failure is higher in men (16.2% vs. 10.5% of women with diabetes experienced HF in the 12 years follow-up period).

Cumulative incidence of HF was higher in those with diabetes than without diabetes (**Supplementary Figure S1**). Results from multivariable adjusted hazard ratios (HR) for diabetes (both types) were 1.73 (1.58–1.89) for all-cause mortality and 2.12 (1.91–2.36) for HF (**Figure 2**).

Individuals with diabetes had a markedly higher risk of HF than those without diabetes, for both men and women (**Figure 3**). This relationship was observed regardless of the diabetes type. It should be noted that the estimates for the subtypes of diabetes are not comparable with results for models that include both types of diabetes. This is due to DAN-MONICA and FINRISK are the only studies that have the type of diabetes defined from documentation as opposed to self-reported data source, whereas the other cohorts include self-reported and documented diabetes, but not the sub-type.

The interaction estimates did not demonstrate differences in the associations of diabetes with relative risk of HF between men and women. Due to limited data, the confidence intervals for subtype-specific estimates were very wide. None of the studies included showed any significant difference in relative risk of HF according to sex (see **Supplementary Table S2** for further details). Sensitivity analyses using models with adjustment for competing risk of non-HF death resulted in slightly smaller estimates (**Supplementary Table S3**) but did not change the conclusions.

Discussion

The results from this study show that the risk of death and heart failure is higher in those with diabetes compared to those without. This confirms the findings seen in the survival analysis performed in the UK Biobank cohort and numerous other epidemiological studies. The focus of this study was to better understand the impact of sex on the outcome of heart failure for people with diabetes.

The results which included all the MORGAM cohorts which fit the inclusion criteria of this paper, showed, as expected, that the absolute risk of heart failure is higher in men. The increased absolute risk of cardiovascular outcomes being higher in men (regardless of diabetes status) has been well documented (18–20). This study was focusing on the increased relative risk of heart failure in women with diabetes compared to men as demonstrated in other studies (9, 10), which suggests that the protection from adverse cardiovascular outcomes offered by the female sex, is attenuated in those with diabetes (21). The results

TABLE 1 Baseline characteristics and HF follow-up of the participants.

	Overall	Diabetes (men)	No diabetes (men)	Diabetes (women)	No diabetes (women)	Missing, *n* (%)
N	88,559	1,763	40,964	1,518	44,314	
N by centre (%)						
DAN-MONICA	6,991 (7.9)	66 (3.7)	3,413 (8.3)	59 (3.9)	3,453 (7.8)	
FINRISK	33,177 (37.5)	524 (29.7)	15,161 (37.0)	601 (39.6)	16,891 (38.1)	
Moli-sani	23,357 (26.4)	863 (49.0)	10,284 (25.1)	648 (42.7)	11,562 (26.1)	
N. Sweden MONICA	10,056 (11.4)	227 (12.9)	4,709 (11.5)	156 (10.3)	4,964 (11.2)	
SHHEC	14,978 (16.9)	83 (4.7)	7,397 (18.1)	54 (3.6)	7,444 (16.8)	
Baseline age, mean (SD)	49.06 (12.64)	60.41 (11.30)	48.94 (12.55)	56.85 (12.86)	48.46 (12.47)	0 (0.0)
Non-European, *n* (%)	294 (1.0)	5 (0.5)	128 (0.9)	7 (1.0)	154 (1.0)	58,444 (66.0)[a]
Baseline coronary disease, *n* (%)	2,152 (2.4)	216 (12.3)	1,424 (3.5)	73 (4.8)	439 (1.0)	181 (0.2)
Hypertension, *n* (%)	37,268 (42.4)	1,319 (75.2)	18,934 (46.6)	996 (66.0)	16,019 (36.4)	637 (0.7)
Hypercholesterolemia, *n* (%)	36,203 (41.3)	559 (32.1)	17,141 (42.3)	612 (40.8)	17,891 (40.8)	943 (1.1)
BMI, mean (SD)	26.46 (4.50)	29.27 (4.74)	26.62 (3.82)	30.09 (6.32)	26.07 (4.87)	797 (0.9)
Smoking, *n* (%)						494 (0.6)
Current	28,010 (31.8)	444 (25.3)	15,284 (37.5)	235 (15.6)	12,047 (27.4)	
Never	38,346 (43.5)	453 (25.8)	12,856 (31.5)	1,005 (66.8)	24,032 (54.6)	
Previous	21,709 (24.7)	860 (48.9)	12,627 (31.0)	265 (17.6)	7,957 (18.1)	
Alcohol use (g/day), mean (SD)	11.01 (18.18)	17.59 (23.37)	17.54 (22.97)	3.79 (7.86)	4.96 (8.32)	2,213 (2.5)
HF follow-up time, mean (SD)	10.04 (2.60)	7.96 (3.24)	9.98 (2.69)	8.83 (2.92)	10.21 (2.42)	0 (0.0)
HF, *n* (%)	2,772 (3.1)	286 (16.2)	1,341 (3.3)	160 (10.5)	985 (2.2)	0 (0.0)

SHHEC, Scottish Heart Health Extended Cohort; SD, standard deviation; HF, heart failure.
[a]Missingness of ethnicity led to exclusion as covariate.

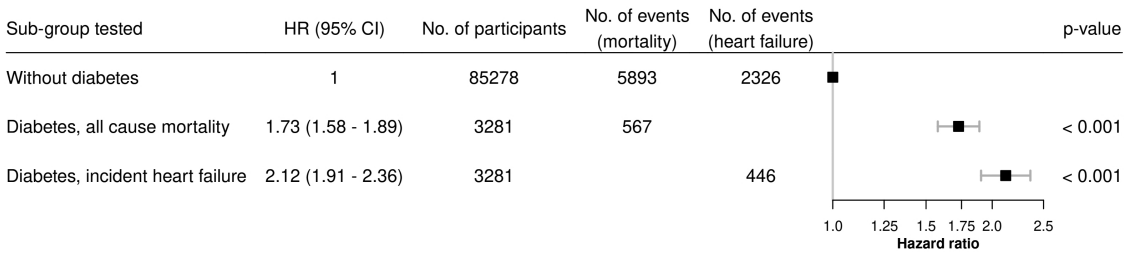

FIGURE 2
Hazard ratios (HR) with 95% confidence intervals (CI) for diabetes (both types) from separate models with all-cause mortality and heart failure as responses. Adjusted for age, sex, hypertension, hypercholesterolemia, smoking, BMI, alcohol use and coronary artery disease at baseline.

in this study did not show any sex-specific differences in the relative risk of heart failure when comparing men and women with diabetes (both types) with their non-diabetic counterparts (HR of 2.22 vs. 1.99 respectively). This deviates from the findings in the UK Biobank study as well as a large meta-analysis which did report an increased relative risk of heart failure in women with diabetes compared to men with diabetes (9, 10).

There are several reasons that could explain our contrasting and negative findings. There is the possibility of missing an existing effect in our population due to insufficient sample size and power of men and women with type 1 diabetes. It is type 1 diabetes that seems to drive the higher relative risk of heart failure in women in the literature rather than type 2 diabetes. In the UK Biobank cohort, the interaction of sex and diabetes was

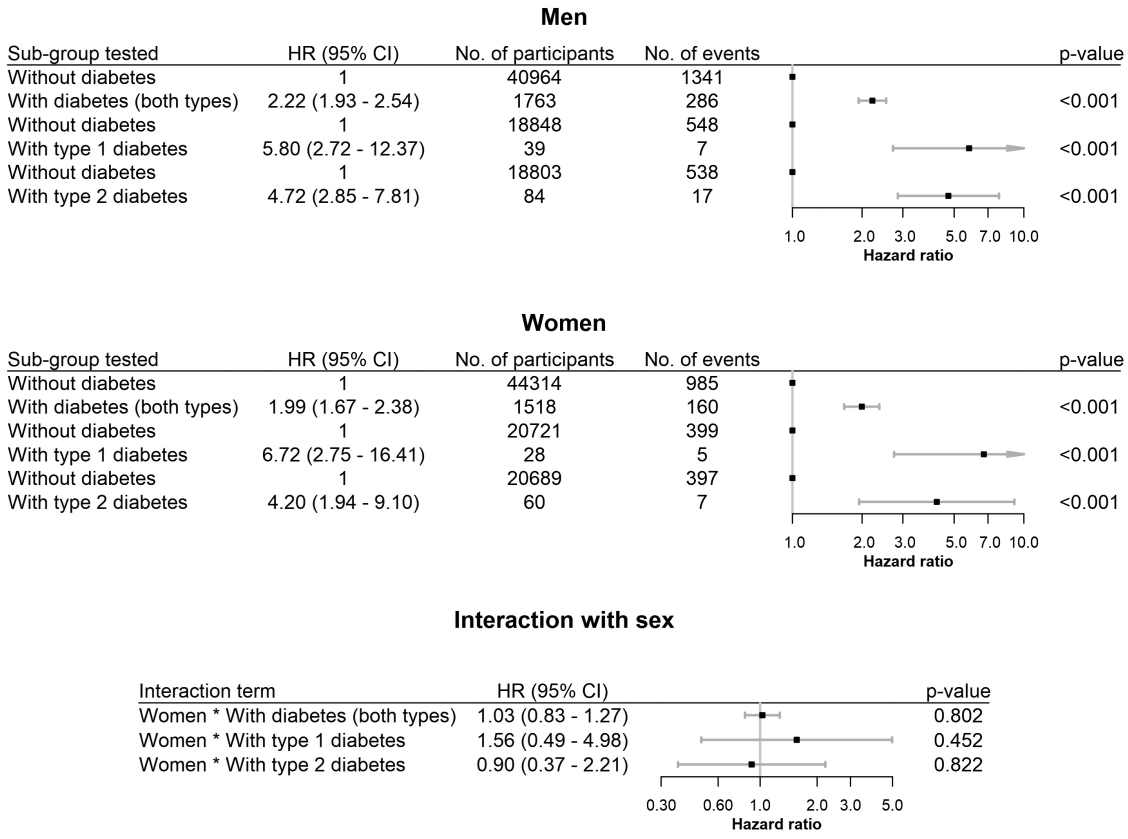

FIGURE 3
Hazard ratios (HR) with 95% confidence intervals (CI) for diabetes and sex and diabetes interactions on heart failure. Results are from separate models for men, women and both sexes and different types of diabetes adjusted for age, hypertension, hypercholesterolemia, smoking, BMI, alcohol use and coronary artery disease at baseline. Models with both types of diabetes combined use all the cohorts, whereas models with separate variables for type 1 and type 2 diabetes use only cohorts from DAN-MONICA and FINRISK Studies.

the strongest and statistically most significant with type 1 diabetes (T1DM), whereas the interaction term was insignificant for type 2 diabetes (10). Findings in the meta-analysis which included 12 million people also reflected this trend, where those T1DM were affected more than those with T2DM, but did not have interaction term analysis to determine statistical significance based on type of diabetes, due to lack of individual level data (9). Our analysis performed in the two MORGAM cohorts with information on type of diabetes (DAN-MONICA and FINRISK) indicated a trend towards an increased relative risk of heart failure in women with T1DM (HR of 6.72 in women with T1DM vs. 5.80 in men with diabetes) despite the interaction term being insignificant. However, our findings may also be negative for reasons other than reduced power. It is possible that there are disparities related to sex in the detection of risk factors such as diabetes and outcomes such as heart failure across different countries and healthcare systems included in the MORGAM consortium, which may partly explain the negative findings in this study. Additionally, many of the studies in the MORGAM consortium derive their data from as early as 1980s when the diagnosis of conditions such as diabetes (including sub-types) and heart failure were not as well established as they are in contemporary studies. The UK Biobank differs from the data in this study as the UK Biobank is comprised of more recent data collected prospectively within a single country with a more standardized healthcare provider. Similarly, the metanalysis of 12 million people may reflect epidemiological association between sex and heart failure, which are not seen in more heterogenous and historic populations like those included in this study.

One reason for why those with T1DM are possibly affected more may be due to the duration of diabetes, which would typically be longer than those with T2DM. Prolonged period of exposure to hyperglycemia could activate and sustain the inflammatory pathways implicated in an altered metabolism which could lead to adverse cardiac remodeling known as diabetic cardiomyopathy (22, 23). A recent study has demonstrated that a deterioration in strain measurements (E/e′ and GLS), which are thought to be a hallmark of diabetic cardiomyopathy are associated with major adverse cardiovascular events (MACE) in women but not in men (24). Hyperinsulinemia has also been implicated as a contributor to adverse cardiac modeling (22, 25, 26) which could explain the differences in observed cardiovascular consequences between those with T1DM and T2DM.

Further studies need to be performed which distinguishes not only by the type of diabetes, but also glycemic control, insulin treatment and the duration of diabetes. These studies could provide further evidence to support or refute the hypothesis that those with T1DM, in particular women, are disproportionately affected by the processes that lead to an increased risk of heart failure in diabetes.

Strengths and limitations

A major strength of our study is the multicenter, multi-country, individual-level harmonized data. One of the limitations of this validation study is that despite the overall large sample there were only 3,281 participants with diabetes compared to 22,300 in the UK Biobank study. The smaller sample size also didn't allow for mediation analysis to be performed to further assess to what extent risk factors such as coronary disease are mediating the increased risk of heart failure, which would further inform potential underlying mechanisms. In particular, there were only 67 participants with T1DM in this study, which is the principal sub-group of interest, compared to 2,626 participants with type 1 diabetes in the UK Biobank study. This reflects the historic nature of the data represented in this study where some studies were established when the detailed sub-typing of diseases was not a standard practice.

Another limitation is the lack of information on ethnicity. This is a majority white ethnicity population as was the case with the UK Biobank study. It is possible that other ethnicities may be more sensitive to the cardiovascular changes caused by diabetes, but this cannot be studied due to the lack of participants from other ethnicities, which affects the applicability of the findings to the wider world population. On the other hand, the MORGAM study includes data from several countries with different healthcare systems, therefore is perhaps a more representative cohort of the general white population.

Conclusion

A survival analysis performed on harmonized cohorts in the MORGAM study demonstrated that those with diabetes have a significantly higher risk of death and heart failure compared to those without. This is in keeping with the survival analysis performed in UK Biobank and many other epidemiological studies. However, overall, this study was not able to demonstrate the difference in relative risk of heart failure based on sex in those with diabetes. A smaller sub-study which distinguished participants by the type of diabetes suggested that women with T1DM may have a higher relative risk of heart failure, but this difference was not statistically significant. These findings have added support to the theory that the increased relative risk of heart failure seen in women with diabetes in the larger studies may be mostly driven due to the inclusion of larger numbers of participants with T1DM who are possibly disproportionately affected.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: The MORGAM data is not available in a public repository. Access to the data is restricted by the ethical approvals and the legislation of the European Union and the countries of each study. Approval by the Principal Investigator of each cohort study and the MORGAM/BiomarCaRE Steering Group will be required for release of the data. The MORGAM Manual at https://www.thl.fi/publications/morgam/manual/contents.htm gives more information on access. Requests to

access these datasets should be directed to https://www.thl.fi/publications/morgam/manual/contents.htm.

Author contributions

SC and JR are the first authors and were involved in the conceptualization, data collation, data analysis, and manuscript preparation. JA, ADC, LI, PJ, LK, AL, SS, HT-P, NA, MJ, KL and KK have all contributed equally to this work and were involved in data interpretation and manuscript preparation. TN and SP are the senior authors and have supervised all aspects of the study and contributed to the manuscript preparation. 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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2023.1136764/full#supplementary-material.

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Oxidative stress and inflammation distinctly drive molecular mechanisms of diastolic dysfunction and remodeling in female and male heart failure with preserved ejection fraction rats

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Heart failure with preserved ejection fraction (HFpEF) is a complex cardiovascular insufficiency syndrome presenting with an ejection fraction (EF) of greater than 50% along with different proinflammatory and metabolic co-morbidities. Despite previous work provided key insights into our understanding of HFpEF, effective treatments are still limited. In the current study we attempted to unravel the molecular basis of sex-dependent differences in HFpEF pathology. We analyzed left ventricular samples from 1-year-old female and male transgenic (TG) rats homozygous for the rat Ren-2 renin gene (mRen2) characterized with hypertension and diastolic dysfunction and compared it to age-matched female and male wild type rats (WT) served as control. Cardiomyocytes from female and male TG rats exhibited an elevated titin-based stiffness ($F_{passive}$), which was corrected to control level upon treatment with reduced glutathione indicating titin oxidation. This was accompanied with high levels of oxidative stress in TG rats with more prominent effects in female group. In vitro supplementation with heat shock proteins (HSPs) reversed the elevated $F_{passive}$ indicating restoration of their cytoprotective function. Furthermore, the TG group exhibited high levels of proinflammatory cytokines with significant alterations in apoptotic and autophagy pathways in both sexes. Distinct alterations in the expression of several proteins between both sexes suggest their differential impact on disease development and necessitate distinct treatment options. Hence, our data suggested that oxidative stress and inflammation distinctly drive diastolic dysfunction and remodeling in female and male rats with HFpEF and that the sex-dependent mechanisms contribute to HF pathology.

KEYWORDS

diastolic dysfunction, sex differences, mechanisms, oxidative stress, inflammation

Introduction

Worldwide the female sex is more affected after menopause by cardiovascular diseases with high morbidity and mortality rates. However, little is known about sex-dependent differences in mechanisms that drive disease prognosis and therapy outcomes (1). Considering the increasing cardiovascular morbidity and mortality in both sexes and the growing evidence of sex differences in cardiovascular diseases (2), the therapeutic advances in heart failure (HF) apply essentially exclusively to men and have not been investigated sufficiently in women even in breakthrough clinical trials women are underrepresented (3–5). Furthermore, female patients with heart failure with preserved ejection fraction (HFpEF) showed evidence of greater diastolic dysfunction associated with higher left ventricular (LV) filling pressure and diastolic stiffness as compared to male HFpEF patients (6).

Oxidative stress and inflammation are ascribed a central role in HFpEF pathophysiology. Both mechanisms mediate diastolic dysfunction via endothelial, extracellular matrix (ECM), and cardiomyocyte dysfunction (7). Despite the well-established contribution of redox imbalance to cardiomyocyte dysfunction, studies in both sexes are contradictory and with difference in functioning outcomes. For instance, studies on 9-week-old Wistar rats (castrated or sham-operated) have shown that oxidative stress was higher in male than in female rats (8), perhaps due to a lower induction of vascular reactive oxygen species (ROS) levels in female rats (9). Indeed, higher levels of oxidative stress biomarkers were detected in young male compared to female rats of the same age (9). In addition, recent evidence indicated that there are differences between men and women in the expression and activity of antioxidant enzymes (10), although a unified consensus is not yet apparent (11). This implies differences in the speed of the shift between oxidants and antioxidants, but does not explain the differences in oxidants between women and men with HF. Therefore, the question remains whether there are differences between both sexes at the molecular level caused by distinct oxidative stress levels. This would be important for the development of new treatment options with better efficiency for both sexes.

In addition to oxidative stress, inflammation contributes to HF development and progression linking excessive ROS with cytokine formation that results in downstream signaling pathways (12, 13). Like for oxidative stress, it remains unclear whether inflammatory events lead to sex different responses. The female sex is associated with higher susceptibility to inflammatory events and autoimmune diseases (14). On the other hand, women are less affected by inflammation than men, possibly through the protection provided by estrogens (11). We have previously provided evidence on the detrimental effects of redox imbalance on cardiomyocyte function (15, 16). The mechanisms by which oxidative damage occurs include ROS-mediated oxidative modification of myofilament proteins and/or indirect modulation of signaling pathways leading to the accumulation of oxidized proteins (17). Under various stress conditions, cardiomyocyte function is maintained via protein quality control system (PQS), which mediate the correction of misfolded proteins and/or the clearance of aberrant proteins that cannot be rescued (18). However, ROS might induce several impairments in PQS components leading to the accumulation of protein aggregates and cardiomyocyte dysfunction (19, 20). In experimental models an enhanced cardiac protection was demonstrated in female rats compared with males after trauma-induced hemorrhage, which was associated with estrogen-promoted upregulation of myocardial-specific heat shock proteins (HSPs) (21) indicating sex-based differences in PQS. In the current study, we aimed to investigate the sex-dependent differences in molecular pathways that contribute to HF pathology, especially the diastolic compliance in response to hypertensive conditions in male vs. female mRen2 transgenic (TG) rats.

Methods

Animal model

All animal care and experimental procedures were approved by the Ethical Committee of the University of Debrecen (Ethical Statement No. 1/2013/DE MÁB) in accordance with the Directive 2010/63/EU of the European Parliament. Female ($n = 8$) and male ($n = 13$) homozygous transgenic (TG) rats carrying the mouse Ren-2 renin gene (mRen2) at 1 year of age were compared with age-matched female ($n = 12$) and male ($n = 6$) non-transgenic wild type (WT) control rats from our in-bred colonies (22). Parent female and male animals were originally obtained from the Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin-Buch, Germany. No medication (e.g., antihypertensive drug) was administered to study subjects, and animals were fed a standard chow and tap water *ad libitum*. At 1 year of age, animals were sacrificed, hearts and left ventricles (LV) were quickly excised and weighed, and then further dissected in isolating solution (1.0 mM $MgCl_2$, 100.0 mM KCl, 2.0 mM EGTA, 4.0 mM ATP, and 10.0 mM imidazole, pH 7.0; all chemicals from Sigma-Aldrich, St. Louis, MO), snap frozen in liquid nitrogen, and stored at 80°C until further use.

Echocardiography

Transthoracic echocardiography with a General Electric Vivid E9 ultrasound system equipped with a linear 14.1 MHz i13l probe (General Electric, Fairfield, CT, USA) was performed on 1 year old rats under light in cardiomyocytesb y combination of ketamine and xylazine (50 mg/kg and 5 mg/kg body weight, respectively). Parasternal long axis M-mode was obtained at the level of the papillary muscles for the morphology of left ventricular (LV) such as wall thickness and internal diameter and also to assess systolic function such as ejection fraction (EF). Early diastolic filling peak velocity (E), late filling peak velocity (A), E-wave deceleration time (DT) and isovolumetric relaxation time (IVRT) were recorded by pulsed-wave Doppler. All Images were analyzed off-line by EchoPAC clinical workstation software (General Electric).

Quantification of tissue oxidative stress

Total glutathione (GSH) in myocardial homogenates ($n = 6$ LV sample/group) was determined in triplicate with a colorimetric glutathione assay kit (CS0260, Sigma-Aldrich) according to manufacturer's instructions and as previously described (23).

Western blot analysis

LV tissue samples were solubilized in a modified Laemmli buffer (50 mM Tris–HCl at pH 6.8, 8 M urea, 2 M thiourea, 3% SDS w/v, 0.03% ServaBlue w/v, 10% v/v glycerol, 75 mM DTT, all from Sigma-Aldrich, St. Louis, MO, USA), heated for 3 min at 96°C and centrifuged for 3 min at 4°C and 14,000 rpm. From LV supernatant, 20 μg protein/lane was loaded and separated by electrophoresis using 12% or 15% SDS gels, which were run at 90 V for 20 min followed by 125 V for 90 min. After SDS-PAGE, the gels were blotted onto polyvinylidene difluoride (PVDF) membranes (Immobilon-P 0.45 μm; Merck Millipore, Burlington, MA, USA). Blots were blocked with 5% bovine serum albumin (BSA) in Tris-buffered saline with Tween (TBST) for 1 h at room temperature (RT) and subsequently incubated with primary antibodies overnight at 4°C (**Table 1**). We used GAPDH (Sigma, 1:10,000) for comparison of protein load. After washing with TBST, primary antibodies were detected with HRP-conjugated secondary anti-rabbit or anti-mouse antibodies (1:10,000) and enhanced chemiluminescence (Clarity Western ECL Substrate, BioRad, Munich, Germany). Imaging was carried out with a ChemiDoc Imaging system (BioRad). Stained protein bands were quantified by densitometry using the Image Lab software (version 6.1., Bio-Rad, Hercules, CA, USA) and Multi Gauge V3.2 software. Finally, the signals obtained for the amounts of total protein and phosphorylated protein were normalized to signals obtained from GAPDH stains referring to the entire protein amount transferred. Phosphoproteins are shown as ratio of total protein. The obtained density values are expressed in arbitrary units (a.u).

TABLE 1 Primary antibody list.

Antibody	Catalogue number	Company	Dilution
Alpha—B-crystallin	Ab13497	Abcam	1:1,000
Cathepsin L	Sc-32320	Santa Cruz Biotechnology	1:1,000
Calpain 1 Large Subunit (Mu-type)	2556S	Cell Signaling	1:1,000
Caspase 1	2225S	Cell Signaling	1:1,000
Caspase 3	14220S	Cell Signaling	1:1,000
Caspase 9 p10	Sc-7885	Santa Cruz Biotechnology	1:1,000
HSP 27 (rodent preferred)	2442S	Cell Signaling	1:1,000
HSP 70	Ab2787	Abcam	1:1,000
IL 6	P620	Invitrogen	1:1,000
IL 18	PA5-80719	Invitrogen	1:1,000
LC 3 A/B	12741S	Cell Signaling	1:1,000
Total mTor	2983S	Cell Signaling	1:1,000
Phospho—mTor (S2448)	2971S	Cell Signaling	1:1,000
Total NF-κappaB p65	8242S	Cell Signaling	1:1,000
Phospho—NF-κappaB p65 (S536)	3033S	Cell Signaling	1:1,000
Nox2	MA5-35348	Invitrogen	1:1,000
Nox4	MA5-32090	Invitrogen	1:1,000
SQSTM1/P62	39749S	Cell Signaling	1:1,000
TnF alpha	AMC3012	Invitrogen	1:1,000
GAPDH	G9545-200UL	Sigma	1:10,000

Titin expression and phosphorylation

To detect titin expression and phosphorylation, LV samples were solubilized in the modified Laemmli (buffer composition given above). Samples were heated at 96°C for 3 min, centrifuged for 3 min at 4°C at 14,000 rpm, and then separated by agarose strengthened 2% SDS-PAGE (24, 25). Gels were run at 2–4 mA constant current per gel for 16 h. Thereafter, western blotting was performed to measure the expression and total phosphorylation of titin. Following SDS-PAGE, proteins were blotted onto polyvinylidene difluoride (PVDF) membranes (Immobilon-P 0.45 μm; Merck Millipore, Burlington, MA, USA). Blots were preincubated with 5% bovine serum albumin in Tris-buffered saline with Tween (TBST; containing: 10 mM Tris–HCl; pH 7.6; 75 mM NaCl; 0.1% Tween; all from Sigma-Aldrich) for 1 h at RT followed by primary antibody incubation overnight at 4°C. Titin phosphorylation was determined by an anti–phospho-serine/threonine antibody (ECM Biosciences LLC; PP2551; 1:500); for titin oxidation an anti-GSH antibody (ab19534, Abcam, 1:500) and for titin ubiquitination an anti –ubiquitin antibody (43124S, Cell signaling, 1:750) was used. Titin phosphorylation, oxidation and ubiquitination were visualized by HRP-conjugated secondary anti-rabbit or anti-mouse antibodies (1:10,000), which were used next day for 1 h at RT, then blots were treated with ECL (Clarity Western ECL Substrate, BioRad) for developing chemiluminescence signal. Chemiluminescence signals were normalized to signals obtained from Coomassie-stained PVDF membranes referring to the entire protein amount transferred. The results were quantitated by densitometry using Multi Gauge V3.2 software.

Force measurements on isolated cardiomyocytes

Force measurements were performed on single de-membranated cardiomyocytes ($n = 26–30/5–6$ heart/group) as described before (26).

Briefly, LV samples were de-frozen in relaxing solution (containing in mM: 1.0 free Mg2+; 100 KCl; 2.0 EGTA; 4.0 Mg-ATP; 10 imidazole; pH 7.0), mechanically disrupted and incubated for 5 min in relaxing solution supplemented with 0.5% Triton X-100 (all from Sigma-Aldrich). The cell suspension was washed 5 times in relaxing solution. Single cardiomyocytes were selected under an inverted microscope (Zeiss Axiovert 135, 40x objective; Carl Zeiss AG Corp, Oberkochen, Germany) and attached with silicone adhesive between a force transducer and a high-speed length controller (piezoelectric motor) as part of a

"Permeabilized Myocyte Test System" (1600A; with force transducer 403A; Aurora Scientific, Aurora, Ontario, Canada).

Cardiomyocyte Ca^{2+}-independent passive force ($F_{passive}$) was measured in relaxing buffer at room temperature within a sarcomere length (SL) range between 1.8 and 2.4 μm. Force values were normalized to myocyte cross-sectional area calculated from the diameter of the cells, assuming a circular shape. $F_{passive}$ was thereafter measured within a SL range between 1.8 and 2.4 μm as described above.

The forces were recorded at baseline and after incubation with the antioxidant, reduced glutathione (GSH) 30 min (10 mM; Sigma-Aldrich) and/or recombinant human $\alpha\beta$-crystallin or HSP27 or HSP70 concentrations 1 mg/ml and caspase 3 inhibitor concentration 0,5 mg/ml. All incubations were performed for 20 min to 30 min in relaxing solution.

Statistical analysis

Data are given as the mean values ±SEM. For statistical analysis of the two groups of parametric data Student's *t*-test was used, for non-parametric data Mann–Whitney test was used. For analysis of parametric data comparing more than two groups, 2-way ANOVA followed by Tukey's multiple comparisons test was used. *P* values were corrected for multiple comparisons by the Tukey method. For analysis of proportions, Fisher's exact test was used. The analysis was performed using GraphPad Prism 8. *P* values are two-sided and considered statistically significant if $P < 0.05$.

Results

TG male group showed cardiac enlargement and LV hypertrophy appreciated from weight/TL and LV weight/TL ratios, which were significantly higher in TG males (46.84 ± 2.29) than those in either WT males (34.55 ± 1.96) or TG females (29.90 ± 2.63). Nonetheless, pulmonary congestion with the apparent dominancy of males could not be confirmed in TG animals because lung wet/dry weight ratios were unchanged. In addition liver wet/dry weight ratios were similar as well.

Additional data based on echochardiography analysis (ECG) showed for the TG male rats left ventricle diastolic dysfunction and in female TG rats a preserved but in male TG rats a reduced left ventricle ejection fraction. The male TG showed also left ventricle hypertrophy with the absence of LV dilation. Finally, TG animals showed impaired relaxation as evident from the mitral inflow pattern with a prolonged isovolumic relaxation time (IVRT), a prolonged deceleration time (DT), and decreased E/A ratio in both sexes.

Sex and oxidative stress dependent alterations in titin-based cardiomyocyte stiffness

To investigate the effect of sex on diastolic dysfunction observed in TG animals, we measured $F_{passive}$ in single-skinned cardiomyocytes at sarcomere lengths (SL) between 1.8 and 2.4 μm. The cardiomyocytes were obtained from male and female rats and from healthy (WT) and transgenic (TG) rats before and after the treatment with reduced glutathione (GSH). Male TG cardiomyocytes showed significant increase in $F_{passive}$ at SL 2.0 μm and above compared to male WT group. GSH treatment decreased the elevated $F_{passive}$ in male TG cardiomyocytes, however, the reduction in $F_{passive}$ was only significant at SL of 2.4 μm (**Figure 1A**). Similarly, $F_{passive}$ was significantly increased in female TG cardiomyocytes at SL 2.0 and beyond compared with female WT group and could be significantly corrected at SL of 2.3 and 2.4 after GSH treatment (**Figure 1B**). $F_{passive}$ of control cardiomyocytes from WT male as well as WT female rats remained unaltered in response to GSH treatment (**Figures 1A, B**). The direct comparison between female vs. male TG and calculating the difference before and after GSH (ΔFpassive) female vs. male TG showed the great benefit of female TG from GSH compared to male TG (**Figures 1C,D**).

Since GSH treatment was effective in reducing $F_{passive}$, we examined GSH expression level (**Figure 1E**). Interestingly, both female and male TG rats showed reduced GSH level in comparison with WT matched groups. However, both TG and WT female groups exhibited higher GSH expression level when compared to matched male groups (**Figure 1E**). Considering that lower glutathione levels and depletion of antioxidant defense proteins are associated with increased ROS levels, we examined the expression level of NADPH oxidases (NOXs), which contribute mainly to ROS generation. Both proteins, NOX2 and NOX4, showed significantly increased expression in both TG males and females compared with the corresponding WT groups (**Figures 1F,G**). Notably, female sex showed significant NOX2 upregulation and NOX 4 downregulation when compared to matched male groups.

Sex dependent differential HSPs expression and cardiomyocyte passive stiffness

The molecular components of PQS, especially HSPs, can be targeted by oxidative modifications leading to deficient cytoprotective function and thereby cardiac proteotoxicity. Hence, we examined the effect of *in vitro* supplementation of HSP on titin-based myocardial stiffness as well as the expression level of various sHSP proteins (HSP27, $\alpha\beta$-crystallin, and HSP70). In general, treatment with HSP27, $\alpha\beta$-crystallin, and HSP70 reduced the significantly increased $F_{passive}$ in both TG males and females compared to the untreated corresponding TG groups. WT cardiomyocytes from males and females showed no differences in $F_{passive}$ with or without HSP treatment (**Figures 2A,B**,D,E,G,H).

As HSPs are present in cells under physiological conditions and are upregulated under stress conditions, we examined their expression in TG compared to WT animals. While HSP27 expression was significantly reduced in male TG rats compared to WT male rats, the HSP27 expression was significantly elevated in female TG rats compared to both WT female and TG male groups (**Figure 2C**). In contrast, a significant reduction in $\alpha\beta$-crystallin expression was found in male and female TG rats

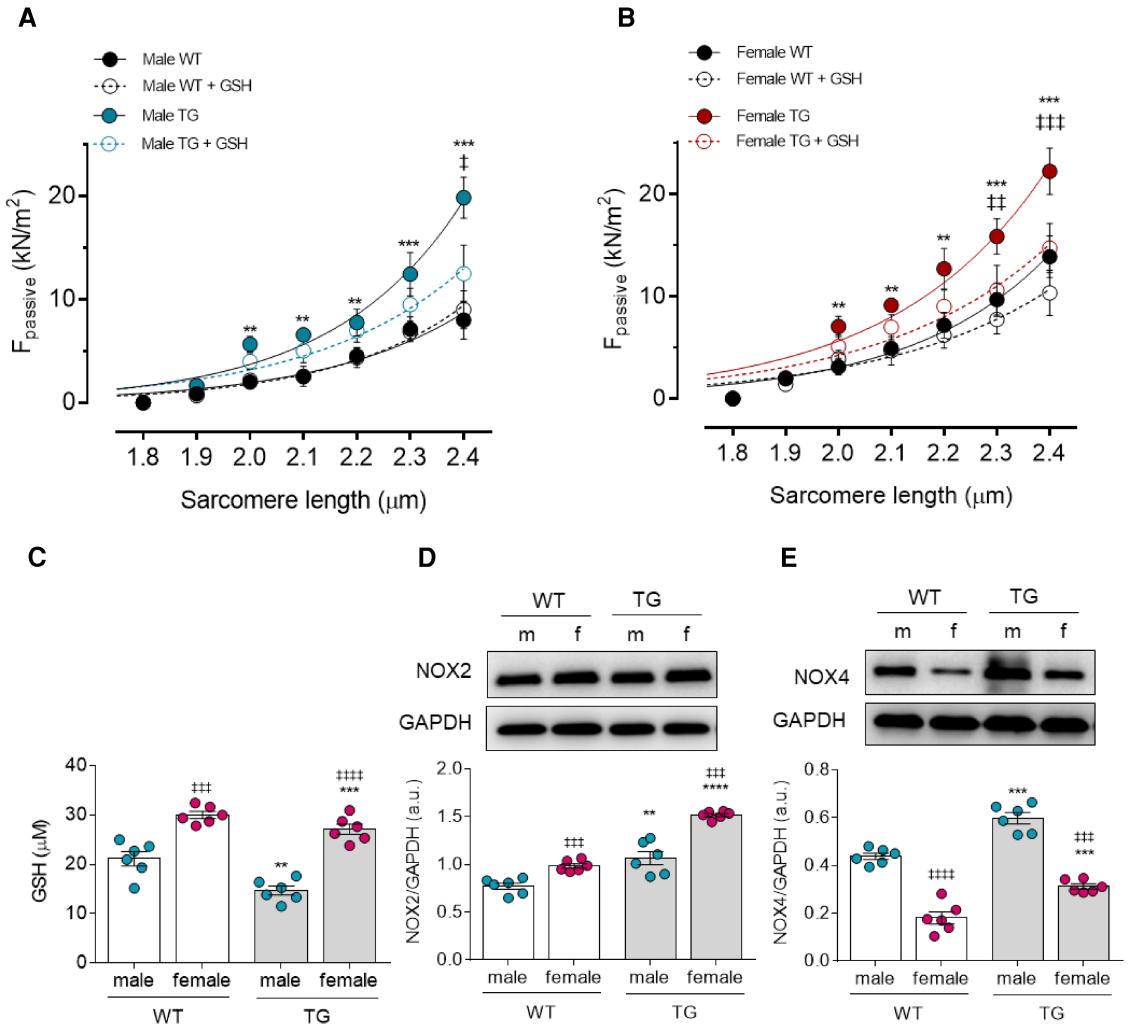

FIGURE 1
Cardiomyocyte passive stiffness ($F_{passive}$) and oxidative stress parameters in male and female mRen2 and WTrats. $F_{passive}$ before (baseline) and after *in vitro* reduced glutathione (GSH) treatment at sarcomere length 1.8–2.4 μm in (**A**) male and (**B**) female TG and WT rats. (**C**,**D**) Comparison of passive stiffness lowering effect (ΔFpassive) of GSH in TG females vs. TG males is shown at sarcomere length of 2.2 μm. (**E**) GSH concentration level, (**F**) Nicotinamideadenine-dinucleotide phosphate oxidase (NOX) 2 expression level and (**G**) NOX4 expression level. Data are shown as mean ± SEM; panels (**A**,**B**), (n = 26–30/5–6 heart/group): Data are shown as mean ± SEM; n = 6. Panel (**A**,**B**): $^{*}P < 0.05/^{**}P < 0.01/^{***}P < 0.001/^{****}P < 0.001$ female WT vs. female TG and male WT vs. male TG; $‡P < 0.05/‡‡P < 0.01/‡‡‡P < 0.001/‡‡‡‡P < 0.001$ female TG vs. female TG + GSH and male TG vs. male TG + GSH. Panel (**C**,**D**): $^{*}P < 0.05/^{**}P < 0.01/^{***}P < 0.001/^{****}P < 0.001$ female WT vs. female TG and male WT vs. male TG; $‡P < 0.05/‡‡P < 0.01/‡‡‡P < 0.001/‡‡‡‡P < 0.001$ female WT vs. male WT and female TG vs. male TG; $\#\#P < 0.01/\#\#\#P < 0.001$/the difference of Fpassive after GSH beween female TG and male TG after GSH by 2-way ANOVA followed by Tukey's multiple comparisons test.

compared to the corresponding WT rats (Figure 2F). For HSP70, however, only a significant increase was found in male TG rats compared to both male WT and female TG groups (Figure 2I). These data suggest that the individual HSPs are differently regulated in a sex-dependent manner.

Altered titin post translational modifications in male and female TG rats

Other modulators of titin-based stiffness include posttranslational modifications that may vary by gender and pathology. Therefore, we investigated the phosphorylation status, oxidation state (S-glutathionylation), and ubiquitination of titin. While N2B phosphorylation of titin was significantly reduced in TG females and males compared with the corresponding WT groups (Figure 3A), N2B glutathionylation and N2B ubiquitination of titin were significantly increased in TG groups (Figures 3B,C). However, both TG and WT female groups showed no significant alterations in titin post translation modifications when compared to their matched male groups.

Altered proinflammatory cytokine levels in male and female TG rats

Based on our findings of oxidative stress-related changes in titin, increased NADPH oxidase expression, and altered HSPs

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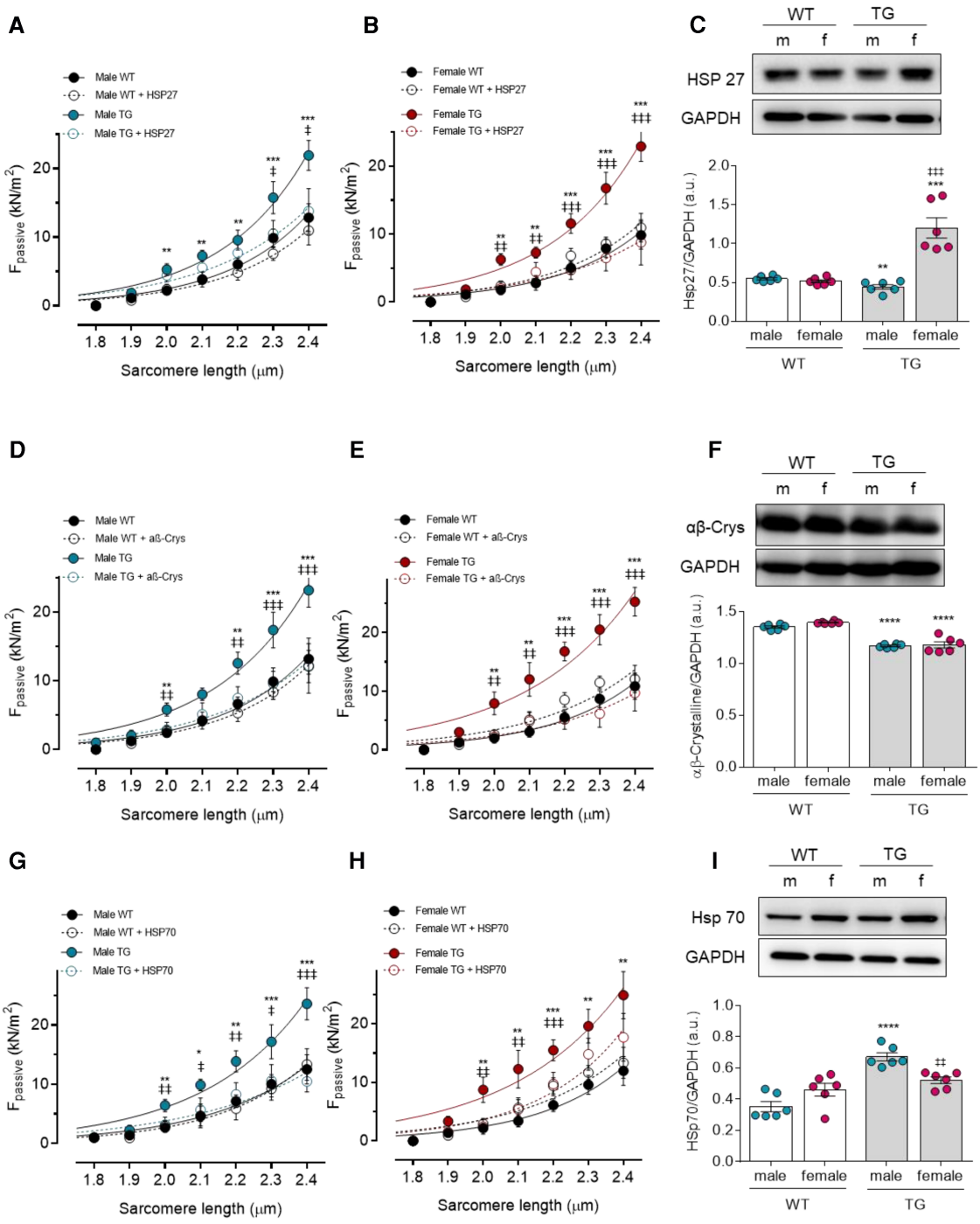

FIGURE 2
Effect of heat shock proteins (HSPs) on cardiomyocyte passive stiffness ($F_{passive}$) in male and female mRen2 and WT rats. $F_{passive}$ before (baseline) and after *in vitro* HSP27 administration at sarcomere length 1.8–2.4 μm in (**A**) male and (**B**) female mRen2 and WT rats. (**C**) Expression level of HSP27 in male and female mRen2 and WT rats. $F_{passive}$ before (baseline) and after *in vitro* $\alpha\beta$-Crystalline ($\alpha\beta$-Crys) administration in (**D**) male and (**E**) female mRen2 and WT rats. (**F**) Expression level of HSP27 in male and female mRen2 and WT rats. $F_{passive}$ before (baseline) and after *in vitro* HSP70 administration in (**G**) male and (**H**) female mRen2 and WT rats. (**I**) Expression level of HSP70 in male and female mRen2 and WT rats. Data are shown as mean ± SEM; $n = 6$. Panel (**A**–**H**): $^{*}P < 0.05/^{**}P < 0.01/^{***}P < 0.001/^{****}P < 0.001$ female WT vs. female TG and male WT vs. male TG; $‡P < 0.05/‡‡P < 0.01/‡‡‡P < 0.001/‡‡‡‡P < 0.001$ female TG vs. female TG + HSP27/ $\alpha\beta$-Crys / HSP70 and male TG vs. male TG + HSP27/ $\alpha\beta$-Crys / HSP70. Panel (**C**, **F**, **I**): $^{*}P < 0.05/^{**}P < 0.01/^{***}P < 0.001/^{****}P < 0.001$ female WT vs. female TG and male WT vs. male TG; $‡P < 0.05/‡‡P < 0.01/‡‡‡P < 0.001/‡‡‡‡P < 0.001$ female WT vs. male WT and female TG vs. male TG 2-way ANOVA followed by Tukey's multiple comparisons test.

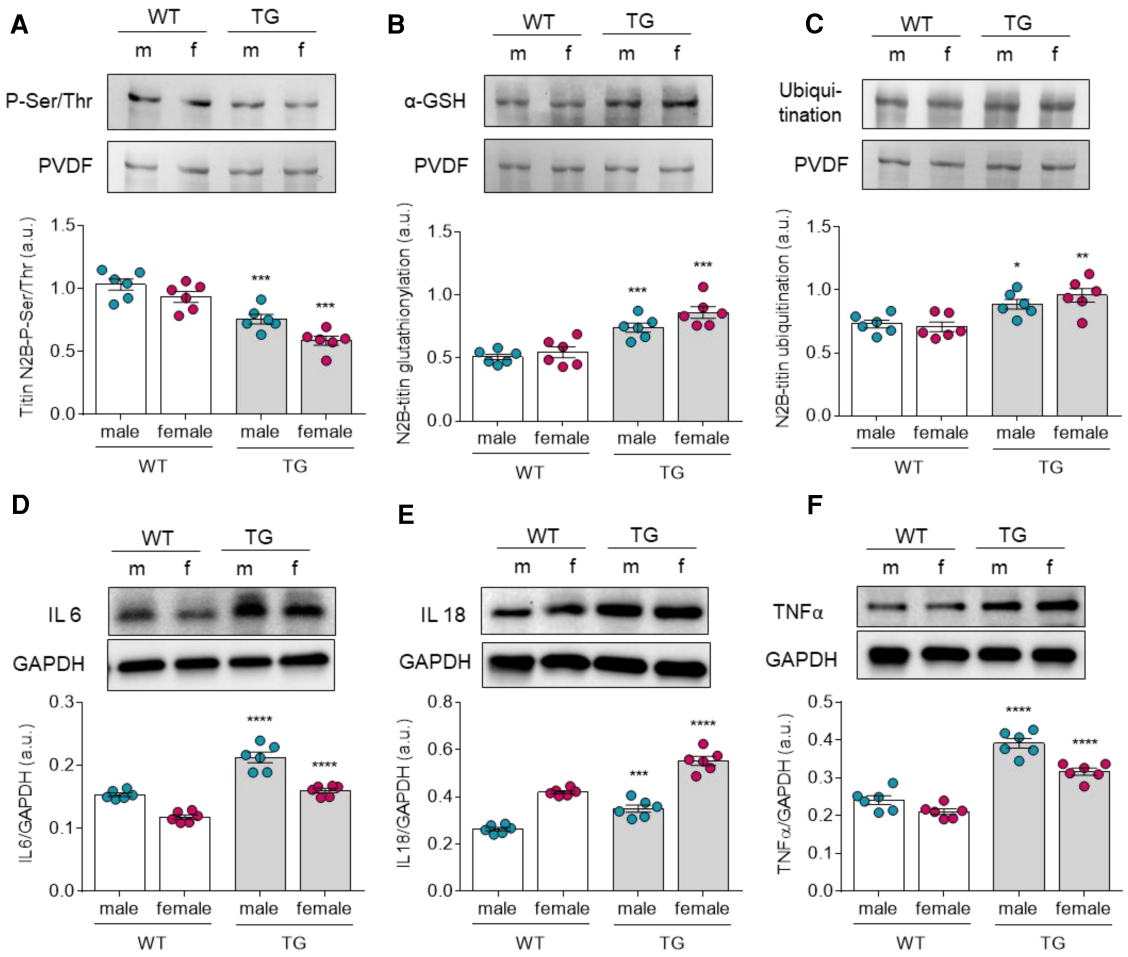

FIGURE 3
Altered titin post-translational modifications and inflammation markers in male and female mRen2 and WT rats. N2B-Titin (**A**) total phosphorylation, (**B**) total glutathionylation and (**C**) total ubiquitination. Expression levels of (**D**) interleukin 6 (IL6), (**E**) IL18 and tumor necrosis factor α (TNFα). Data are shown as mean ± SEM; $n = 6$. Panel (**A-F**): $^{*}P < 0.05/^{**}P < 0.01/^{***}P < 0.001/^{****}P < 0.001$ female WT vs. female TG and male WT vs. male TG; $‡P < 0.05/‡‡P < 0.01/‡‡‡P < 0.001/‡‡‡‡P < 0.001$ female WT vs. male WT and female TG vs. male TG by 2-way ANOVA followed by Tukey's multiple comparisons test.

expressions in TG groups, it was also plausible to investigate inflammatory responses in all groups. Therefore, we examined the expression of proinflammatory cytokines such as IL-6, IL-18, and TNFα. All of which were significantly increased in diastolic dysfunction in TG compared to WT groups (Figures 3D,E,F). In addition, both male and female animals showed comparable tendencies towards higher cytokine levels in TG groups.

Differential regulation of apoptotic pathways and proteases in male and female TG rats

Oxidative stress also plays a pivotal role in apoptosis. Therefore, we investigated the three functional caspase groups involved in (i) inflammatory cytokine processing such as caspase-1 (Figure 4A), (ii) apoptotic effector caspases-3 (Figure 4B), (iii) apoptotic initiator caspases-9 (Figure 4C), and additionally the proteases cathepsin L (Figure 4D) and calpain (Figure 4D). We found the expression level of caspase-1 to be significantly upregulated only in female TG rats compared to the matched control group but unchanged in male TG rats (Figure 4A). Of note, expression of caspase-3 was significantly increased in male TG rats, whereas it was significantly downregulated in female TG rats compared with the corresponding WT groups (Figure 4B). On the other hand, both caspase-1 and caspase-3 were significantly upregulated in TG and WT female groups when compared to the matched male groups (Figures 4A,B). The expression of caspase-9 was significantly upregulated in both male and female in TG rats (Figure 4C). Both proteases, cathepsin and calpain (Figures 4D,E), showed a significant reduction in male TG rats compared to WT rats. In contrast, female TG rats showed only a slight increase in expression of both proteases compared to female WT rats, however, the increase was only statistically significant for cathepsin L. Furthermore, cathepsin L and calpain L were downregulated in female WT compared to male WT group (Figure 4D).

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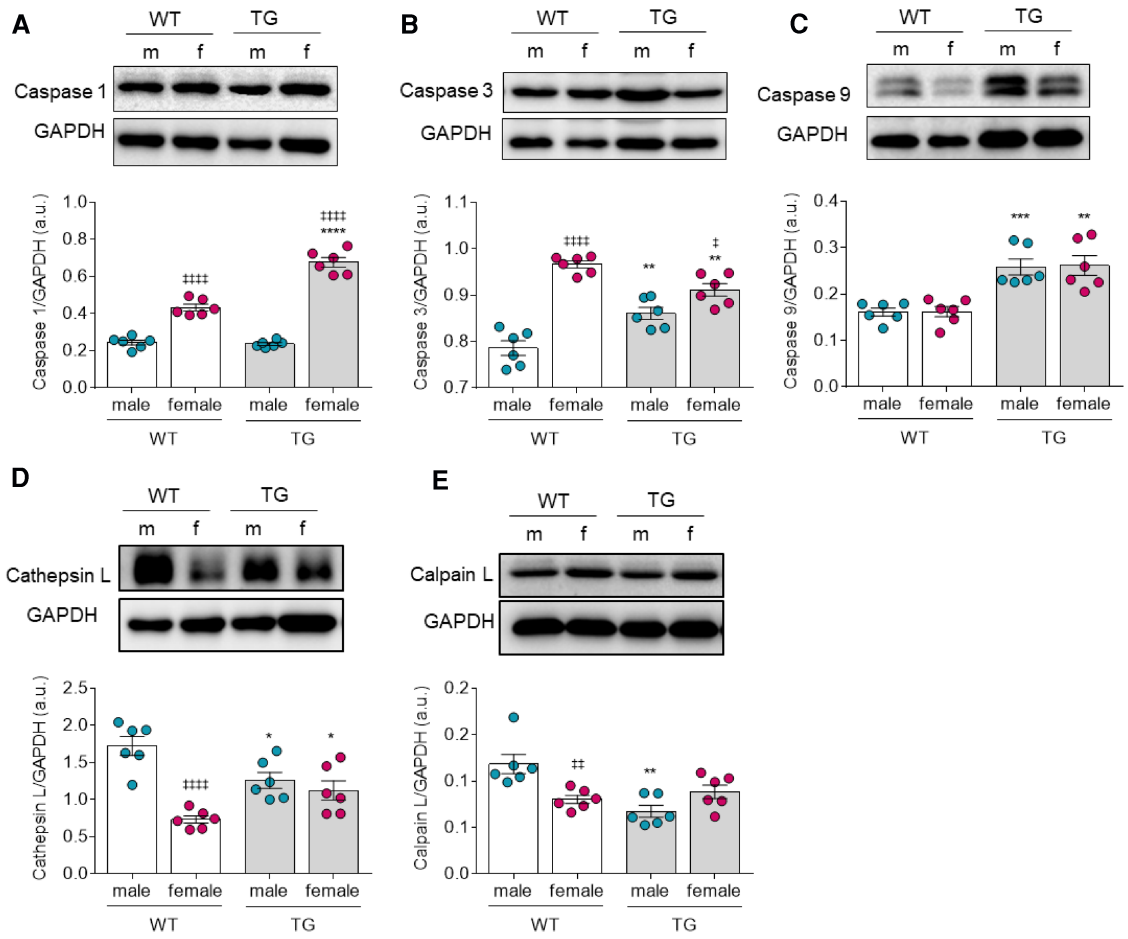

FIGURE 4
Apoptotic factors in male and female mRen2 and WT rats. Levels of (**A**) Caspase 1, (**B**) Caspase 3, (**C**) Caspase 9, (**D**) Cathepsin L and (**E**) Calpain L. (**F**,**G**) $F_{passive}$ before (baseline) and after *in vitro* Caspase 3 inhibitor (Caspase3-i) administration at sarcomere length 1.8–2.4 μm in (**F**) male and (**G**) female TG and WT rats. Data are shown as mean ± SEM; $n = 6$. Panel (**A**–**E**): $^{*}P < 0.05/^{**}P < 0.01/^{***}P < 0.001/^{****}P < 0.001$ female WT vs. female TG and male WT vs. male TG; $‡P < 0.05/‡‡P < 0.01/‡‡‡P < 0.001/‡‡‡‡P < 0.001$ female WT vs. male WT and female TG vs. male TG; for Fpassive: $^{*}P < 0.05/^{**}P < 0.01/^{***}P < 0.001/^{****}P < 0.001$ female WT vs. female TG and male WT vs. male TG; $‡P < 0.05/‡‡P < 0.01/‡‡‡P < 0.001/‡‡‡‡P < 0.001$ female TG vs. female TG + Caspase 3-inhibitor and male TG vs. male TG + Caspase 3-inhibitor by 2-way ANOVA followed by Tukey's multiple comparisons test.

Altered autophagy response in male and female TG rats

Furthermore, we examined the phosphorylation status of NF-κB, which is involved in stress responses and plays a central role in mediating immune and inflammatory responses along with regulating cell proliferation, apoptosis, and autophagy. We found that NF-κB phosphorylation level was significantly increased in both TG groups compared to their matched WT groups (**Figure 5A**). However, the total amount of NF-κB in female TG rats also showed a significant increase compared with the corresponding female control group (**Figure 5B**). Therefore, the ratio of NF-κB phosphorylation over total NF-κB was only significantly elevated in TG male rats compared with matched control rats. Of note, both TG and WT female groups exhibited greater NF-κB phosphorylation over total NF-κB when compared to the matched male groups.

In addition, mTOR (mammalian target of rapamycin) is also a regulator of various signaling pathways such as autophagy, apoptosis, and cell growth, hence we further investigated changes in its phosphorylation level. mTOR phosphorylation was significantly downregulated only in female TG rats and remained unchanged in male TG rats compared to WT male rats (**Figure 5D**). The expression level of total protein among all groups was unchanged. Consequently, the ratio of phosphorylated to total protein in female TG rats was significantly decreased but remained unchanged in male rats compared to control groups (**Figure 5F**). However, when compared to their matched male groups, both TG and WT female groups exhibited higher phosphorylated to total mTOR ratio (**Figure 5F**). In addition, we examined downstream effectors that play an important role in cellular autophagy, such as the ubiquitin-binding protein p62 (sequestosome 1), which is an autophagosome cargo protein, together with the autophagy marker light chain 3 (LC3). The expression level of p62 was significantly elevated in both male and female TG groups compared with the control groups. However,

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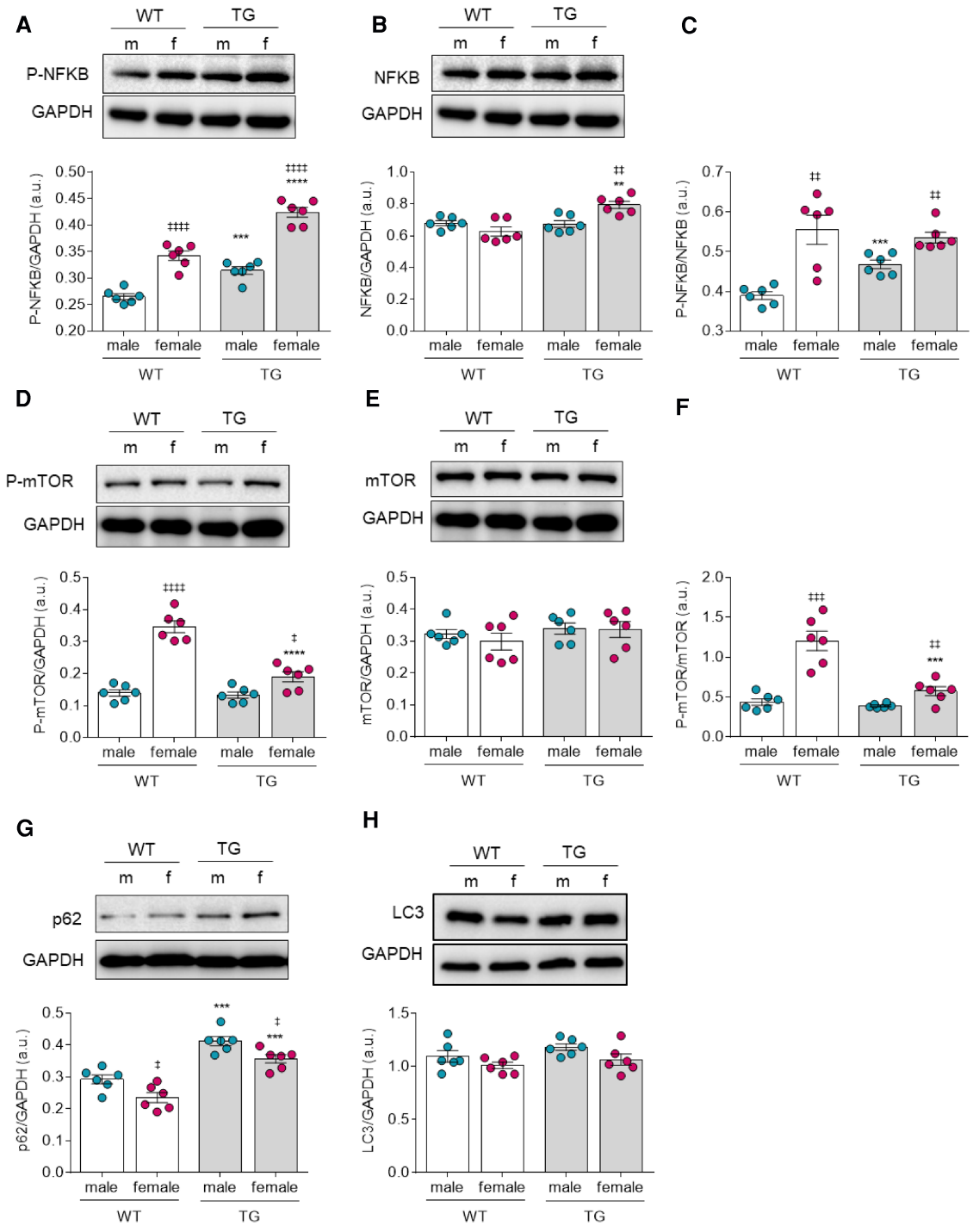

FIGURE 5
Markers of autophagy in male and female mRen2 and WT rats. (**A**) Phosphorylation, (**B**) expression and (**C**) ratio of phosphorylated over total Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). (**D**) Phosphorylation, (**E**) expression and (**F**) phosphorylated/total mammalian target of rapamycin (mTOR)-ratio. Expression level of (**G**) Sequestosome 1 (p62) and (**H**) autophagy marker light chain 3 (LC3). Data are shown as mean ± SEM; $n = 6$. Panel (**A**–**F**): $^{*}P < 0.05/^{**}P < 0.01/^{***}P < 0.001/^{****}P < 0.001$ female WT vs. female TG and male WT vs. male TG; $‡P < 0.05/‡‡P < 0.01/‡‡‡P < 0.001/‡‡‡‡P < 0.001$ female WT vs. male WT and female TG vs. male TG by 2-way ANOVA followed by Tukey's multiple comparisons test.

both TG and WT male groups showed greater elevation in p62 level compared to matched female groups (Figure 5G). LC3 expression level remained unchanged all groups (Figure 5H).

Discussion

A wide variety of comorbid conditions and risk factors correlate significantly with HFpEF phenotype. Longstanding

arterial hypertension, among others like type 2 diabetes, is well known as a potential cofounder of myocardial structural and functional changes and the subsequent diastolic dysfunction (27). Although, our understanding of diastolic dysfunction as a fundamental contributor in HFpEF pathology has been substantially advanced, the treatment options remained limited due to heterogeneity in the mechanisms arising from the comorbid conditions and risk factors (28). Female sex is differentially recognised in the context of disease prevalence, functional parameters, and treatment outcomes (29, 30). However, a deep understanding of the sex-dependent alterations in molecular mechanisms that drive the diverse mechanical and functional abnormalities is still lacking. In the current study we analysed sex-specific differences of key molecular mechanisms involved in cardiac remodelling and diastolic dysfunction.

In both sexes, we demonstrated a significant elevation in $F_{passive}$ of TG cardiomyocytes, which is a major determinant of diastolic dysfunction. In agreement with previous research highlighting the association between oxidative stress and increased myocardial stiffness, we detected high levels of oxidative stress in TG rats with prominent effects in female rats. *In vitro* supplementation with sHSPs reversed the elevated $F_{passive}$ indicating restoration of their cytoprotective function. Furthermore, TG rats exhibited high levels of proinflammatory cytokines in addition to significant alterations in apoptotic and autophagy pathways in both sexes.

Elevated titin-based myocardial stiffness due to oxidative stress in TG animals

Hypertension is a well-characterized risk factor for diastolic dysfunction. The chronic systemic pressure overload correlates with maladaptive cardiac remodelling processes including LV hypertrophy and fibrosis, thereby leading to reduced myocardial relaxation and diastolic compliance (27). The increased myocardial stiffness is a primary feature of diastolic dysfunction (31). Consistently, we found in both sexes a significant elevation of $F_{passive}$ in TG cardiomyocyte, indicative of diminished diastolic compliance. Since oxidative modifications of myofilament proteins and kinases are known to modulate myocardial stiffness (32), the reduction in cardiomyocyte passive stiffness upon GSH supplementation and in TG cardiomyocytes suggests a subtle role of oxidative stress in the modulation of myocardial stiffness, perhaps via altering titin post-translational modifications in TG cardiomyocytes such as phosphorylation and oxidations. This is in line with the significant decrease in GSH content in cardiomyocytes of TG rats compared to matched control groups and the upregulation of NADPH oxidases (NOX) the crucial mediators of ROS generation and inflammatory responses (33). Indeed, in TG animals, angiotensin-2 (ANG II) was found to stimulate NADPH oxidase-dependent-ROS production mainly through the activation of the MAPK signalling pathways (34). Of note, the NOX2 expression level showed a pronounced elevation in female TG cardiomyocytes compared to all groups. In agreement with this result, female sex has been suggested to exhibit higher tendencies towards increased levels of oxidative stress and inflammation (14). Both mechanisms mediate diastolic dysfunction via endothelial, ECM and cardiomyocyte dysfunction (16).

Effects of oxidative stress on titin and PQS components in TG animals

When under stress conditions ROS generation exceeds the antioxidative capacity, a direct oxidative modification of the proteins can cause functional and/or structural impairments that lead to protein misfolding, aggregation, and increased myocardial stiffness. Small HSPs (sHSPs) are fundamental components of the PQS serving as a first line of defence against protein misfolding (7). In agreement with previous studies reporting the upregulation of sHSPs upon various stress conditions (35), our data showed distinct regulation of HSP27 and HSP70 in female compared to male TG rats. This distinct regulation suggests the existence of sex-specific regulation of the PQS pathways. Despite the upregulation of endogenous sHSP in TG animals, cardiomyocyte passive stiffness remained elevated and was reversed to control levels only after *in vitro* supplementation of sHSPs in both male and female TG cardiomyocytes. Such observation can be explained by direct and/or indirect effect of oxidative modifications perhaps of HSPs, translocation of HSP away from sarcomeres or HSPs proteins malfunction (15–20). Previously, we reported that sHSPs can be targeted by ROS leading to a reduction of their cytoprotective function and hence protein aggregation (19, 20). In human hypertrophic cardiomyopathy (HCM), we detected oxidative-stress induced impairments in PQS as anticipated from the S-glutathionylation of HSP 27 and $\alpha\beta$-crystallin (20). Furthermore, we and other reported a oxidative stress-induced translocation of HSP27 and $\alpha\beta$-crystallin away from the Z-disk and A-band in HCM (19, 36). Conversely, sHSPs supplementation reduced the elevated $F_{passive}$ in HCM cardiomyocyte (19, 20), further confirming the direct effect of oxidative modifications on PQS.

Through its mechano-sensing properties, titin represents the main determinant of the cardiomyocyte passive stiffness. Previous research by us and others demonstrated post-translational modifications of titin such as phosphorylation, ubiquitination, and oxidation within cardiomyocyte regulate or modify the myocardial stiffness. Oxidative modifications of titin have been linked to impaired diastolic stiffness in HF patients (15, 19, 23). In the current study, we detected high levels of titin S-glutathionylation and ubiquitination in both male and female TG rats compared to matched control groups. Titin can be oxidized in different ways, either forming disulphide bridges, by S-glutathionylation, or S-nitrosylation leading either to an increase or reduction of the acrdiomyocyte stiffness (37, 38). An important mechanism that regulates titin elasticity under physiological and oxidative conditions is the mechanical unfolding of I-band Ig-domains (39). Through increased mechanical strain on the sarcomeres, Ig domains unfold, thereby exposing the cryptic cysteines for redox modifications and resulting in the formation of disulphide bridges or S-glutathionylation (38). S-glutathionylation of the cryptic cysteines at the Ig domains prevents their refolding,

decreases their mechanical stability, and reduces the passive tension (38). On the other hand, disulphide bonding in the N2B-us domain reduces its extensibility resulting in elevated cardiomyocyte passive tension (40). Upon stress, molecular chaperons such as HSP27 and $\alpha\beta$-crystallin, translocate to sarcomeres and bind at specific I-band regions of titin protecting thereby the unfolded Ig domains from aggregation and the consequent myocardial stiffening (36). However, direct oxidative modifications of these chaperons and/or their binding partners prevents the correction of misfolded proteins and hinders their clearance by the proteasome machinery (19, 20). The accumulation of protein aggregates may result in elevated myocyte stiffness, aggravated oxidative stress, and augmented cell death pathways (35, 41). These mechanisms might explain the elevation oftitin ubiquitination and cardiomyocyte stiffness in TG animals despite the upregulation of sHSPs expression. Notably, differential upregulation of sHSPs in male vs. female TG animals was observed, suggesting sex-specific regulation of sHSP.

Oxidative stress may also modulate cardiomyocyte function via indirect effects on several signalling pathways involved in posttranslational modifications of myofilament proteins (17). Oxidation of kinases and/or their downstream targets results in dysregulated phosphorylation of several proteins (17). Among which, the deranged phosphorylation status of titin due to oxidative-stress induced impairments in several kinases that phosphorylate titin spring elements (26, 42). In failing human hearts, PKA and PKG dependent hypo-phosphorylation of titin is associated with increased myocardial stiffness (25), which could be reversed upon kinase supplementation and anti-oxidant treatment (17, 23). In TG animals we detected in addition to titin S-glutathionylation, a significant reduction in total titin phosphorylation, suggesting potential alterations in kinase/phosphatase-titin interactions, which might result in dysregulated phosphorylation/dephosphorylation processes and hence altered myocyte stiffness. Thus, titin oxidation could play a major role in modulating passive stiffness, similar to or togetherwith the effects of titin phosphorylation.

Elevated pro-inflammatory cytokines in TG animals

In HFpEF patients, and upon various comorbid conditions, the systemic inflammatory state and microvascular inflammation have been linked to myocardial dysfunction (43). Pro-inflammatory cytokines induce ROS generation thereby exacerbating oxidative stress. In addition, pro-inflammatory signals contribute to myocardial fibrosis and stiffness, mainly via macrophage stimulation and/or collagen formation by fibroblast activation (12, 13). In TG animals, pro-oxidant agonists, such as Ang II and tumour necrosis factor- α (TNF-α) are known to induce the expression of pro-inflammatory molecules. Indeed, both IL-6 and IL-18 showed significant elevation in both sexes. However, distinct elevation patterns were found in male vs. female TG animals confirming the sex-specific differences in pro-inflammatory responses to hypertensive conditions.

Altered apoptotic and autophagy pathways in TG animals

The activation of apoptotic cascades occurs in response to the accumulation of oxidized, aberrant proteins upon PQS dysfunction (44). The diminished ability of sHSPs to inhibit apoptosis may also contribute to the increase in apoptotic events (45, 46). Furthermore, chronic mechanical overload associates with increased ROS generation, hypertrophic remodelling, and apoptosis. Therefore, we checked the expression level of apoptotic markers and found caspase-3 and caspase-9 to be significantly upregulated in both male and female TG animals compared to control groups. In addition, the expression levels of proteases such as cathepsin and calpain were differentially regulated in male and female TG animals compared to matched control groups. These results suggest a contribution of dysregulated apoptotic and proteolytic pathways to diastolic dysfunction. Interestingly, caspase-1 showed significant upregulation in female but not in male TG animals compared to matched control groups. Consistently, previous studies reported more frequent apoptotic event in female compared to male sex under both physiological and pathological conditions (47, 48). Caspase-1 is activated in inflammasomes and was shown to trigger both programmed necrosis (pyroptosis) and apoptotic pathways (49). In addition, caspase-1 activation promotes IL-18 release and NF-κB activation (50). Both of which showed higher upregulation/activation tendencies in female compared to male TG animals. These observations can be attributed to the sex-specific differences in immune responses, as the susceptibility to inflammatory events and autoimmune diseases is generally higher in females (14).

It is evident that dysregulated autophagy plays a pivotal role in PQS dysfunction. Down or upregulated, autophagic responses were shown to be involved in pathological cardiac remodelling upon various stress conditions (51). In the current study, female TG animals exhibited a significant decrease in mTOR phosphorylation level, compared to matched control group. However, both TG sexes showed significant upregulation in sequestosome-1, also known as ubiquitin-binding protein p62, which is an autophagosome cargo protein, suggesting the contribution of alteredNFkB and mTOR in PQS dysfunction and diastolic impairments in TG animals (52).

Molecular mechanisms underlying sex-dependent differences in diastolic dysfunction

Clinical data from HFpEF cohorts demonstrate the existence of sex-dependent differences in terms of disease progression, prognosis, and therapy outcomes (53). In addition, sex-dependent comorbid conditions have been suggested to influence the diverse HF phenotypes (53). Although the mechanisms underlying these variations have yet to be explained, it is evident that multiple factors contribute to the sex-specific differences in disease development such as sex-hormones, immune response, risk factors, and the Y chromosome (54). Testosterone was suggested to be the main driving force for hypertension in men (48). Moreover, postmenopausal women exhibit higher prevalence of LV diastolic

dysfunction than men suggesting a cardio-protective role of female sex-hormones, however inconsistent findings were reported about the impact of hormone replacement therapy on the elevated blood pressure in women (55, 56). In both sexes, activation of the Renin-Angiotensin-Aldosteron-System (RAAS) is associated with hypertension, cardiac hypertrophy, cardiac fibrosis, and impaired cardiomyocyte relaxation. Mounting amount of evidence demonstrate the regulatory role of estrogen on RAAS activity (57), NO bioavailability (58), and myocardial substrate metabolism (59). However, it is evident that hypertension increases the risk of HF by 3 times in women compared to only twice in mRen2 (60). Furthermore, women frequently develop diastolic HF, and men more often systolic HF (61, 62). A plausible explanation for such tendencies despite the protective role of female hormones is the complex interaction between oxidative stress, inflammation, hormones, and sex-specific gene regulation (54).

A classic example of such complex interplay is LV hypertrophy. LV hypertrophy, a major causative factor in reduced diastolic compliance, is mediated by AKT signalling, which is known to have higher activity in women compared to men hearts (63). Other estrogen-sensitive pathways include mTOR, GSK3ß, MAPK-ERK1/2. All of which can be dysregulated in the presence of inflammation and oxidative stress impairing thereby PQS activity (20). Therefore, the degree of redox-imbalance, inflammation, and PQS dysfunction potentially contribute to the observed sex differences in terms of diastolic dysfunction.

Conclusion

In summary, the data presented in the current study provide evidence ofdiastolic dysfunction in TG animals, which is associated with impaired cardiomyocyte function and impaired vasodilator responses through increased systemic inflammation and oxidative stress. TG animals also exhibited PQS impairment as anticipated from sHSPs malfunction and the alterations of many signal transduction pathways that are involved in autophagy and apoptosis. Our work provided further evidence of sex-specific mechanisms in the development of diastolic dysfunction in HF animals. Therefore, future research is needed to unravel the sex-dependent mechanisms contributing to HF pathology in order to design sex-specific and thus more effective therapies for female or male HF-patients.

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 Ethical Committee of the University of Debrecen (Ethical Statement No. 1/2013/DE MÁB).

Author contributions

Conceptualization, NH; methodology, SZ, RH, MH, HB, ÁK, AT; validation, NH, ÁK; formal analysis, SZ, RH, MH, HB; investigation, NH; re-sources, NH; data curation, NH; writing—original draft preparation, SZ, RH, HB, editing, MH, ÁK, HM, IE, WS, AM, NH; project supervision and administration, NH; funding acquisition, NH. 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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Sex differences in the renin-angiotensin-aldosterone system and its roles in hypertension, cardiovascular, and kidney diseases

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Cardiovascular disease is a pathology that exhibits well-researched biological sex differences, making it possible for physicians to tailor preventative and therapeutic approaches for various diseases. Hypertension, which is defined as blood pressure greater than 130/80 mmHg, is the primary risk factor for developing coronary artery disease, stroke, and renal failure. Approximately 48% of American men and 43% of American women suffer from hypertension. Epidemiological data suggests that during reproductive years, women have much lower rates of hypertension than men. However, this protective effect disappears after the onset of menopause. Treatment-resistant hypertension affects approximately 10.3 million US adults and is unable to be controlled even after implementing ≥3 antihypertensives with complementary mechanisms. This indicates that other mechanisms responsible for modulating blood pressure are still unclear. Understanding the differences in genetic and hormonal mechanisms that lead to hypertension would allow for sex-specific treatment and an opportunity to improve patient outcomes. Therefore, this invited review will review and discuss recent advances in studying the sex-specific physiological mechanisms that affect the renin-angiotensin system and contribute to blood pressure control. It will also discuss research on sex differences in hypertension management, treatment, and outcomes.

KEYWORDS
cardiovascular, hypertension, kidney, renin-Angiotensin system, sex differences

Introduction

Hypertension, defined as blood pressure greater than 130/80 mmHg, has been firmly established as a primary risk factor associated with cardiovascular disease, stroke, and kidney diseases (1–4). In the United States alone, nearly 48% of American men and 43% of American women suffer from hypertension (2). Currently, most if not all available data from clinical studies in humans have consistently shown that premenopausal women are generally protected from the development of hypertension compared with age-matched men, but the prevalence of hypertension increases drastically in women during postmenopausal years. The mechanisms underlying these sex differences or sex dimorphism in the pathogenesis of hypertension in men vs. women remain incompletely understood. Historically, however, biological, physiological, and clinical research were

conducted primarily on male cells, male animal models, and male human subjects, largely based upon the assumption that they are genetically, molecularly, and physiologically identical to their female counterparts (5–7). To further promote biomedical research in sex differences in all physiological and diseased models, the National Institute of Health (NIH) in 2014 began to mandate that all recipients of NIH funding are required to consider sex as biological variables in their experimental approaches to test their hypotheses. This policy has led to an explosion of the research on sex differences or sex dimorphism and the mechanisms involved across the board on the disease development and health outcomes (8).

Although hypertension is a multifactorial medical disorder, the renin-angiotensin-aldosterone system (RAAS) is recognized as one of the most important regulators of basal blood pressure homeostasis and a major contributor in the development of hypertension. This recognition is not only supported by extensive biomedical research in animal models of hypertension, but also by numerous clinical trials using the inhibitors of renin, angiotensin-converting enzyme (ACE), or type 1 angiotensin II (Ang II) receptor (AT_1) or aldosterone receptor blockers to treat hypertension in human subjects (1–4). However, the RAAS is not only the targets for the development and treatment of hypertension, as many hypertensive patients require dual or multidrug therapy with a diuretic, calcium channel blocker, and an α or β blocker to control their blood pressure. Even then, appropriate >10 million Americans still suffer from resistant hypertension even treated with ≥3 antihypertensive medications with blood pressures persisting above the treatment threshold (1–4). The mechanisms underlying the development of resistant hypertension and the difficulty in treating resistant hypertension remain poorly understood. One of major problems may involve sex differences in the pathogenesis, mechanisms, and treatment of resistant hypertension between aging men and postmenopausal women. Thus, there is an urgent need for further studies of the sex differences in the mechanisms of hypertension and the contributions of the RAAS, which may offer more tailored or precision hypertensive treatments and achieve better therapeutic outcomes.

Against this background, the objective of this invited article is to review and discuss recent advances in studying sex differences or dimorphism in the RAAS and its contributions to the physiological regulation of blood pressure and in the development of hypertension, cardiovascular and kidney diseases. Our emphases will include sex differences in the RAAS and the mechanisms by which sex hormones and the RAAS contribute to normal blood pressure control and the development of hypertension, sex differences in the hypertension treatment and outcomes, as well as potential strategies for sex-specific treatment of resistant hypertension in humans.

Overview of the localization and roles of the RAAS in cardiovascular and kidney tissues

To help better understand the sex differences in the RAAS and its contributions to the regulation of cardiovascular and renal physiology and the development of hypertension and cardiovascular and kidney diseases, it is important to first review the localization and roles of the RAAS briefly. The RAAS has been delineated as a primary effector of the development of hypertension and two main axes responsible for blood pressure control have been established. The angiotensinogen (AGT)/renin/angiotensin-converting enzyme (ACE)/angiotensin II (Ang II)/AT_1 receptor (AGT/renin/ACE/Ang II/AT_1R) axis is the predominant pathway for Ang II formation and responsible for most if not all classic effects of Ang II in the development of hypertension and cardiovascular and kidney diseases (9) (**Figure 1**). The juxtaglomerular apparatus of the kidney tightly regulates renin release from the kidney via two important mechanisms—a baroreceptor mechanism that senses decreased blood pressure or blood volume loss within the renal vasculature and an osmoreceptor mechanism that senses NaCl delivery from the proximal nephron to the macula densa (10–14). Renin comprises the rate-limiting step in the activation of the RAAS, converting AGT to Ang I, so its expression levels are in constant balance via a variety of biological mechanisms (15). Ang I is then converted to the biologically active peptide Ang II by ACE. In addition to renin- and ACE-dependent pathways, non-renin/ACE independent pathways may also contribute to the formation and metabolism of Ang II in cardiovascular and kidney tissues (**Figure 1**). Chymase, a serine endopeptidase, is highly expressed in the heart of patients with cardiovascular diseases compared to ACE (16, 17), and reportedly ~75% of Ang II is estimated to be generated from Ang (1–8, 10–13) in cardiac tissues by chymase rather than ACE (18, 19). The catalytic activity of chymase is reportedly about 20-fold higher compared to ACE (19, 20). In rats with pressure-overload, the expression of chymase was significantly increased in female than male rats (21). In the kidney, neprilysin (NEP), an endopeptidase, is highly expressed that directly cleaves Ang I into Ang (1-7) and shows much higher catalytic activity for Ang I compared ACE2 (22, 23). The expression of NEP in kidney is reportedly higher in female than male hypertensive mRen (2). Lewis rats (24). Thus, both renin/ACE-dependent and non-renin/ACE-dependent pathways may contribute to Ang II formation or metabolism in cardiovascular and kidney tissues in health and diseases (25, 26) (**Figure 1**).

The most pertinent G protein-coupled receptors with which Ang II activates are AT_1 and AT_2 receptors. AT_1 receptors can be classified further into two subtypes: AT_{1a} and AT_{1b}. In humans, there is only one AT_1 receptor that is expressed, corresponding to the AT_{1a} receptor found in rodents (27–29). The AT_1 receptor is generally considered to have pro-hypertensive, pro-growth, and pro-proliferative downstream effects. Activation of the AT_1 receptor promotes vasoconstriction, increased oxidative stress, aldosterone release, and renal sodium absorption which all contribute to the regulation of blood pressure and fluid homeostasis, as well as the development of hypertension and cardiovascular and kidney diseases (30, 31) (**Figure 2**). In the kidney, activation of AT_1 receptors especially induces the sodium-hydrogen exchanger 3 (NHE3) expression in the proximal tubules and the ascending limp of loop of Henle, resulting in the impairment of the pressure-natriuresis response

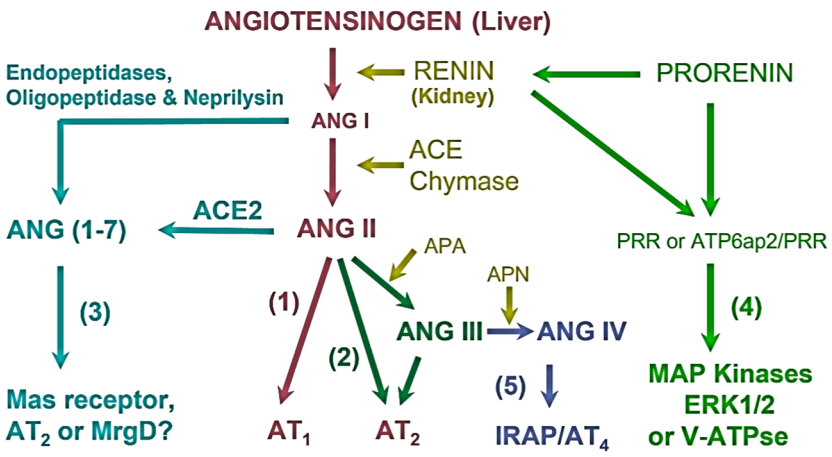

FIGURE 1
Classical renin/ACE-dependent and non-renin/ACE-dependent pathways for Ang II formation, metabolism, and actions in cardiovascular and kidney tissues. (1) The classical angiotensinogen/renin/ACE/ANG II/AT_1 receptor axis. (2) The ANG II/APA/ANG III/AT_2 receptor/NO/cGMP axis. (3) The ANG I/ANG II/ACE2-Neprilysin/ANG (1–7)/*Mas* receptor axis. (4) The prorenin/renin/prorenin receptor (PRR or ATP6ap2)/MAP kinases ERK1/2/V-ATPase axis. (5) The ANG III/APN/ANG IV/AT_4 receptor/IRAP axis. Note that not only ACE but also chymase generate ANG II from ANG I, whereas neprilysin also cleaves ANG I to generate ANG (1-7). ACE, angiotensin-converting enzyme; ACE2, angiotensin-converting enzyme 2; APA, aminopeptidase A; APN, aminopeptidase N; IRAP, insulin-regulated aminopeptidase; PRR, prorenin receptor. Modified from reference (9) with permission.

and an increase in blood pressure (32–36). Conversely, Ang II activation of AT_2 receptors works against the pro-hypertensive, pro-growth, and proliferative effects of AT_1 activation, causing vasodilation and increased natriuresis (**Figure 2**) (34, 37–40). However, Ang III, a biologically active metabolite of Ang II, also acts to increase the natriuresis response reportedly by regulating Na^+/K^+-ATPase activity and reducing NHE3 activity (41–44).

The final cascade of the RAAS is the release and function of aldosterone from the adrenal glands. Ang II and Ang III both contribute to the stimulation of aldosterone release from the adrenal glands via binding to and activation of AT_1 and AT_2 receptors (**Figure 3**) (45, 46). Aldosterone is a mineralocorticoid that increases blood pressure by inducing the expression and activity of the epithelial sodium channel (ENaC) (47, 48). Previous studies have shown that Ang II stimulates aldosterone secretion in the zona glomerulosa cells (ZG) of the adrenal cortex and catecholamine release from chromaffin cells of the adrenal medulla. The catecholamines may stimulate aldosterone secretion via a paracrine mechanism (49, 50). Most if not all Ang II-induced aldosterone biosynthesis and release from the adrenal glands are mediated by AT_1 (AT_{1a}) receptors. Ang III has been demonstrated to have significant, if not equivocal aldosterone stimulating effects, to Ang II, but is hypothesized to primarily work through AT_2 receptor activation (46, 51–54). Aldosterone acts to stimulate ENaC expression to increase sodium reabsorption primarily in the distal nephron and collecting tubules, resulting in blood pressure elevation (55). Additionally, increased levels of circulating aldosterone have been found to contribute to the pathogenesis of hypertension by causing endothelial dysfunction via increased production of reactive oxygen species (56).

In addition to the AGT/renin/ACE/Ang II/AT_1 receptor axis, there exits an alternative counteracting angiotensin-converting enzyme 2 (ACE2)/Ang (1-7)/Mas receptor/AT_2 receptor (ACE2/Ang (1-7)/MasR/AT_2R) axis in the cardiovascular and kidney tissues, which is responsible for inducing vasorelaxation, lower blood pressure, and natriuretic responses (9, 57, 58) (**Figure 2**). Ang (1-7) is a biologically active derivative of Ang I and Ang II that are enzymatically cleaved by ACE2 (57, 58). The primary effects of Ang (1-7) are to counter the effects of the AGT/renin/ACE/Ang II/AT_1 receptor axis by binding to G-protein coupled Mas receptors (MasR) and inducing the release of nitric oxide (NO), prostaglandin E_2, and bradykinin to promote vasodilation (59–64). Ang (1-7) infusion was also found to reduce plasma renin activity, which may contribute to its antihypertensive effect (65).

In the kidney, the (pro)renin receptor (PRR) is another receptor that has been established as an important RAAS modulator in the cardiovascular and kidney tissues. PRR is encoded by the ATP6AP2 gene on the X chromosome and has been localized to many tissues including adipose, heart, brain, vessel wall, placenta, and kidney (66–71). Three forms of the protein exist including PRR, soluble PRR (sPRR), and truncated PRR (tPRR). sPRR is released into the plasma, while tPRR remains within the cellular membrane. PRR binds to renin and prorenin resulting in approximately a 5-fold increase in angiotensinogen conversion to angiotensin I (72). PRR has been implicated in both water and sodium homeostasis, as well. During water deprivation trials, PRR and sPRR expression is markedly increased and animal models with principal cell specific PRR deletion have demonstrated significant reductions in AQP2 expression and urine osmolality (73–76). Ang II has also

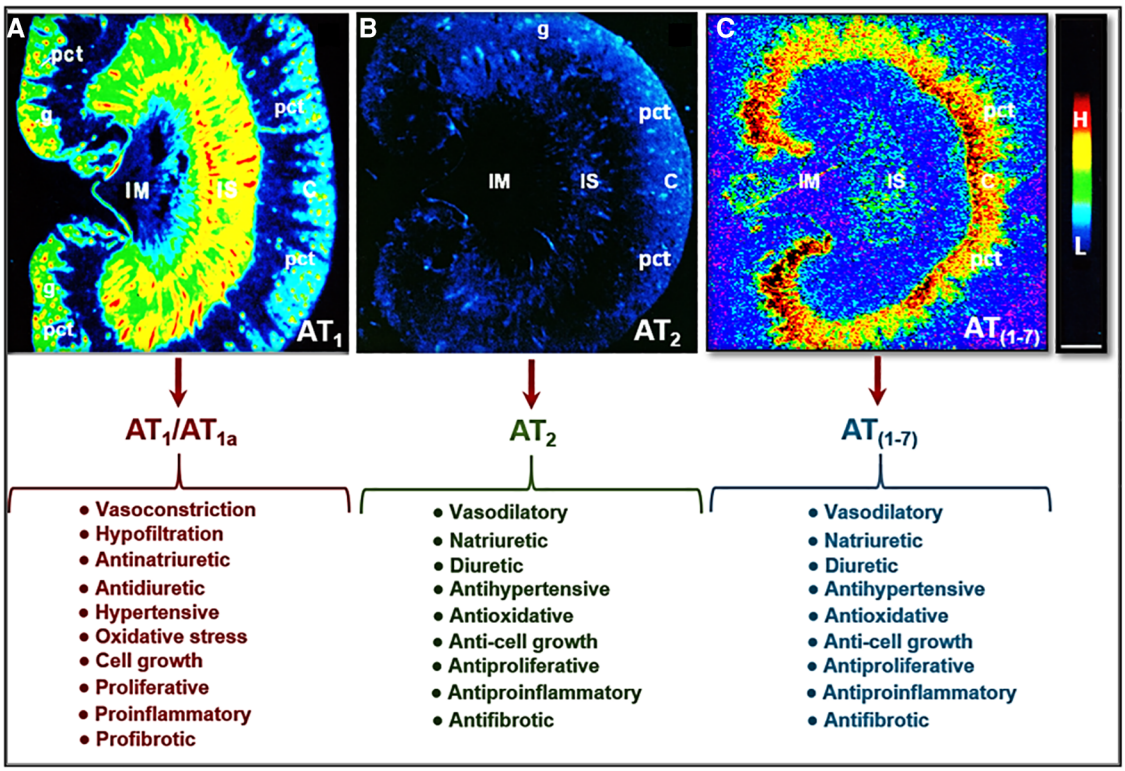

FIGURE 2
Localization of Ang II type 1 (AT_1 or AT_{1a}) and type 2 receptors (AT_2) in the rat kidney using ^{125}I-labeled Ang II receptor autoradiography and opposing actions of AT_1 (AT_{1a}), AT_2, and/or AT (1-7) receptor activation in the kidney. (**A**) Shows the localization of AT_1 or AT_{1a} receptors with high levels in the glomerulus (g) and the inner stripe of the outer medulla corresponding to vasa recta bundles, and moderate levels in the proximal convoluted tubules (pct) in the cortex (C) and renomedullary interstitial cells (RMICs) in the inner stripe of the outer medulla between vasa recta bundles. The inner medulla (IM) expresses a very low level of AT_1 or AT_{1a}. (**B**) Shows the localization of AT_2 receptors with low levels in the outer cortex, corresponding to the glomeruli and the proximal tubules, and the inner stripe of the outer medulla, corresponding to vasa recta bundles and RMICs. (**C**) Shows the localization of the receptor binding for Ang (1-7) in the kidney primarily in the inner cortex corresponding to the proximal tubules. Red represents high level (H), whereas dark blue represents background levels (L). Modified from reference (30) with permission.

been found to increase AQP2 expression within the collecting duct through several intracellular signaling pathways (77). However, animal studies have demonstrated that chronic Ang II infusion augments sPRR expression which in turn augments water reabsorption via AQP2 demonstrating a positive feedback mechanism within the collecting duct (78). PRR in the collecting duct may cause a marked increase in blood pressure via increasing ENaC expression (76, 79, 80). The precise mechanisms and downstream effects of PRR and its derivatives on water, sodium, and blood pressure have been thoroughly reviewed elsewhere (81).

It is now well-recognized that multiple RAAS axes are working concomitantly to regulate blood pressure and tissue perfusion (32, 34, 43, 82–86). The circulating or classical RAAS including all major components that have well-recognized endocrine effects (15, 32, 34). By contrast, the RAAS in the kidney may represent an important paracrine/autocrine/intracrine system, eliciting a more local and intracellular effect within the kidney tissue, especially within the proximal tubules (32, 34, 43, 82–86). Notably, the intrarenal RAAS has been found to have markedly higher concentrations of Ang II when compared to circulating plasma concentrations (87–93). Chronic Ang II exposure typically causes a down-regulation of AT_1 receptors in different cardiac and vascular tissues; however, within the intrarenal RAAS, AT_1 receptor expression is either constant or upregulated during the development of hypertension, cardiovascular and kidney diseases (94, 95).

Recently, there is evidence supporting a functional role for an intracellular and mitochondrial RAS as well. Initial animal studies demonstrated the presence of Ang II binding sites within hepatic cells (33, 92, 96–99). Since then, significant progress has been made in characterizing intracellular RAS within other tissue types. Within the kidney, high-density specific receptors for Ang II and Ang (1-7) were localized to cortical nuclei in sheep and rats (100–103). A fully functional RAS has also been demonstrated within the mitochondria (33, 104, 105). The exact origin of the intracellular RAS and its role in blood pressure homeostasis is yet to be determined, but there is evidence suggesting that they both serve physiological functions in the context of Ang II-induced hypertension (106, 107).

Clearly, recent studies in delineating the vasoconstrictive properties of the AGT/renin/ACE/Ang II/AT_1 receptor and the vasodilatory properties of the counteracting ACE2/Ang (1-7)/Mas receptor/AT_2 receptor axes have greatly expanded the therapeutic

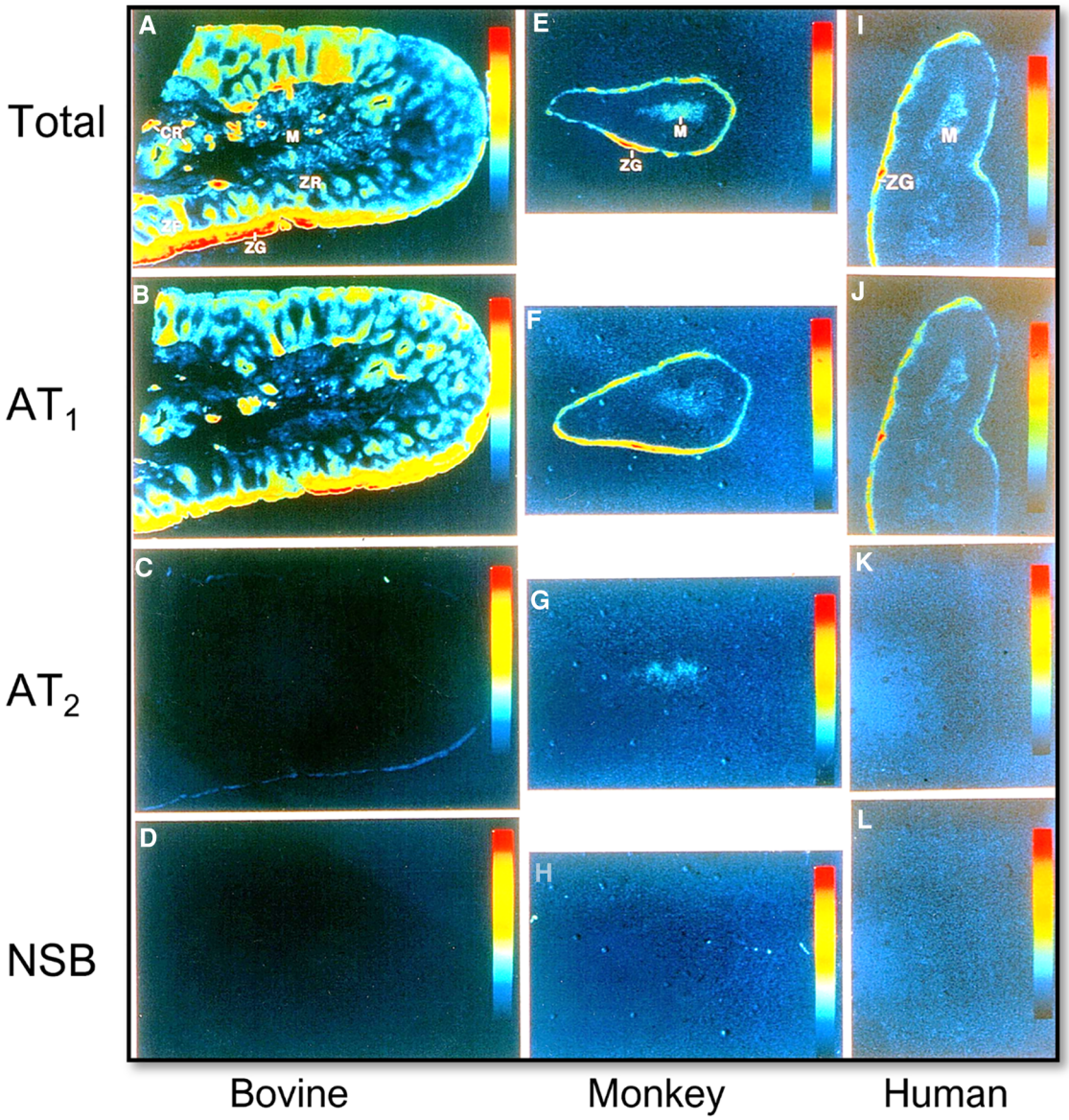

FIGURE 3
Localization of Ang II type 1 (AT_1 or AT_{1a}) and type 2 receptors (AT_2) in the bovine, monkey, and human adrenal glands using quantitative ^{125}I-labeled Ang II receptor autoradiography. (**A,E,I**) Represent total Ang II receptor binding; (**B,F,J**) represent AT_1 receptor binding in the presence of an excess concentration of the AT_2 receptor blocker PD123319 (10 μM); (**C,G,K**) represent AT_2 receptor binding in the presence of an excess concentration of the AT_1 receptor blocker losartan (10 μM); and (**D,H,L**) represent nonspecific binding in the presence of an excess concentration of unlabeled Ang II (10 μM), respectively. AT_1 receptors predominate in the zona glomerulosa cells (ZG) of the adrenal cortex where aldosterone is synthesized and release into the circulation (**B,F,J**), and the adrenal medulla (M). AT_2 receptors are low in the adrenal glands of bovine, monkey, and human adrenal glands (**C,G,K**). Red represents the highest level, whereas dark blue represents the background level of receptor binding. Modified from reference (32) with permission from the copyright holder.

targets available to treat hypertension and cardiovascular and kidney diseases. Currently, first-line pharmacological treatments for hypertension include monotherapy or combination therapy using ACE inhibitors and angiotensin AT_1 receptor blockers (ARBs), thiazide diuretics, and long-acting dihydropyridine calcium channel blockers (108, 109). Alpha- and β-blockers have also been identified as adjunctive treatments for hypertension, but they have additional side effects that may make them intolerable to patients including asthma exacerbations, insomnia, worsening glucose intolerance, bradycardia, and sick sinus syndrome (110, 111). Treatment-resistant hypertension is defined as hypertension that is unable to be controlled after the implementation of three antihypertensives with complementary mechanisms (1–4). Now affecting nearly 10.3 million Americans, it has become increasingly prevalent in the United States, indicating a need for alternative or additional therapies (2). Since the classical RAAS has been expanded in recent years, various new drugs have been developed to target these new substrates and receptors. Preclinical data has supported Ang (1-7) and AT_2 agonists as viable treatment targets, but whether they are

effective therapeutic targets in hypertension, cardiovascular and kidney diseases remains to be confirmed in clinical trials (112–114).

Sex differences in the RAAS and their roles in cardiovascular and renal physiology and hypertension

Sex differences in vascular dysfunction

Evidence has repeatedly demonstrated that there is an age-dependent difference in the prevalence of hypertension between men and women. Until age 45, women are less likely to develop hypertension than men, while this difference is not present between ages 46 and 64 (2, 115, 116). After age 65, the prevalence among women increases significantly. It is estimated that 85% of women over 75 have hypertension compared with 79% of men within the same age group (2, 115, 116). Recent studies are ongoing to further characterize these differences and underlying mechanisms in the RAAS between males and females, which may contribute to this age-dependent difference in the prevalence of hypertension between men and women.

There are several baseline physiological differences that contribute to the development of hypertension that have been observed in male and female subjects. Nitric oxide (NO), which has vasodilatory effects, has been established as a key mechanism of blood pressure homeostasis (117, 118). NO plays a protective role in the development of hypertension because of its vasodilatory effects and ability to quickly react with superoxide to counteract the latter's effects (119). Animal studies have shown that females have greater NO bioavailability compared with males due to higher NO-generating capacity in females and increased oxidative stress levels in males (120–125). Oxidative stress causes endothelial dysfunction due to vasoconstriction and the activation of the RAAS in blood vessels. *In vivo* studies have shown that Ang II causes mesangial cells in the kidney to produce superoxide, while the inhibition of the RAAS has been shown to reduce oxidative stress (126, 127). More recent data has demonstrated that mice treated with buthionine sulfoximine (BSO), a substance that induces oxidative stress, had higher levels of AT_1 receptors within the proximal tubules. Additionally, they demonstrated a more dramatic downstream signaling effect, indicating that oxidative stress sensitizes kidney cells to produce an amplified RAS response (128). An inflammatory response to oxidative stress is also activated by Ang II via AT_1 receptors, leading to nuclear factor-κB (NF-κB) transcription factor expression (128, 129).

Sex differences and the cardioprotective roles of estrogen

In view of the age differences well-recognized in hypertension prevalence between males and females, the interactions between estrogen and the RAAS have become an important research focus (130). Estrogen is a steroid hormone that binds to two nuclear receptors, estrogen receptor-α (ER-α) and estrogen receptor-β (ER-β), and G protein-coupled estrogen receptor 1 (GPER1) (130–133). ER-α is abundantly expressed in the vascular endothelium and helps promote vasodilation, endothelial repair, and NO production (134). ER-β activation primarily results in NO production (134, 135). Together, the binding of estrogen to these two receptors increases vasodilation and has a protective effect against hypertension. Esqueda et al. demonstrated that after ovariectomy, estrogen-supplemented, salt-sensitive rats had restored ER-β expression levels. The same was not demonstrated for ER-α, implying that the imbalance between ER-α and ER-β might contribute to the development of hypertension after menopause (136).

In animal studies, estradiol has been found to have a role in protecting against hypertension. In spontaneously hypertensive rats (SHRs), young male rats have demonstrated higher mean blood pressures than young female rats (137–140). This difference was eliminated through pharmacological RAS inhibition and the cessation of estrous cycling, implicating estrogen as the cardioprotective factor and accounting for the sex and age-related differences (139, 141, 142). Aging SHRs have been established as a model for postmenopausal hypertension due to their non-cycling, low serum estradiol and the ensuing increase in blood pressure (142, 143).

In human studies, 17β-estradiol (E_2) has been determined to regulate the RAS via the changes in this enzyme expression. For example, Proudler et al. investigated the effect of estrogen/progesterone combined hormone replacement therapy (HRT) on ACE activity in postmenopausal women. They determined that ACE activity was reduced by 20% in treated women when compared to their untreated controls; however, this study was limited by sample size, including only 28 women in the treatment group and 16 in the untreated group (144). Soon after, Schunkert et al. measured and compared renin and angiotensinogen levels between women treated with estrogen replacement therapy (ERT) and those who were not. Renin levels were found to be significantly increased in women without ERT, measuring 16.6 ± 0.9 mU/L compared to 12.0 ± 0.7 mU/L in the treated group. Angiotensinogen levels were found to be higher in women with ERT, compared to those without, indicating a reduced rate of conversion by renin (145). Thus, these studies provide the evidence for estrogen's cardioprotective effects in part by regulating the expression or activity of the RAS.

Sex differences in the classical RAS and the role of estrogen

New data has recently built upon these previous studies to elucidate the mechanisms by which estrogen modulates the classical RAS. Essentially, estrogen can alter RAS activities by regulating the levels of key substrate, enzyme, and receptor expression, and protein production. Animal studies have shown that the expression of the RAS enzymes was significantly altered in the presence or absence of estrogen. In young male SHRs,

ACE mRNA expression in the kidneys was significantly increased when compared to their female counterparts (146, 147). Similar results were found in two-kidney, one-clip (2K1C) renal hypertension animal models (147). This difference in intratubular enzyme concentrations is attenuated between aging SHR male and female rats (148). In aging SHRs, plasma renin activity (PRA) and concentrations of AGT and Ang II, which are measures of the circulatory RAS activation, were not significantly different between aging male and female SHRs. However, intratubular AGT expression was increased in males when compared to females, whereas aging females were found to have higher Ang II expression (148). These data suggest that in young rats, males have higher levels of intratubular RAS enzyme expression and cascade activation compared to females. In aging rats, when the protective effect of estrogen has diminished, females have increased intrarenal RAS activation and higher levels of Ang II. In addition to the regulation of renin and ACE, estrogen also regulates the renin- and ACE-independent enzymes in the RAS. Ahmad et al. and others compared the metabolic pathway for Ang II formation in cardiac tissues of gonadal-intact and ovariectomized (OVX) adult Wistar Kyoto (WKY) and SHR rats, and found that estrogen depletion significantly increased chymase activity, but not ACE activity (24, 25). Li et al. demonstrated that estrogen inhibits chymase release from cardiac mast cells to prevent pressure overload-induced adverse cardiac remodeling (20). The latter studies suggest that estrogen status may play an important role in the regulation of cardiac chymase expression and cardiovascular protection in adult female animals (20, 24, 25).

Estrogen also plays an important role in regulating the RAS through the modulation of AT_1 and AT_2 receptor expression (141). In animal studies comparing arterial AT_1 expression in male rats, ovariectomized rats, and estrogen-supplemented ovariectomized rats, AT_1 receptor density was found to be significantly increased in the males and ovariectomized rats when compared to those supplemented with estrogen (140, 149). In aging SHRs, this difference is eliminated and AT_1 expression was found to be the same between male and female rats (148). Silva-Antonnialli et al. demonstrated that AT_2 receptor expression was similar among male, female, oophorectomized females, and estrogen-replaced females, causing the AT_1/AT_2 ratio in estrogen-treated females to be higher (140). These studies suggest that estrogen's protective role can be partially attributed to its ability to downregulate AT_1 receptor expression. Indeed, these differences are supported by the studies showing a significant difference in the response to AT_1 blockers. For instance, aging male rats were observed to have 52% decrease in mean arterial blood pressure, while females only had a 37% drop (148). Increased Ang II or its AT_1 receptor expression in the kidneys of postmenopausal female rats may explain why postmenopausal women are more susceptible to the development of hypertension and the roles of estrogen in sex differences in hypertension.

The third mechanism by which estrogen can influence blood pressure via the classical RAS is by regulating aldosterone secretion. Aldosterone is known to cause increased salt retention and blood pressure. In animal studies, estrogen was found to reduce AT_1 receptor expression in the adrenal glands, which in part contribute to reduced aldosterone secretion (150). More recent clinical studies have shown that when consuming high salt diets, men had significantly higher plasma aldosterone, extracellular volume, and systolic blood pressure than women (151). These two studies further suggest that aldosterone secretion may be a key contributor to the sex differences in hypertension prevalence between men and women.

However, the sex differences or the sexual dimorphism of PRR and its role in the development of hypertension remain poorly understood. A study on type 2 diabetic men and women reported that plasma sPRR was significantly higher in women compared to men and that sPRR concentrations appeared to correlate with age, BMI, eGFR, and plasma renin activity in female subjects, though not statistically significant in the male subjects (152). The finding that increased age correlates with increased sPRR and systemic RAS activation suggests that the transition to an estrogen-deficient state of menopause causes increased sPRR expression and RAS activation. However, more work is necessary to characterize the mechanism by which estrogen and PRR interact in further studies.

Sex differences in the vasoprotective axis of the RAS and the role of estrogen

In addition to inhibitory effects on the classical RAS system, estrogen exerts antihypertensive effects via upregulation of the substrate and enzymes in the counterregulatory RAS pathways. Lee et al. studied ACE2 expression in control and 2K1C male and female rats and demonstrated that female rats showed increased intratubular ACE2 expression regardless of 2K1C treatment status, suggesting estrogen's protective role in increasing Ang II metabolism to Ang (1-7) (147). In studies using human umbilical vein endothelial cells (HUVEC), estrogen activation of ER-α receptors was shown to elevate intracellular ACE and ACE2 mRNA expression and ACE protein expression. This increased ACE2 expression is expected to increase intracellular Ang (1-7) formation (153). This data supports the hypothesis that the intracellular RAS, especially ACE2 and Ang (1-7), and estrogen cooperate in a manner that protects against the development of 2K1C renal hypertension, most likely due to increased Ang (1-7) production and AT_2 receptor activation.

The MasR is another component of the alternative vasoprotective RAS pathway that demonstrates sex-dependent properties. Previous studies have solidified the hypothesis that NO release is mediated by Ang (1-7) activation of MasR (64, 154, 155). Sobrino et al. used HUVEC to demonstrate that estradiol increased the intracellular expression of enzymes responsible for Ang (1-7) and NO production (156). Their data showed that estradiol treatment increased ACE and cathepsin A expression which are ultimately responsible to produce Ang (1-7). These authors also reported that eNOS and cytosolic guanylate cyclase expression was increased, indicating that NO synthesis was promoted by estradiol treatment. When MasR was blocked, they found that NO levels were decreased, supporting

their hypothesis that estradiol mediates increased NO production via the activation of MasR (156). Mompéon et al. also used HUVEC to show that estradiol increased Ang (1-7) production via ER-α activation and increased ACE2 mRNA expression (153). One limitation of these studies, however, is the tissue-specific characteristics of intracellular RAS. It would be beneficial to utilize human or animal kidney cells to fully determine the relationship between estrogen treatment and intracellular RAS responses in the kidney.

In addition to *in vitro* cell culture studies, animal studies have also demonstrated estrogen effects on MasR function. When subjected to Ang II infusion, female rats demonstrated reduced renal blood flow responses, but only in the context of dual MasR and AT_1 blockade (157, 158). With AT_1 blockade, there is an increased concentration of circulating Ang II, possibly allowing for increased Ang (1-7) formation via the ACE2 pathway. Saberi et al. compared the effects of estrogen supplementation in response to Ang (1-7) infusion and MasR blockade. They found that estradiol-treated ovariectomized rats had decreased renal blood flow in response to Ang (1-7) after MasR blockade when compared to their untreated counterparts (159). These studies suggest that one of estradiol's antihypertensive mechanisms operates via MasR activation. When MasR is blocked, there are fewer opportunities for estrogen to exert protective effects leading to decreased renal blood flow and worsening hypertension.

Finally, an additional protective axis of the RAAS consisting of Ang III/AT_2 receptor activation is also modified by estrogen. Female mice have been shown to utilize the AT_2 receptor pathway to attenuate the effects of Ang II via AT_1 receptors; however, this effect diminishes with increased age (160, 161). Another study demonstrated that exogenous estrogen replacement reinstituted this protective pathway and attenuated Ang II-induced hypertension (162). Together, these studies support the hypothesis that estrogen affects the RAS primarily through activation of the vasoprotective signaling pathways, rather than the attenuation of the classical RAAS signaling pathway. This evidence could result in novel therapeutics for estrogen-deficient individuals who are suffering from resistant hypertension.

Sex differences in Ang II-induced hypertension and the roles of testosterone and estrogen

There is no question that testosterone contributes to sex differences in cardiovascular and kidney diseases and hypertension, but its contribution to sex differences is not as well-studied as that of estrogen. Historically, there are animal studies showing mild adverse effects of testosterone on hypertensive outcomes in young spontaneously hypertensive rats (138, 139, 163, 164). Dalmasso et al. have suggested that in aging SHRs, testosterone supplementation causes a reduction of blood pressure, indicating that age, in concordance with testosterone status, affects hypertensive outcomes rather than testosterone alone (164). A more recent animal study determined that testosterone played a permissive role in the development of hypertension since Ang II-induced hypertension was worsened when castrated males were supplemented with exogenous testosterone (165). They also noted that castrated males demonstrated a reduced AT_1/AT_2 receptor ratio, which favors the vasoprotective axis of the RAS. This ratio was restored when testosterone was re-administered (165). A mendelian randomization model concluded that high testosterone states could lead to increased rates of hypertension (166). Studies utilizing human subjects present only mildly convincing data. In women specifically, one study showed some evidence that high testosterone states were correlated with increased carotid-femoral pulse wave velocities, which is an indicator of arterial stiffness (167). One review article summarizing the effects of testosterone therapy on various laboratory markers of transgender men concluded that there was only weak evidence supporting the correlation between increased blood pressure and testosterone administration (168). Interestingly, some studies have correlated testosterone-deficient states to the development of hypertension, which would appear to be contrary to the trends observed in previous studies. One such study investigated the effects of free testosterone and biologically available testosterone on blood pressure. It found that free testosterone is essentially inversely correlated with systolic and diastolic blood pressure in men (169). Given the evidence, it is likely that increased testosterone levels in conjunction with decreased estrogen levels, like those found in PCOS, work synergistically to facilitate the development of hypertension. Further research is necessary to characterize the mechanisms by which testosterone regulates blood pressure and its role in the development of hypertension.

Whether there are sex differences in Ang II-dependent or Ang II-induced hypertension remains to be further studied. Some inconsistencies have been reported in the roles of sex differences in Ang II-induced hypertension in animal models (160–162, 170, 171). These inconsistencies range from complete reversal, attenuated responses, or no effect at all in female rats or mice, based on the doses of Ang II infusion (low pressor or high pressor), animal models (rat or mouse, global AT_{1a} or AT_2 receptor knockout), or routes of administration (subcutaneous or intraperitoneal infusion) (160–162, 170, 171). It is difficult to directly compare these studies and draw a clear conclusion on whether sex differences contribute to the development of Ang II-induced hypertension. Indeed, no significant sex differences in basal blood pressure levels in age-matched adult male and female Sprague-Dawley rats, wild-type, or AT_2 receptor knockout mice in which Ang II induced similar increases in blood pressure, natriuretic, or diuretic responses (172–175).

Recently, we have determined whether there are sex differences in the blood pressure, renal excretory, and fibrotic responses to Ang II between male and female wild-type mice, and between male and female proximal tubule-specific AT_{1a} receptor knockout mice (PT-*Agtr1a*$^{-/-}$) (170, 171). Although we found sex differences in some minor phenotypic responses, deletion of AT_{1a} receptors

selectively in the proximal tubules decreased basal arterial blood pressure similarly in both male and female wild-type and PT-*Agtr1a*$^{-/-}$ mice. Both male and female wild-type and PT-*Agtr1a*$^{-/-}$ mice responded to Ang II infusion and developed hypertension to the similar magnitudes (**Figure 4**) (170, 171). The maximal pressor responses remained to be ~20 mmHg lower in male and female PT-*Agtr1a*$^{-/-}$ mice than male and female wild-type mice. Furthermore, concurrent blockade of AT_1 receptors with losartan decreased the pressor response to Ang II to similar extents in male and female wild-type and PT-*Agtr1a*$^{-/-}$ mice (170, 171). Thus, no significant sexual dimorphism or sex differences in blood pressure phenotypes were discovered in wild-type and PT-*Agtr1a*$^{-/-}$ mice in response to Ang II or AT_1 receptor blockage. However, we did uncover sex differences in Ang II-induced hypertension in a mutant mouse model with deletion of the Na^+/H^+ exchanger 3 (NHE3) selectively in the proximal tubules of the kidney (PT-*Nhe3*$^{-/-}$) (36). In male wild-type and PT-*Nhe3*$^{-/-}$ mice infused with a high pressor dose of Ang II, systolic, diastolic, and mean arterial blood pressure increased in a time-dependent manner reaching a peak response within a week of Ang II infusion (**Figure 5**). In female PT-*Nhe3*$^{-/-}$ mice, however, systolic, diastolic, and mean arterial blood pressure responses to Ang II began to decrease 4 days after Ang II infusion, suggesting that estrogen (and/or other female hormones) may contribute to these sex differences in Ang II-induced hypertension in this mutant mouse model (**Figure 5**).

Sex differences in antihypertensive treatments or managements

In 2017, the American College of Cardiology published new guidelines for the treatment of hypertension. They stratified blood pressure into five categories with different treatment strategies or approaches. Non-pharmacological interventions are an integral part of controlling hypertension of all categories. Lifestyle changes that promote blood pressure reduction include weight loss, DASH diet, sodium intake reduction, dietary potassium supplementation, increased physical activity, and reduced alcohol consumption (1–4). These lifestyle changes are recommended to every patient, regardless of blood pressure status. Patients are initiated on BP-lowering medications once they are diagnosed with Stage 1 and have ASCVD or a 10-year CVD risk ≥10% (1–4). Primary agents for the treatment of hypertension include thiazide diuretics, ACE inhibitors, angiotensin II receptor blockers (ARBs), and calcium channel blockers (CCBs). Secondary agents include loop diuretics, potassium-sparing diuretics, aldosterone antagonists, beta-blockers, direct renin inhibitors, alpha-blockers, and direct vasodilators (1–4).

The INTERHEART study established that elevated blood pressures presented an increased risk for adverse cardiac events for female subjects when compared to male subjects (176). Regarding control, there has been an ongoing debate about the risks and benefits of intensive vs. less intensive therapy. The 2021

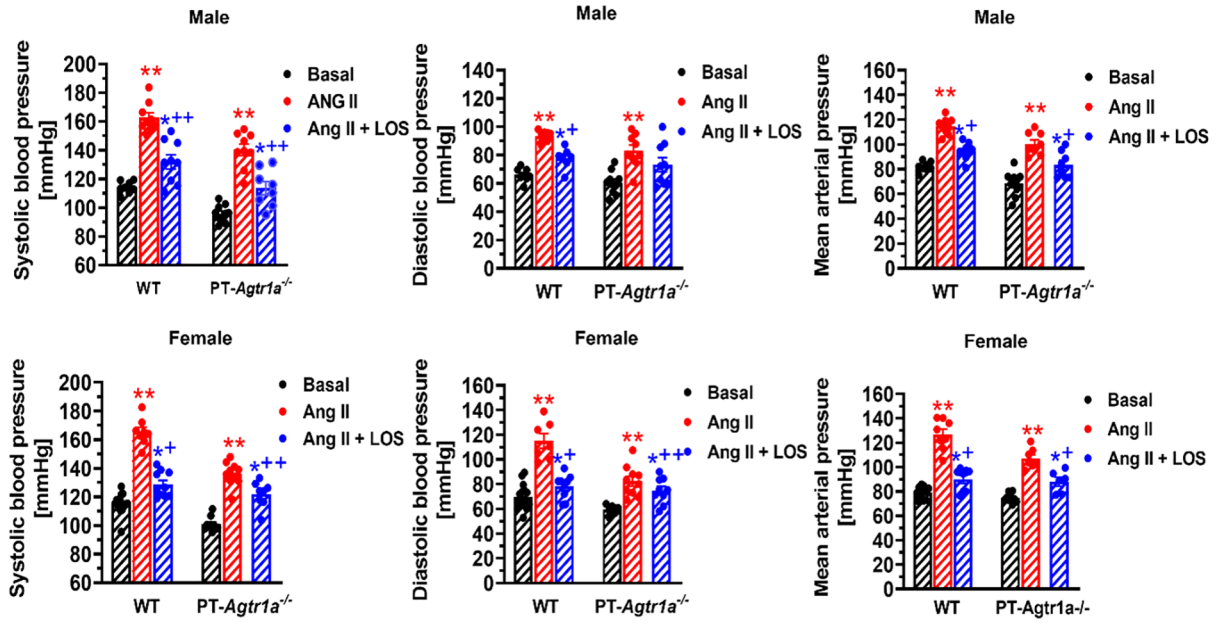

FIGURE 4
Comparisons of basal systolic, diastolic, and mean arterial blood pressure and their responses to Ang II infusion with or without AT_1 (AT_{1a}) receptor blocker losartan between male and female wild-type (WT) and PT-*Agtr1a*$^{-/-}$ mice. Proximal tubule-specific deletion of AT_{1a} receptors significantly decreased basal blood pressure similarly in male and female PT-*Agtr1a*$^{-/-}$ mice under basal conditions, and significantly attenuated the hypertensive responses to Ang II similarly in both male and female PT-*Agtr1a*$^{-/-}$ mice. No significant sex differences were found in basal blood pressure and its responses to Ang II with or without losartan treatment between male and female WT or between male and female PT-*Agtr1a*$^{-/-}$ mice. $^{*}P < 0.05$ or $^{**}P < 0.01$ vs. control WT or PT-*Agtr1a*$^{-/-}$ mice; $^{+}P < 0.05$ or $^{++}P < 0.01$ vs. Ang II-infused male or female wild-type or PT-*Agtr1a*$^{-/-}$ mice. Reproduced from reference (171) with permission.

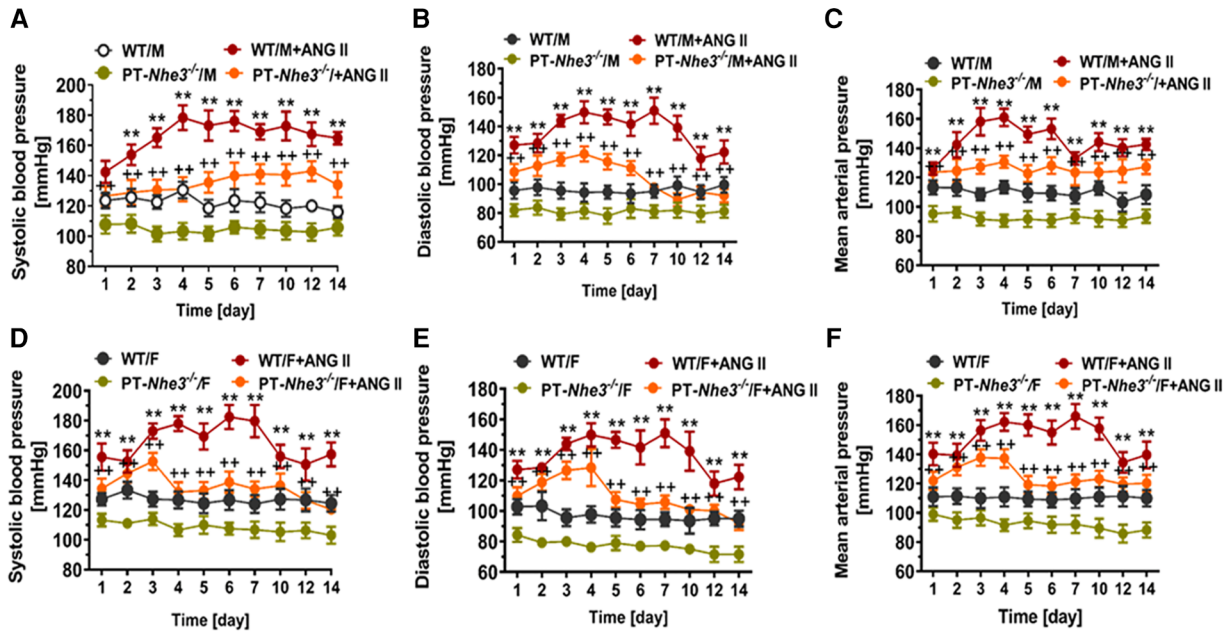

FIGURE 5
Sex differences in basal systolic, diastolic, and mean arterial blood pressure and their responses to a high pressor dose of Ang II infusion, 1.5 mg/kg per day, intraperitoneal via osmotic minipump in conscious, adult male and female wild-type (WT) and PT-*Nhe3*$^{-/-}$ (proximal tubule-specific NHE3 knockout) mice, as measured using the direct implanted telemetry technique. Please note the time-dependent increases in systolic, diastolic, and mean arterial blood pressure responses to Ang II infusion in male WT mice and significantly attenuated hypertensive responses to Ang II in male PT-*Nhe3*$^{-/-}$ mice. However, systolic, diastolic, and mean arterial blood pressure responses to Ang II began to decrease 4 days after Ang II infusion in female PT-*Nhe3*$^{-/-}$ mice, revealing significant sex differences in these mutant mice. (A–C) Male mice; whereas (D–F) female mice. $^{**}P < 0.01$ vs. WT time-control group; $^{++}P < 0.01$ vs. PT-*Nhe3*$^{-/-}$ time-control group, respectively. Reproduced from reference (36) with permission.

SPRINT trial concluded that patients with increased cardiovascular risk were less likely to experience a major adverse cardiac event when their target systolic blood pressure was <120 mmHg when compared to the less intensive <140 mmHg target that was previously established by clinical guidelines (177). When the data is analyzed by sex, the hazard ratio is not statistically significant in the female subgroup. It is important to note that this outcome could be attributed to small female sample size within the trial and lower baseline cardiovascular risk (177). Although the data on blood pressure control is not unanimous, it is generally accepted in clinical practice that a more intensive approach to BP control yields better long-term outcomes (178). Indeed, a study examining worldwide rates of hypertensive control found that blood pressure control rates were significantly worse in women (34.0%) when compared to men (37.7%) (179).

However, current guidelines still do not have sex-specific recommendations when it comes to hypertension management, with an exception for women who are pregnant, breastfeeding, or of childbearing age. One meta-analysis comparing the treatment benefits of ACE inhibitors, CCBs, ARBs, and diuretics/beta-blockers concluded that these blood pressure-lowering regimens all have similar protection against major cardiovascular events between men and women (180). Another study determined that women who have been prescribed losartan were more likely to be hospitalized for angina than their male counterparts receiving the same treatment (181). The ACCOMPLISH trial compared multidrug therapy consisting of ACE inhibitors + CCBs to ACE inhibitors + HCTZ. Their data demonstrated that the ACEI + CCB combination was more effective in reducing adverse cardiovascular events and death, but this same significance was not demonstrated in the female subject subgroup. These findings were likely limited by the fact that only 39.5% of study subjects were women (182). Generally, data demonstrating the relationship between specific antihypertensive regimens and cardiovascular outcomes is lacking when it comes to comparing female and male subjects.

Sex differences have been identified in drug bioavailability, an important factor when it comes to dosing considerations. Women generally have higher gastric pH, slower gastric emptying, and longer gastrointestinal transit time (183). All these features would promote absorption, causing increased drug absorption in women compared to men. After a drug is absorbed, it is distributed around the body into different compartments which can alter bioavailability. Sex differences in body composition such as higher body fat percentage and decreased plasma volume in females could affect drug availability and create higher levels of lipid-soluble drugs in men and hydrophilic drugs in women. Increased bioavailability usually results in increased risk of adverse outcomes, when not accounted for in dosing regimens.

Adverse outcomes to hypertension treatment are an important consideration when trying to optimize cardiovascular outcomes in patients. Rabi et al. reviewed controlled trials of ACE inhibitors and ARBs and found that only 43% of studies reported sex-specific

outcomes (184). A comparative study by Rydberg et al. concluded that women had an increased prevalence of adverse drug reactions to ACEIs, thiazides, diuretics, and potassium-sparing agents. When it comes to ACE inhibitor adverse drug reactions (ADRs), female patients were 1.31 times more likely to report adverse reactions (185). The most reported symptoms in both sexes were cough and angioedema (185). Male subjects were more likely to report adverse drug reactions while taking aldosterone antagonists, with the most common reported reaction being hyperkalemia (186). No statistical difference was found between males and females for ARBS, sulfonamides, and selective beta-blockers in the prevalence of adverse drug reactions (187). Overall, female patients are more likely to experience adverse drug reactions while undergoing treatment for hypertension (187–192).

Concluding remarks

In summary, hypertension remains a critical area of research due to its prevalence and strong association with adverse cardiovascular events. Historically, female subjects have been excluded from *in vivo* animal experiments and clinical trials in humans, leaving half of the population unaccounted for in health, hypertension, cardiovascular, and kidney research. However, recent efforts have increased our understanding of sex differences in the physiological and pathological development of hypertension.

The data summarized in this review highlights the protective effect of estrogen on hypertension. After menopause, women are more likely to develop hypertension due to decreased estrogen levels. Estrogen exerts inhibitory effects on the classical RAAS while promoting non-classical RAS pathways, resulting in an overall vasodilatory and antihypertensive response. However, the mechanisms through which testosterone influences blood pressure remain unclear, and further research is necessary to elucidate its interaction with the RAAS.

Regarding clinical management, there has been some progress in including female subjects in clinical trials. However, research on the clinical outcomes of female and male subjects on specific antihypertensive regimens remains limited. Female patients have been shown to be more prone to adverse drug reactions while undergoing treatment, likely due to sex differences in pharmacokinetics and pharmacodynamics. As such, hypertension treatment that accounts for biological sex might provide better patient outcomes and fewer adverse drug reactions.

Looking towards the future, sex differences in hypertension, cardiovascular and kidney pathogenesis might provide new opportunities to develop novel therapies that not only suppress the classical AGT/renin/ACE/Ang II/AT_1 receptor responses, but also restore the vasoprotective axis of the ACE2/Ang (1-7)/MasR/AT_2 receptor responses. For example, therapies that promote Ang (1-7) binding with MasR or activate AT_2 receptors might be beneficial for postmenopausal women with poorly controlled hypertension, cardiovascular and kidney diseases. Several clinical trials are currently underway to investigate these as viable treatment targets for hypertension.

In conclusion, while some progresses have been made in studying and understanding sex differences in hypertension, cardiovascular and kidney diseases, further research is necessary to develop more effective and personalized treatments that account for biological sex. Inclusion of female subjects in clinical studies is especially critical to help promote clinical decisions that take into account sex-specific factors in the future.

Author contributions

SN, AL, XL, and JZ: contributions to the conception or design of the work; draft manuscript, and interpretation of data, revision of the manuscript, and approval of manuscript submission, and agreement to be accountable for all aspects of the work. 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.

The handling editor HW declared a past co-authorship with the author JZ.

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Sex differences in patterns of referral and resource utilization in the cardiology clinic: an outpatient analysis

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Aims: Women may have different management patterns than men in specialised care. Our aim was to assess potential sex differences in referral, management and outcomes of patients attending outpatient cardiac consultations.

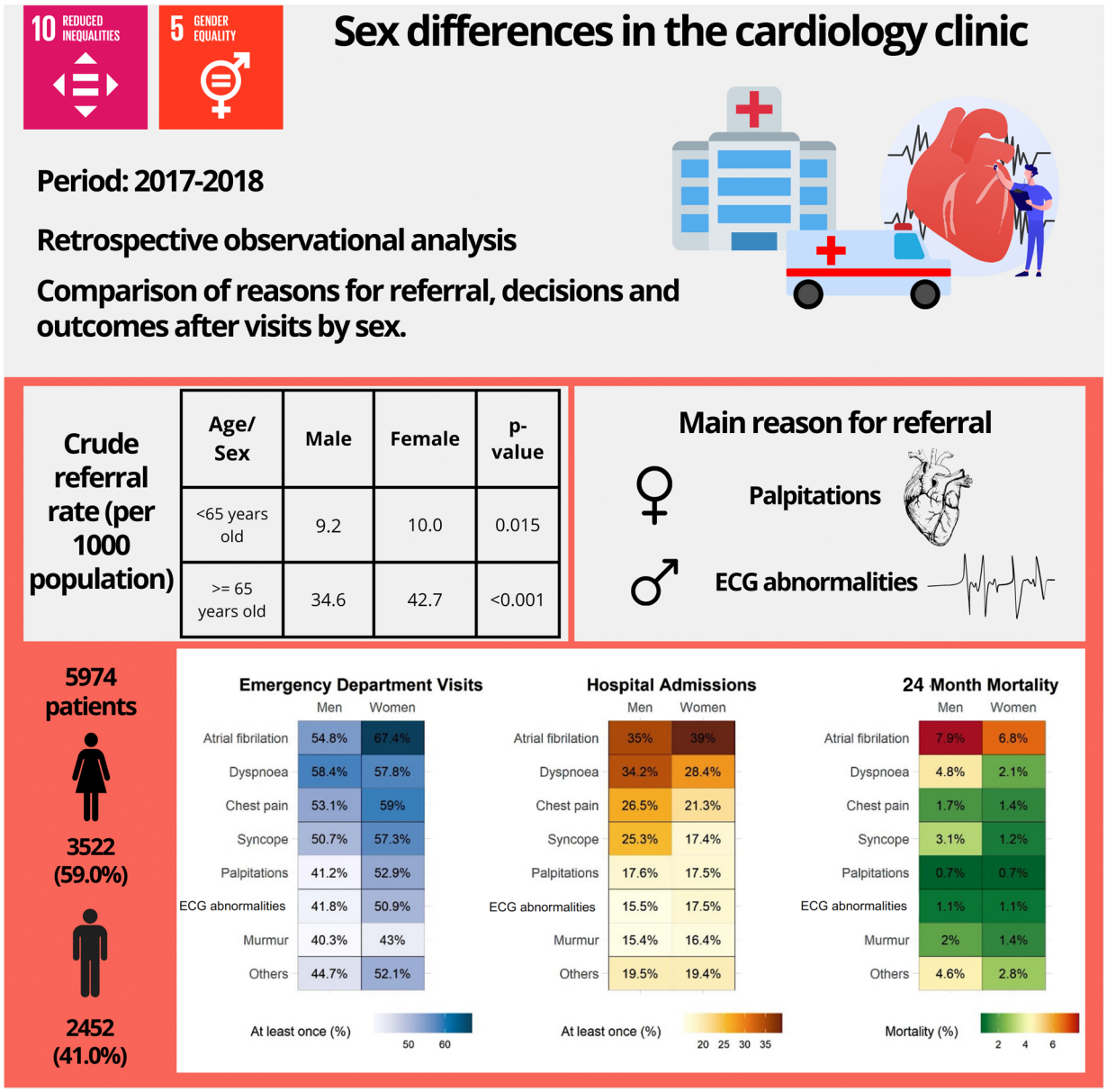
Methods and results: Retrospective observational analysis of patients ≥18 years referred for the first time from primary care to a tertiary hospital cardiology clinic in 2017–2018, comparing reasons for referral, decisions and post-visit outcomes by sex.

A total of 5,974 patients, 2,452 (41.0%) men aged 59.2 ± 18.6 years and 3,522 (59.0%) women aged 64.5 ± 17.9 years ($P < 0.001$) were referred for a first cardiology consultation. The age-related referral rates were higher in women. The most common reasons for consultation were palpitations in women (n = 676; 19.2%) and ECG abnormalities in men (n = 570; 23.2%). Delays to cardiology visits and additional tests were similar. During 24 months of follow-up, women had fewer cardiology hospitalisations (204; 5.8% vs. 229; 9.3%; $P = 0.003$) and lower mortality (65; 1.8% vs. 66; 2.7%; $P = 0.028$), but those aged <65 years had more emergency department visits (756; 48.5% vs. 560; 39.9%, $P < 0.001$) than men.

Conclusion: There are substantial sex differences in primary care cardiology referral patterns, including causes, rates, decisions and outcomes, which are only partially explained by age differences. Further research is needed to understand the reasons for these differences.

KEYWORDS

cardiovascular disease, gender inequity, cardiology consultations, primary care, symptoms, mortality, sex differences



GRAPHICAL ABSTRACT
Study design and clinical outcomes. Sex differences in the pattern of referral, complementary examination and outcomes (emergency department visits, hospitalizations, and mortality) in the cardiology consultation.

Background

Cardiovascular disease (CVD) is the leading cause of death in women (1). However, while cardiovascular mortality has decreased in men in recent years, it has increased in women (2–4). There is a misconception that CVD, particularly coronary heart disease (CHD), is a man's disease. This erroneous assumption has led to inequalities in the care of women with CVD, starting with a lack of initial clinical suspicion and different interpretations of symptoms and signs by women themselves and by those around them (5). Also, different decisions by healthcare professionals for the same cardiovascular signs and symptoms in men and women may lead to inadequate management of CVD in women, both in terms of diagnosis and treatment (6). Higher mortality and poorer outcomes in women have largely been attributed to demographic (older age and life expectancy) and clinical differences, although other factors such as psychosocial stress or adoption of unhealthy habits also play a role (7, 8).

Cardiovascular disease is one of the main reasons for consulting a general practitioner (9); however, the reasons for consulting primary care can vary widely depending on the health care system, geographical location, social class and socioeconomic level (9, 10). To date, most studies evaluating cardiovascular symptoms, such as chest pain or dyspnoea, have been conducted

in emergency departments or during hospitalisation (11–13). There are some previous investigations that have looked at the gender differences in the management of specific cardiac symptoms in primary care, particularly chest pain (12, 14). However, there is limited information on the frequency of different symptoms and the reasons for referral from primary care to cardiology.

The aim of this study was to investigate the presence of potential sex differences in the management of the most common cardiological signs and symptoms in outpatient and primary care settings, including differences in referral patterns, management and clinical outcomes.

Methods

This is a retrospective observational study that included all patients aged ≥18 years referred from primary care for a first cardiology consultation to the outpatient cardiology clinic of the Hospital Universitario 12 de Octubre, a public tertiary hospital belonging to the national health system in Madrid, Spain, using administrative data from primary care referrals and clinical data from the hospital's electronic health record. Exclusion criteria were cases with a previous hospital cardiology history or a previous cardiology consultation, patients who did not attend the medical visit, or cases with missing or inconsistent data. A small number of patients came from outside the hospital catchment area (3.5%). These were included in all analyses as the small numbers should not affect the population rates and calculations.

Consultations were stratified according to the symptom leading to the referral, which was classified by the research team on the basis of the GP's description of the reason for the consultation into 8 main categories: palpitations, dyspnoea, chest pain, ECG abnormalities, syncope, heart murmur, atrial fibrillation and other/miscellaneous.

All patients undergo an ECG at their first consultation. In addition, the consulting cardiologist has an ultrasound machine at his or her disposal. This model of care has been shown to be effective in a previous study (15). All consultations were face-to-face.

The following information was collected: (a) demographic data (sex, age); (b) reason for consultation, as standardised categorical variables; (c) indication for complementary tests or examinations, discharge from consultation or further revision; (d) outcomes during the 24-month follow-up: all-cause mortality, emergency department visits or hospital admissions. Waiting time for cardiology consultation and waiting time for complementary tests were both analysed. A stratified analysis by sex (male and female) and age (over and under 65 years) was performed. The project was approved by the local Research Ethics Committee (CEIm 21/437).

Statistical analysis

An exploratory descriptive analysis was carried out. Categorical variables were expressed as absolute numbers and percentages, and quantitative ones as mean and standard deviation. Significant differences were assessed using the Chi-square test or the Fisher test in the first case, and the Wilcoxon rank sum test in the second one. Crude attendance rates for cardiology consultations were calculated based on the total reference population of the hospital. All analysis were performed using R software (R Core Team, 2021).

Results

A total of 5,974 patients (2,452 [41.0%] men; 3,522 [59.0%] women) attended the cardiology consultation as their first cardiology visit between 2017 and 2018. On average, women were older than men (64.5 ± 17.9 vs. 59.2 ± 18.6 years; $P < 0.001$). The catchment area of the hospital is metropolitan and consists of 384,958 individuals aged ≥18 years [202,202 women and 182,756 men (**Table 1**)], with a medium-low or low socioeconomic status and a high proportion of immigrants (up to 20%, depending on the neighbourhood), mainly born in Latin America, China, Romania and Morocco.

The mean time from GP referral to cardiology consultation was 48.5 ± 34.1 days in men and 49.5 ± 34.7 days in women ($P = 0.270$). Age-stratified analysis showed that referral to cardiology was higher in women than in men both in patients aged ≥65 years (1,962 women [32.8%] and 1,049 men [17.8%], $P < 0.001$) and in younger patients (1,560 women [26.1%] and 1,403 men [23.5%], $P = 0.015$) [**Supplementary Figure S1**].

Reasons for consultation

The most common symptoms presenting to cardiology consultations were palpitations in women (676 patients; 19.2%) and ECG abnormalities in men (570 patients; 23.2%) (**Table 1**). There were important differences in the reasons for consultation according to age and sex [**Table 1**, **Supplementary Figure S2, S3**]. In the younger population (<65 years), palpitations were the most common reason for consultation in women, whereas ECG abnormalities were the most common in men. In the group aged ≥65 years, dyspnoea was the most common situation leading to a cardiology consultation in women, and ECG abnormalities remained the most common in men. Two reasons for referral were most common in women: palpitations (969 patients, 676 women, 69.8%) and dyspnoea (858 patients, 626 women, 72.8%).

Additional investigations

An electrocardiogram (ECG) was performed in all patients and a bedside echocardiogram was performed in more than half of the patients as part of the initial assessment in the cardiology clinic, with no difference by sex or reason for presentation (1,327 [54.1%] men and 1,944 [55.2%], $P = 0.573$). Additional tests were ordered in 917 (37.4%) men and 1,243 (35.3%) women ($P = 0.09$). The time from the cardiology visit to the performance

TABLE 1 Crude population referral rates to cardiology consultations by age group and sex (per 1,000 population).

Age Groups	Under 65			Over 65		
Sex	Men	Women	*P*-value[a]	Men	Women	*P*-value[a]
Reference population	152,475	156,293		30,281	45,909	
Referrals by symptom	*n* = 1,403 (9.2%)	*n* = 1,560 (10%)		*n* = 1,049 (34.6%)	*n* = 1,962 (42.7%)	
Chest pain	295 (21%)	286 (18%)	0.065	170 (16%)	292 (15%)	0.3
Population adjusted (per 1,000)	1.9	1.8	0.474	5.6	6.4	0.132
ECG abnormalities	360 (26%)	208 (13%)	<0.001	207 (20%)	248 (13%)	<0.001
Population adjusted (per 1,000)	2.4	1.3	<0.001	6.8	5.4	0.007
Palpitations	222 (16%)	438 (28%)	<0.001	67 (6.4%)	229 (12%)	<0.001
Population adjusted (per 1,000)	1.5	2.8	<0.001	2.2	5.0	<0.001
Dyspnea	101 (7.2%)	137 (8.8%)	0.11	130 (12%)	479 (24%)	<0.001
Population adjusted (per 1,000)	0.7	0.9	0.019	4.3	10.4	<0.001
Syncope	140 (10%)	152 (9.7%)	0.8	152 (14%)	169 (8.6%)	<0.001
Population adjusted (per 1,000)	0.9	1.0	0.720	5.0	3.7	0.003
Atrial fibrillation	31 (2.2%)	20 (1.3%)	0.053	146 (14%)	216 (11%)	0.019
Population adjusted (per 1,000)	0.2	0.1	0.065	4.8	4.7	0.999
Heart murmur	74 (5.3%)	135 (8.7%)	<0.001	75 (7.1%)	158 (8.1%)	0.4
Population adjusted (per 1,000)	0.5	0.9	<0.001	2.5	3.4	0.010
Others	180 (13%)	184 (12%)	0.4	102 (9.7%)	171 (8.7%)	0.4
Population adjusted (per 1,000)	1.2	1.2	0.999	3.4	3.7	0.358
Total	9.2	10.0	0.015	34.6	42.7	<0.001

[a]Calculated using the Chi-square test.

of the additional tests was 93.5 ± 85.9 days in men and 95.9 ± 103.9 days in women ($P = 0.474$). **Supplementary Table S1** shows the most common tests ordered after the consultation by sex and reason for the consultation. Coronary angiography was ordered in 19 [4.1%] men and 8 [1.4%] women with chest pain ($P = 0.006$). Non-invasive testing for ischaemia was performed more often in women aged ≥65 years than in men (239 [34%] vs. 106 [27%], $P = 0.018$). In patients who underwent an ischaemia test, 4 deaths were observed during follow-up, both in the >65 age group (3 in men and 1 in women).

Follow-up outcomes

Of the total cohort of patients seen in the cardiology clinic, 1,306 men (53.3%) and 1,952 women (55.4%, $P = 0.062$) were discharged to primary care without further tests or visits (men aged ≥65 years were more likely than women to be referred for a subsequent cardiology visit [41.8% men vs. 37.4% women aged ≥65 years ($P = 0.016$)].

During a mean follow-up of 23.7 ± 1.7 months after the cardiology consultation, emergency department visits were more frequent in women (55.0% vs. 47.7%; $P < 0.001$), especially in younger (<65 years) women (48.5% vs. 39.9%; $P < 0.001$; **Figure 1**, **Table 2**). In contrast, hospital admissions were more frequent in men aged ≥65 years (22.6% vs. 21.6%, $P < 0.001$), especially to the cardiology department in the overall study population (9.3% vs. 5.8%, $P < 0.001$), but the difference in hospital admissions to the cardiology department between men and women was statistically significant for specific reasons for consultation [chest pain 15.5% vs. 8.8%, $P < 0.001$; and syncope 10.2% vs. 2.5%, $P < 0.001$ (**Table 2**)]. All-cause mortality was higher in men than in women (2.8% vs. 1.9%, $P = 0.035$). However, this difference was statistically significant only in the group of patients aged ≥65 years (6.0% vs. 3.3%; $P < 0.001$), but not in younger patients (0.2% vs. < 0.1%; $P = 0.4$).

Discussion

There are several differences in the management of CVD between women and men. Although previous studies have addressed disparities in acute CV care and preventive therapies, there is little information on the frequency of presentation and management of specific CV symptoms or signs in the outpatient setting according to patients' sex. Our study aimed to assess the presence of gender differences in cardiac care in this setting and suggests that there are differences in cardiac outpatient care, including referral patterns, management of some symptoms and clinical outcomes. In particular, more women than men without a history of CV disease were referred to a cardiologist by general practitioners, most commonly for palpitations in women and for ECG abnormalities in men, with marked differences by age. On the other hand, we did not find any sex differences in the time it took to evaluate the heart and to perform additional tests.

CV causes are one of the main reasons for consulting the general practitioner (9). Previous studies have shown that general practitioners are less aware of CV diseases in women (2, 16, 17), so women are less likely to have access to specialist advice on CVD (18), to have a cardiovascular risk assessment and to be prescribed preventive medication (2). Contrary to previous observations, we found that women from a medium-low income, metropolitan area evaluated by general

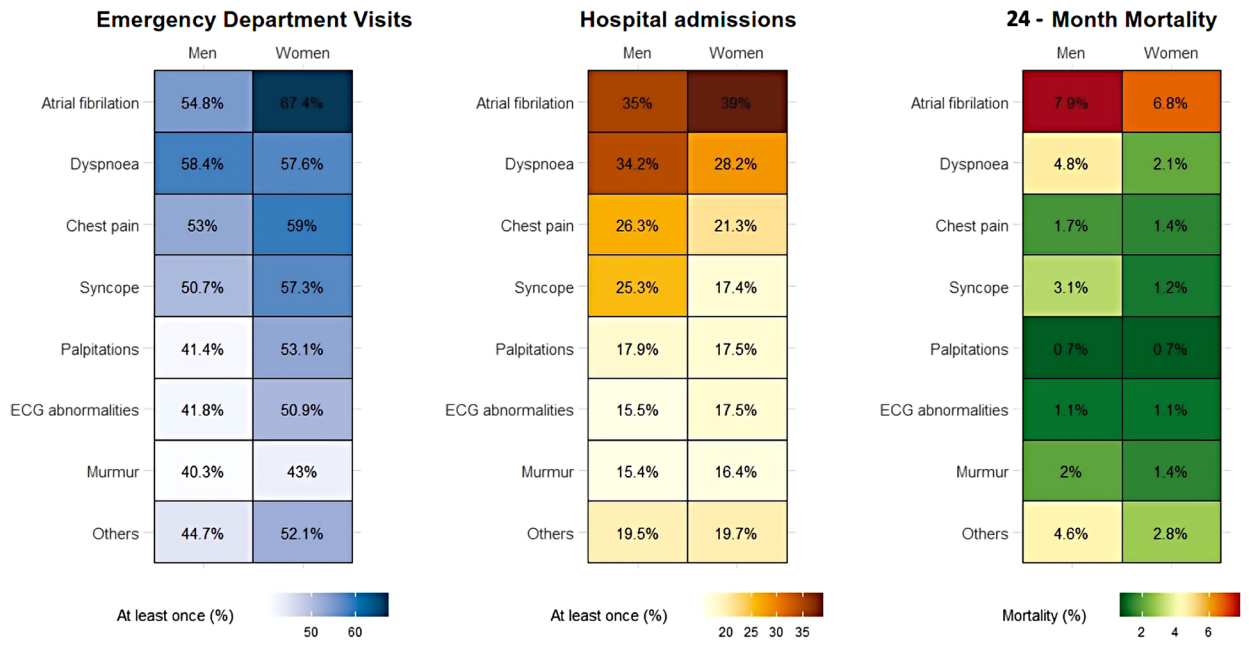


FIGURE 1
Main clinical outcomes according to sex and reason for consultation from primary care.

practicioners have a similar or even higher access to the cardiology specialist in a public tertiary care hospital, even after adjusting for age. However, whether this finding can be extrapolated to other areas is unknown. It has been suggested in a previous study that men seek medical advice later and therefore "under-react" to severe symptoms (19, 20). One of the reasons that could explain the higher frequentation rate among women in the outpatient setting is a greater symptom awareness and disease concern with symptoms of subacute/chronic evolution. It is possible that in the acute context of emergency visits or hospital admissions, women may minimize symptoms due to family pressures. Women are often the carers, they have to do the housework and are responsible for looking after children and relatives, so women may consciously or unconsciously minimize symptoms if they have to return home.

Women consistently use health services more than men, as they have a poorer state of health, poorer quality of life and a greater number of symptoms (21, 22). When considering the differences in the frequency of visits to the primary care physician, it is important to take psychosocial factors into account. A prior study found that a history of affective disorders increased frequentation, as did belonging to socioeconomically disadvantaged areas in the case of women (23). The women in the health care catchment area of our center belong to a medium-low socioeconomic level, and indeed consulted more often with the family physician than men.

Reasons for consultation

We found significant differences according to patient age and sex. Palpitations are a very common reason for consulting a general practitioner (15% of primary care visits) (24, 25) and the main reason for referral in younger women, whereas it was much less common in men. Palpitations are a benign symptom in the majority of cases, but cause significant discomfort and disability in patients (26, 27). Although palpitations are occasionally related to emotional or psychosomatic causes (>30%) (28), and women with palpitations are even more likely than men to be diagnosed with anxiety disorders (26, 29), in our experience palpitations remain a cause of concern for our primary care physicians and referral for cardiology consultation. Unfortunately, we have not been able to determine the proportion of men and women seen by general practitioners with palpitations who are not referred to cardiology.

Dyspnea was a more frequent cause for referral in older patients, particularly among women. A previous study also reported that women have a higher prevalence and severity of dyspnea than men (30). The reasons for this difference are probably multifactorial, including greater prevalence of obesity, anemia, physical deconditioning, a reduced maximum ventilatory capacity in women and, eventually, a role for emotional factors (30–33). Since dyspnea is an individual and subjective sensation, it is affected by emotional factors (33). Women more commonly suffer from anxiety and mood disorders, and these may worsen the frequency and intensity of dyspnea (33–35). However, dyspnea is an important symptom with prognostic impact since it may reflect underlying heart disease, such as ischemic heart disease, also in women. In a large cohort of patients referred for cardiac noninvasive imaging tests, those presenting with dyspnea had a much higher CV and all-cause mortality than patients without dyspnea (36). As mentioned, dyspnea is more common in women and its assessment should be incorporated into the

TABLE 2 Outcomes after the first cardiology visit by age, sex and reason for referral.

	<65 years old		*P* value	≥65 years old		*P* value
	Women, *n* (%)	Men, *n* (%)		Women, *n* (%)	Men, *n* (%)	
ECG abnormalities	*N* = 208	*N* = 360		*N* = 248	*N* = 207	
End of cardiology study. Discharge	135 (65%)	246 (68%)	0.4	183 (74%)	134 (65%)	**0.036***
Cardiology follow-up	58 (28%)	83 (23%)	0.2	49 (20%)	64 (31%)	**0.006***
Death during follow-up	0	1 (0.3%)	>0.9	5 (2%)	5 (2.4%)	>0.9
ED visit	94 (45%)	120 (33%)	**0.005***	138 (56%)	117 (57%)	0.9
≥2 ED visits	39 (19%)	61 (17%)	0.6	77 (31%)	66 (32%)	0.8
Hospital admission	20 (9.6%)	38 (11%)	0.7	60 (24%)	50 (24%)	>0.9
Hospitalization in cardiology	4 (1.9%)	17 (4.7%)	0.089	16 (6.5%)	16 (7.7%)	0.6
≥2 hospitalizations	4 (1.9%)	8 (2.2%)	>0.9	18 (7.3%)	19 (9.2%)	0.5
Palpitations	*N* = 438	*N* = 222		*N* = 229	*N* = 67	
End of cardiology study. Discharge	215 (49%)	103 (46%)	0.5	123 (54%)	35 (52%)	0.8
Cardiology follow-up	188 (43%)	100 (45%)	0.6	87 (38%)	27 (40%)	0.7
Death during follow-up	0	0	-	5 (2.2%)	2 (3%)	0.7
ED visit	207 (47%)	84 (38%)	**0.021***	146 (64%)	35 (52%)	0.089
≥2 ED visits	118 (27%)	40 (18%)	**0.011***	88 (38%)	19 (28%)	0.13
Hospital admission	63 (14%)	25 (11%)	0.3	54 (24%)	26 (39%)	**0.014***
Hospitalization in cardiology	20 (4.6%)	13 (5.9%)	0.5	17 (7.4%)	9 (13%)	0.13
≥2 hospitalizations	13 (3%)	9 (4.1%)	0.5	22 (9.6%)	12 (18%)	0.061
Dyspnea	*N* = 137	*N* = 101		*N* = 479	*N* = 130	
End of cardiology study. Discharge	77 (56%)	44 (44%)	0.054	255 (52%)	66 (51%)	0.6
Cardiology follow-up	50 (36%)	50 (50%)	**0.044***	189 (39%)	51 (39%)	>0.9
Death during follow-up	0	0	-	13 (2.7%)	11 (8.5%)	**0.003***
ED visit	70 (51%)	47 (47%)	0.5	286 (60%)	88 (68%)	0.10
≥2 ED visits	38 (28%)	24 (24%)	0.5	180 (38%)	66 (51%)	**0.007***
Hospital admission	14 (10%)	20 (20%)	**0.037***	161 (34%)	59 (45%)	**0.013***
Hospitalization in cardiology	4 (2.9%)	9 (8.9%)	**0.044***	41 (8.6%)	17 (13%)	0.12
≥2 hospitalizations	4 (2.9%)	6 (5.9%)	0.3	45 (9.4%)	28 (22%)	**<0.001***
Chest pain	*N* = 286	*N* = 295		*N* = 292	*N* = 170	*P*
End of cardiology study. Discharge	134 (47%)	134 (45%)	0.7	100 (34%)	57 (34%)	0.9
Cardiology follow-up	131 (46%)	137 (46%)	0.9	168 (58%)	94 (55%)	0.6
Death during follow-up	0	0	-	8 (2.7%)	8 (4.7%)	0.3
ED visit	169 (59%)	146 (49%)	**0.020***	172 (59%)	101 (59%)	>0.9
≥2 ED visits	97 (34%)	61 (21%)	**<0.001***	102 (35%)	60 (35%)	>0.9
Hospital admission	42 (15%)	55 (19%)	0.2	68 (40%)	68 (40%)	**0.007***
Hospitalization in cardiology	15 (5.2%)	36 (12%)	**0.003***	36 (21%)	36 (21%)	**0.028***
≥2 hospitalizations	8 (2.8%)	21 (7.1%)	**0.017***	23 (14%)	23 (14%)	0.2
Heart murmur	*N* = 135	*N* = 74		*N* = 158	*N* = 75	
End of cardiology study. Discharge	93 (69%)	41 (55%)	0.052	90 (57%)	32 (43%)	**0.041***
Cardiology follow-up	30 (22%)	26 (35%)	**0.044***	61 (39%)	41 (55%)	**0.021***
Death during follow-up	1 (0.7%)	0	>0.9	3 (1.9%)	3 (4%)	0.4
Consultation in the ED	54 (40%)	27 (36%)	0.6	72 (46%)	33 (44%)	0.8
≥2 consultations in the ED	34 (25%)	15 (20%)	0.4	38 (24%)	17 (23%)	0.8
Hospital admission	17 (13%)	8 (11%)	0.7	31 (20%)	15 (20%)	>0.9
Hospitalization in cardiology	1 (0.7%)	3 (4.1%)	0.13	10 (6.3%)	5 (6.7%)	>0.9
≥2 hospitalizations	3 (2.2%)	5 (6.8%)	0.13	11 (7%)	7 (9.3%)	0.5
Atrial fibrillation	*N* = 20	*N* = 31		*N* = 216	*N* = 146	
End of cardiology study. Discharge	14 (70%)	14 (45%)	0.082	130 (60%)	94 (64%)	0.4
Cardiology follow-up	5 (25%)	14 (45%)	0.15	67 (31%)	47 (32%)	0.8
Death during follow-up	0	1 (3.2)	>0.9	16 (7.4%)	13 (8.9%)	0.6
ED visit	14 (70%)	18 (58%)	0.4	145 (67%)	79 (54%)	**0.012***
≥2 ED visits	5 (25%)	10 (32%)	0.6	97 (45%)	47 (32%)	**0.015***
Hospital admission	5 (25%)	16 (52%)	0.059	87 (40%)	46 (32%)	0.089
Hospitalization in cardiology	3 (15%)	8 (26%)	0.5	17 (7.9%)	14 (9.6%)	0.6
≥2 hospitalizations	2 (10%)	6 (19%)	0.50	34 (16%)	18 (12%)	0.4
Syncope	*N* = 152	*N* = 140		*N* = 169	*N* = 152	
End of cardiology study. Discharge	96 (63%)	89 (64%)	>0.9	97 (57%)	55 (36%)	**<0.001***

(*Continued*)

TABLE 2 Continued

	<65 years old		*P* value	≥65 years old		*P* value
	Women, *n* (%)	Men, *n* (%)		Women, *n* (%)	Men, *n* (%)	
ECG abnormalities	*N* = 208	*N* = 360		*N* = 248	*N* = 207	
Cardiology follow-up	47 (31%)	44 (31%)	>0.9	58 (34%)	81 (53%)	**<0.001***
Death during follow-up	0	1 (0.7%)	0.5	4 (2.4%)	8 (5.3%)	0.2
ED visit	76 (50%)	54 (39%)	**0.050***	108 (64%)	94 (62%)	0.7
≥2 ED visits	44 (29%)	25 (18%)	**0.026***	67 (40%)	57 (38%)	0.7
Hospital admission	17 (11%)	18 (13%)	0.7	39 (23%)	56 (37%)	**0.007***
Hospitalization in cardiology	0	5 (3.6%)	**0.024***	8 (4.7%)	25 (16%)	**<0.001***
≥2 hospitalizations	3 (2%)	3 (2.1)	>0.9	11 (6.5%)	23 (15%)	**0.012***
All patients	*N* = 1,560	*N* = 1,403	*P*	*N* = 1,962	*N* = 1,049	*P*
End of cardiology study. Discharge	868 (56%)	769 (55%)	0.7	1,084 (55%)	537 (51%)	**0.033***
Cardiology follow-up	577 (37%)	521 (37%)	>0.9	733 (37%)	439 (42%)	**0.016***
Death during follow-up	1 (<0.1%)	3 (0.2%)	0.4	64 (3.3%)	63 (6%)	**<0.001***
Consultation in the ED	756 (48%)	560 (40%)	**<0.001***	1,180 (60%)	609 (58%)	0.3
≥2 consultations in the ED	409 (26%)	265 (19%)	**<0.001***	716 (36%)	367 (35%)	0.4
Hospital admission	198 (13%)	199 (14%)	0.2	562 (29%)	356 (34%)	**0.003***
Hospitalization in cardiology	49 (3.1%)	97 (6.9%)	**<0.001***	155 (7.9%)	132 (13%)	**<0.001***
≥2 hospitalizations	42 (2.7%)	63 (4.5%)	**0.008***	190 (9.7%)	152 (14%)	**<0.001***

*Refers to statistical significance.
ED, emergency department.
Bold values refer to statistical significance.

routine clinical care of these patients, in view of its important prognostic value.

ECG abnormalities were the main reason for consultation in men at all ages. The higher incidence of ECG abnormalities in men compared with women has been described previously (37, 38), and may be partly explained by the higher incidence of cardiovascular disease in men, particularly ischaemic heart disease, which occurs at a younger age (39). The reason for the higher referral rate may be that general practitioners may be more concerned about the presence of structural heart disease in men with ECG abnormalities.

Additional examinations

Apart from the ECG, which was performed on all patients, and the bedside echocardiogram, which was performed by the attending cardiologist, we found significant differences in the proportion of additional tests ordered, especially in younger patients. Overall, the pattern of additional tests may reflect a greater concern about CVD in men compared with women. One study found that general practitioners considered women to be less likely than men to have ischaemic heart disease, despite having equivalent symptoms or Framingham risk scores to men, and this was associated with a lower indication for diagnostic testing in women (40).

Although the greater use of coronary angiography in male compared with female patients with chest pain and coronary artery disease has been consistently reported for decades (41–45), it is concerning to find this difference 30 years after the description of the Yentl syndrome (41). Gender stereotypes drive differences in the indication for tests such as coronary angiography (43), device implantation (cardioverter defibrillator, cardiac resynchronisation therapy, or mechanical circulatory support) (46), or the performance of invasive procedures such as percutaneous coronary intervention, coronary artery bypass grafting (43), or heart transplantation (47), leading to health inequities. However, it is unlikely that a lower perceived risk of coronary heart disease in women (44) by the consulting cardiologist explains this difference, especially now that the proportion of female cardiologists is high (45% in our department). Rather, this difference may be interpreted as a different perception of patient risk or a higher likelihood of angina with normal coronary arteries in women (45), but an implicit gender bias among cardiologists caring for women with CVD, favouring men who are seen as more robust and willing to take the risk of invasive procedures and interventions than women, has also been described (7, 48). This persistent difference warrants prospective investigation and future corrective action.

Men with ECG abnormalities were more likely than women to undergo a specialised echocardiogram, reinforcing the idea of a higher perceived risk of heart disease in men.

Clinical outcomes

Although mortality was generally low, there were differences between women and men and in other outcomes. Women, especially younger women and those with palpitations, had more emergency department visits than men. In fact, more than half of the people who go to the emergency department for palpitations are women (49). An alternative explanation for a gender bias in the referral rate and use of additional tests in the

palpitations group is that in a tax-funded health care system with universal free access to specialist care at the discretion of the general practitioner, women with palpitations may be more likely to consult a physician. Finally, given that many palpitations are benign and/or non-cardiac in origin, the proportion of true positives among those referred for evaluation may be lower in women than in men. Therefore, the current indication for additional testing could be considered efficient, as fewer tests are indicated in women, who ultimately have fewer hospital admissions and lower mortality. It could also be argued that men are referred to the cardiologist less often than necessary and that this may have a negative impact on hospitalisation and mortality, which are higher in men than in women.

Interestingly, despite having more emergency department visits, women were less likely to be admitted to hospital than men, a finding consistent with previous reports (50). The reasons for this difference are unclear. Given that patients in our cohort did not have previous CV comorbidities requiring specific cardiology consultation, the lower cardiology admission in women is unlikely to be justified by a more favourable clinical profile, so it may be necessary to look at social aspects to explain these differences. A Danish study found similar results, with men having more hospital admissions but higher mortality (19, 20). It has been suggested in previous studies that men seek medical advice later and therefore "under-react" to severe symptoms (19, 20). One of the reasons that could explain the higher frequentation rate among women in the outpatient setting is a greater symptom awareness and disease concern with symptoms of subacute/chronic evolution. It may be possible that women minimized symptoms due to social factors (i.e., family burden, people under their care) particularly in the acute context of emergency visits or hospital admissions. As women most often take most of the housework and are the caregivers for most relatives, they may consciously or unconsciously minimize symptoms or delay in seeking care after they have coped with what they may consider their main responsibilities.

Mortality during follow-up was higher in men. The shorter life expectancy of men is known to be due to a higher incidence of coronary heart disease and cancer (51).

However, CVD in women should not be neglected, as it is also the leading cause of death in women (52), and women also have worse functional status, symptom control and disability than men, known as the "female disadvantage" (6). Despite the fact that CVD is the leading cause of mortality in women, clinicians routinely underestimate the risk of heart disease in them (6, 16, 40, 41, 53, 54). A number of underlying factors lead to inequities in health care between men and women, with a final disadvantage for women ("female disadvantage"). These are found in two main dimensions, The first one, is biological (sex differences), where a lack of understanding of pathophysiological mechanisms and the natural history of CVD in women occurs, with CVD risk assessment being often incomplete and specific female gender factors not taken into account. The second dimension is socio-cultural (gender gap), where stereotypes and social roles of women lead healthcare professionals to consider women less likely to suffer from heart disease, less able to make decisions about their health, and to be treated differently by physicians who routinely treat women (17, 40). Gender biases in the healthcare of CVD start in research, are uncritically transmitted in teaching pathways (health sciences, medical schools), and reproduced in daily clinical practice (17, 40, 53). Interestingly, it has been suggested that women with CVD seen by female cardiologists may have better outcomes than those seen by a male cardiologist (55).

The results of this work help to highlight differences in health care between men and women. Actions are needed at several levels to reduce health inequalities. Healthcare professionals should receive medical training that is sensitive to the differences between men and women. A gender-sensitive medicine approach needs to be promoted to change the medical culture to be more female-friendly, including an increase in the leadership of female cardiologists and other specialists. The socio-economic context of patients needs to be considered in daily clinical practice. More studies are needed to promote women's participation in clinical trials and to deepen the understanding of sex- and gender-related differences and its causes. The empowerment of women as patients should be promoted through education and cultural change that minimise gender stereotypes.

There are some limitations to this study. The reason for consultation was obtained from the GPs' interpretation of the patients' symptoms or reasons for consultation, and the final diagnosis was not available. It was not possible to assess differences in consultation, emergency department visits or hospital admission according to socio-economic variables (education level, income, occupation) because of the lack of such information. Further analysis using this approach is needed to fully understand the complexity of these processes. In addition, our data refer to a specific metropolitan health area, from a tertiary care hospital, so there could be differences in other health areas and other clinics. External validity may be limited and further multicenter analyses are needed. No information was available on the uncertainty of the patients' symptoms.

It is desirable that future studies include sex-specific analyses in order to assess and reduce inequities and the gender gap that is still present in the management of CVD.

Conclusion

We have identified significant sex differences in patterns of cardiology referral from primary care, including causes, rates, clinical decisions and outcomes, which are only partially explained by differences in age. The reasons for these differences are unclear, and further research is needed to understand the reasons for these differences.

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 human participants were reviewed and approved by Research Ethics Committee Hospital 12 de Octubre (CEIm 21/437). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

Conceptualization, LV, NR, HB, RS-B, GM, GS, CG, and JB; methodology, LV, NR, HB, RS-B, GM, GS, CG, and JB; software, NR, CG, JB; validation, LV, NR, HB, RS-B, GM, GS, CG, and JB; formal analysis, NR, CG; investigation, LV, NR, HB, RS-B, GM, GS, CG, and JB; resources, HB; data curation, LV, NR; writing—original draft preparation, LV, NR; writing—review and editing, LV, NR, HB, RS-B, GM, GS, CG, and JB; visualization, LV, NR, HB, RS-B, GM, GS, CG, and JB; supervision, LV, NR, HB, RS-B, GM, GS, CG, and JB; project administration, LV, HB and GM; funding acquisition, HB. All authors contributed to the article and approved the submitted version.

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Conflict of interest

HB receives research funding from AstraZeneca, Janssen, and Novartis; has received consulting/speaking fees from Astra-Zeneca, Novartis, Novo Nordisk and Organon: and is a scientific advisor for MEDSCAPE-the heart.og.

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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2023.1202960/full#supplementary-material

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Cardiovascular sex-differences: insights via physiology-based modeling and potential for noninvasive sensing via ballistocardiography

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In this study, anatomical and functional differences between men and women in their cardiovascular systems and how these differences manifest in blood circulation are theoretically and experimentally investigated. A validated mathematical model of the cardiovascular system is used as a virtual laboratory to simulate and compare multiple scenarios where parameters associated with sex differences are varied. Cardiovascular model parameters related with women's faster heart rate, stronger ventricular contractility, and smaller blood vessels are used as inputs to quantify the impact (i) on the distribution of blood volume through the cardiovascular system, (ii) on the cardiovascular indexes describing the coupling between ventricles and arteries, and (iii) on the ballistocardiogram (BCG) signal. The model-predicted outputs are found to be consistent with published clinical data. Model simulations suggest that the balance between the contractile function of the left ventricle and the load opposed by the arterial circulation attains similar levels in females and males, but is achieved through different combinations of factors. Additionally, we examine the potential of using the BCG waveform, which is directly related to cardiovascular volumes, as a noninvasive method for monitoring cardiovascular function. Our findings provide valuable insights into the underlying mechanisms of cardiovascular sex differences and may help facilitate the development of effective noninvasive cardiovascular monitoring methods for early diagnosis and prevention of cardiovascular disease in both women and men.

KEYWORDS

cardiovascular sex differences, noninvasive sensing, ballistocardiography, cardiovascular modeling, sex differeces, physiology-based modeling

1. Introduction

Women and men exhibit anatomical and functional differences in their cardiovascular systems. For example, women have smaller heart sizes, stronger ventricular contractility, smaller blood vessels, and smaller overall blood volume in the circulation (1–3). The manifestations of cardiovascular diseases also differ by sex. During a heart attack, men often present crushing chest pain, spreading pain in arms, nausea and cold sweat, whereas women mostly exhibit pain under the breastbone, abdominal pain, shortness of breath, nausea, and extreme fatigue (4, 5). In recent years, clinical studies have raised awareness of these differences along with the need to account for them to improve patient outcomes (6).

In this work, we contribute to this important area of research by investigating the effect of sex anatomical and functional differences on the circulation by means of a validated closed-loop mathematical model of the cardiovascular system (7–9). The model here is used as a virtual laboratory to simulate and compare multiple scenarios where parameters associated with sex differences are varied, such as ventricular contractility and arterial geometry. The model is also used to quantify the impact of sex-related parameter differences on the distribution of blood volume through the cardiovascular system. The model-predicted volumetric outputs are found to be consistent with published clinical data. Interestingly, the model indicates that the balance between the contractile function of the left ventricle (LV) and the load opposed by the arterial circulation, represented by the ventricular-arterial coupling (VAC) ratio, attains similar levels in females and males. This balance, however, is achieved through a different combination of factors. In females, the higher LV contractility is met by reduced arterial diameters, and this coupling ultimately leads to similar VAC ratios as in males.

Another interesting aspect of this work is that the mathematical model used to simulate cardiovascular sex differences also allows us to predict the shape of the ballistocardiogram (BCG) pertaining to the specific simulated scenario. As a matter of fact, the BCG signal is directly related to cardiovascular volumes. At each heartbeat, the blood ejected from the ventricles moves across the vascular compartments of the body which, as a consequence, host different amounts of blood at different instants along the cardiac cycle. The repetitive motion of blood volumes within the cardiovascular system results in the repetitive motion of the center of mass of the human body at each cardiac cycle, which is the motion captured by the BCG (10). Since the body motion is transmitted to the objects with which the body is in contact, the BCG offers a natural opportunity for noninvasive, unobtrusive monitoring of cardiovascular function. In the last decades, many devices for BCG sensing have been proposed, such as bed sensors (11, 12), weighing scales (13, 14), and accelerometers (15, 16).

A recent study showed that different BCG waveforms may be indicative of different cardiovascular baseline characteristics (15). Given the differences that the cardiovascular system exhibits in women and men, it is reasonable to conjecture that the baseline shape of the BCG signal may also be different depending on sex. Within this work, we test this conjecture by comparing the shape of BCG waveforms predicted by the mathematical model upon sex-related changes in cardiovascular parameters with BCG waveforms experimentally acquired on healthy males and females. Our study indicated that sex-related differences in arterial diameter and length are the major determinants of sex-related BCG differences, which manifest primarily through a decrease in amplitude and an earlier occurrence of the peaks, especially in the systolic phase.

The approach proposed in this work consists of utilizing mathematical modeling to interpret clinical and experimental data on the grounds of fundamental principles of cardiovascular physiology. This approach provides valuable insights on how different factors contribute to determine the healthy baseline conditions in women and men, which is a fundamental step towards a deeper understanding of sex differences in cardiovascular disease. Furthermore, we envision that the sex-related analysis of the BCG waveform will facilitate the effective design and implementation of noninvasive cardiovascular monitoring based on BCG sensing that will enable early diagnosis and prevent the worsening of cardiovascular disease in both women and men. The work is organized as follows. Sex-related cardiovascular differences observed in clinical studies are reviewed in Section 2. The main features of the mathematical model for the cardiovascular system are illustrated in Section 3, with particular emphasis on its inputs, outputs, and methods for BCG computing. The details of the experimental BCG acquisition are also provided in Section 3. The comparison between model predictions and clinical and experimental data is presented in Section 4, while conclusions and perspectives are outlined in Section 5.

2. Overview: sex-related cardiovascular differences

The cardiovascular system exhibits a similar structure in women and men, but its dimensions and functions are distinctly different depending on sex (5). The female heart size is, on average, one-fourth smaller than the male heart (3). Independently from the body size, women showed to have smaller ventricular chambers and smaller arterial diameter and length compared to men of the same age and race (1, 17). **Table 1** summarizes the main findings related to differences in healthy hearts and arteries of males and females. The findings are also discussed below.

The Left Ventricle (LV) in women is typically smaller than in men, leading to lower end-diastolic volume (EDV) and end-systolic volume (ESV). The stroke volume (SV) is also smaller in women, being approximately 22.9% less than that in men (3, 18). The higher heart rate (HR) typically observed in women reduces the gap difference in the cardiac output (CO) to approximately 12.5% (1, 3, 18, 19). LV ejection fraction (EF), a meaningful indicator of ventricular efficiency, is found to be approximately 6.5% higher in women than in men (20). LV end-systolic elastance (Ees), an important marker of LV contractility, is also found to be higher in the female heart, with Ees being

TABLE 1 Cardiovascular parameters for males and females.

Parameter	Male	Female	Reference
HR [beat/min]	74.3 ± 8.9	79.1 ± 8.2	(3)
Left ventricle			
EDV [mL]	168.4 ± 27.2	124.0 ± 27.1	(18)
ESV [mL]	78.6 ± 20.3	53.5 ± 11.9	(18)
SV [mL]	89.8 ± 15.3	69.3 ± 19.7	(18)
CO [L/min]	5.6 ± 1.4	4.9 ± 1.5	(18)
EF [%]	53.7 ± 6.5	57.2 ± 5.1	(18)
Ees [mm Hg/mL]	1.74	2.13	(22)
Ea [mm Hg/mL]	1.20	1.45	(22)
Ea/Ees ratio	0.69	0.68	(22)
Ed [mm Hg/mL]	0.063	0.081	(22)
Right ventricle			
EDV [mL]	157.9 ± 47.5	132.5 ± 46.7	(32)
ESV [mL]	80.9 ± 43.3	65.2 ± 44.0	(32)
SV [mL]	95.0 ± 26.0	74.0 ± 18.0	(33)
CO [L/min]	5.6 ± 1.4	4.4 ± 1.0	(33)
EF [%]	57.0 ± 8.0	60.0 ± 7.0	(33)
Ees [mm Hg/mL/m^2]	0.7 ± 0.2	0.8 ± 0.2	(25)
Ea [mm Hg/mL/m^2]	0.5 ± 0.2	0.6 ± 0.3	(25)
Ees/Ea ratio	1.4 ± 0.4	1.7 ± 0.9	(25)
Main arteries			
Diameter:			
Ascending aorta [cm]	3.4 ± 0.4	3.1 ± 0.5	(17)
Aortic arch [cm]	3.0 ± 0.3	2.7 ± 0.3	(17)
Thoracic aorta [cm]	2.5 ± 0.3	2.3 ± 0.3	(17)
Abdominal aorta [cm]	1.9 ± 0.3	1.6 ± 0.3	(17)
Carotid artery [cm]	0.65 ± 0.10	0.61 ± 0.10	(34)
Length:			
Ascending aorta [cm]	8.4 ± 1.1	7.6 ± 1.0	(17)
Aortic arch [cm]	3.7 ± 0.8	3.2 ± 0.7	(17)
Thoracic aorta [cm]	23.5 ± 2.9	21.2 ± 2.2	(17)
Abdominal aorta [cm]	14.4 ± 2.2	12.8 ± 2.0	(17)
Carotid artery [cm]	13.6 ± 1.5	12.3 ± 1.6	(30)
BCG amplitude [N]	5.56 ± 1.74	3.56 ± 1.06	(31)

Values are reported as mean ± standard deviation, when such information was available in the referenced articles.
HR, heart rate; EDV, end-diastolic volume; ESV, end-systolic volume; SV, stroke volume; CO, cardiac output; EF, ejection fraction; Ees, end-systolic elastance; Ea, arterial elastance; Ed, diastolic elastance; BCG, ballistocardiogram.

approximately 22.4% higher in women than in men (21). The increased value of Ees in females is accompanied by an increased value of diastolic elastance, Ed, and arterial elastance, Ea. Specifically, Ed and Ea are found to be approximately 28.6% and 20.8% higher in women than in men (22, 23). In a healthy heart, an increase in Ees is usually accompanied by an increase in Ea; this maintains the ventricular-arterial coupling (VAC) ratio (Ea/Ees ratio) within the healthy human range, approximately between 0.6 and 1.2, to ensure overall cardiovascular efficiency and performance (24). Interestingly, despite the many differences in anatomy and function, both the female and male hearts are characterized by similar VAC ratios very close to 0.7 (25).

The Right Ventricle (RV) in women is also characterized by smaller size and higher contractility compared to men (20, 26). Similarly to the LV, EDV and ESV in the RV are lower in females by 16.1% and 19.4%, respectively. The SV and the CO in the right ventricle are also lower in females. The female RV is characterized by values of EF, Ees, and Ea that are higher than those found in males by 5.2%, 14.3% and 20%, respectively (18, 22, 27). The end-diastolic elastance Ed, on the other hand, has not been found to be significantly different between the right ventricle of males and females (26). The RV-Pulmonary Arterial (RV-PA) coupling estimated by RV Ees/Ea ratio is considered to be an indicator of RV efficiency. Its healthy range falls between 1.0 and 2.0 for healthy individuals; in males and females it is reported at 1.7 and 1.4, respectively (25, 28).

The Main Arteries also present important differences among women and men. Vessel diameters and lengths are usually smaller in females compared to males (1, 29). Diameter and length of the arterial root are, on average, approximately 10% smaller in females than in males. This difference, however, seems to vary along the aortic segments. Specifically, the female-male differences in the diameters of the ascending aorta, the aortic arch, the thoracic aorta, and the abdominal aorta are found to amount approximately to 8.8%, 10%, 8.0%, and 15.8%, respectively (17). The diameter of the carotid artery is approximately 6.2% smaller in females compared to males (30). Similarly, arteries are typically shorter in women, for whom the length of ascending aorta, the aortic arch, the thoracic aorta, and the abdominal aorta are approximately 9.5%, 13.5%, 9.8% and 11.1% smaller than in men (17). The carotid length is reported to be 9.6% smaller in women (30).

It is reasonable to assume that the female-male differences in the cardiovascular system will also manifest in BCG signals. We recall that the BCG waveform results from the motion of the center of mass of the human body as the blood volume redistributes within different vascular compartments at each heartbeat (10). Thus, anatomic and functional differences in the cardiovascular system may lead to different patterns in blood volume distribution, which could be picked up by the BCG. Indeed, it has been observed that the mean BCG amplitude in females is lower than in males by nearly 36% (3.56 N vs. 5.56 N) (31).

3. Methods

This study utilizes a validated mathematical model for the cardiovascular system to predict and quantify how the distribution of blood volume within the body is impacted by differences in specific cardiac and vascular parameters associated with males and females (Section 2). The cardiovascular model leverages the analogy between the flow of a fluid in a hydraulic network and the flow of current in an electric circuit and translates fundamental principles of cardiovascular physiology into mathematical equations, a comprehensive description of the cardiovascular model, with full details, is provided in (7). Here we focus on describing which parameters are used as model inputs (Section 3.1), which quantities are computed as model outputs (Section 3.3), and which numerical strategies are used to solve the equations (Section 3.4). The model-predicted BCG waveforms are compared with experimental BCG waveforms acquired on human subjects by means of an accelerometer placed on a suspended bed. The details of the experimental BCG acquisition are given in Section 3.5.

3.1. Cardiovascular model inputs

The cardiovascular model schematized in the central panel of Figure 1 is described in detail in Guidoboni et al. (7). The pumping action of the ventricles, represented by a voltage source and a time-varying capacitor connected in series, is driving the blood flow through the systemic and pulmonary circulations. Resistances and inductances along the circuit represent viscous and inertial effects, respectively, of the blood flowing through. Capacitances capture the compliance of blood vessels, which can deform and accommodate different levels of blood volume throughout the cardiac cycle.

In the following, we illustrate how specific parameters exhibiting sex-related differences are accounted for in the model, referring the interested reader to (7) for the full model details.

- *Heart rate.* The HR can be used as a direct input of the model through the parameters that define the activation functions $a_L(t)$ and $a_R(t)$ for the left and right ventricles, respectively (see Eq. (6g) in (7)). The functions $a_L(t)$ and $a_R(t)$ are periodic and their period T_c can be determined from HR as

$$T_c = \frac{60}{\mathrm{HR}} \quad [\mathrm{s}] \tag{1}$$

where HR is measured in beats/min. In order to account for the higher HR that is typically observed in females (see Section 2), we have assumed the HR in our female model to be 5% higher than in our male model. This led us to adopt the values of 75 beats/min and 78.75 beats/min for the HR in our male and female models, respectively.

- *Ventricular properties.* Each ventricle in the cardiovascular model is described via a pressure generator capturing the isovolumic contraction, connected in series with a time-varying elastance accounting for the tension-length curve of activated fibers and the ventricular geometry. In particular, the elastances for the left and right ventricles, denoted by $E_L(t)$ and $E_R(t)$, respectively, are related to the activation functions $a_L(t)$ and $a_R(t)$ via the following constitutive equations:

$$E_L(t) = ELD + ELS\, a_L(t), \quad E_R(t) = ERD + ERS\, a_R(t) \tag{2}$$

where *ELD*, *ELS*, *ERD* and *ERS* are parameters that can be set to different values for women and men. Specifically, we have assumed *ELS*, *ERS*, and *ELD* to be higher in the female model (see Table 2). Conversely, *ERD* was assumed to be the same for both the female and male models, since no significant differences were reported in the literature for the values of Ed in the right ventricle.

- *Arterial diameter and length.* Diameter and length of the main arteries are a direct input for the cardiovascular model, as they enter explicitly in the formulas to compute the vessel resistance R, inductance L, and capacitance C reported below for ease of reference:

$$R = \frac{128 l \eta}{\pi d^4}, \quad L = \frac{4\rho_b l}{\pi d^2}, \quad C = \frac{3l\pi d^2 h(d+2h)^2}{16E(d+h)}. \tag{3}$$

In these formulas, d and l represent the vessel diameter and length, respectively, whereas η and ρ_b represent the blood viscosity and

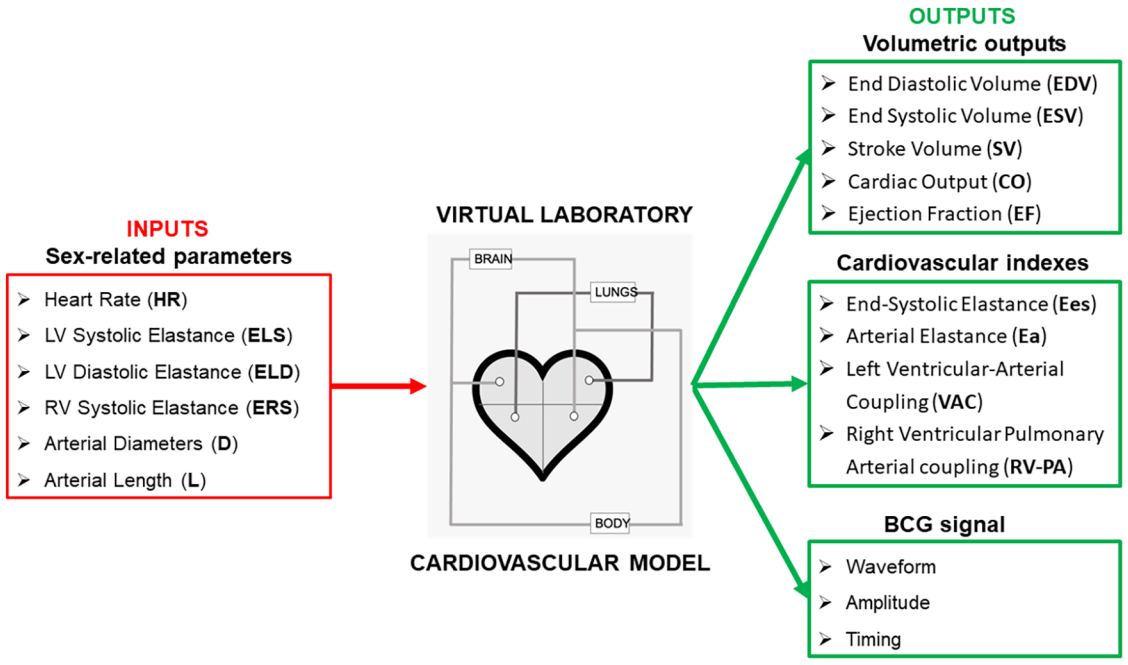

FIGURE 1
Selected parameters that have been found to be significantly different among women and men are used as inputs for the cardiovascular model proposed in Guidoboni et al. (7) to estimate volumetric outputs of the left and right ventricles, along with cardiovascular indeces and the ballistocardiogram (BCG) waveform.

TABLE 2 Input parameter values for the 8 different versions of the cardiovascular model considered in this work, from the original parameters employed in (7), to represent the idealized male to the parameters used to describe the idealized female.

	Model versions							
	Idealized male	HR	ELS	ELD	ERS	Arterial diameter	Arterial length	Idealized female
Heart Rate [beat/min]	75	**79**	75	75	75	75	75	**78.75**
ELS [mmHg cm^{-3} s^{-1}]	1.375	1.375	**1.581**	1.375	1.375	1.375	1.375	**1.581**
ELD [mmHg cm^{-3}]	0.04	0.04	0.04	**0.05**	0.04	0.04	0.04	**0.05**
ERS [mmHg cm^{-3} s^{-1}]	0.23	0.23	0.23	0.23	**0.27**	0.23	0.23	**0.27**
Arterial diameter [cm]								
Ascending aorta	1.44	1.44	1.44	1.44	1.44	**1.30**	1.44	**1.30**
Aortic arch	1.14	1.14	1.14	1.14	1.14	**1.03**	1.14	**1.03**
Thoracic aorta	0.96	0.96	0.96	0.96	0.96	**0.86**	0.96	**0.86**
Abdominal aorta	0.85	0.85	0.85	0.85	0.85	**0.77**	0.85	**0.77**
Iliac artery	0.52	0.52	0.52	0.52	0.52	**0.47**	0.52	**0.47**
Carotid artery	0.39	0.39	0.39	0.39	0.39	**0.35**	0.39	**0.35**
Arterial length [cm]								
Ascending aorta	4.0	4.0	4.0	4.0	4.0	4.0	**3.6**	**3.6**
Aortic arch	5.9	5.9	5.9	5.9	5.9	5.9	**5.31**	**5.31**
Thoracic aorta	15.6	15.6	15.6	15.6	15.6	15.6	**14.04**	**14.04**
Abdominal aorta	15.9	15.9	15.9	15.9	15.9	15.9	**14.31**	**14.31**
Iliac artery	5.8	5.8	5.8	5.8	5.8	5.8	**5.22**	**5.22**
Carotid artery	20.8	20.8	20.8	20.8	20.8	20.8	**18.72**	**18.72**

Boldface fonts highlight the changes from the original model values retrieved from (7).

density, and h and E represent the thickness and the Young modulus of the vessel wall, respectively. In order to account for the smaller vessel diameters and lengths that are typically observed in females (see Section 2), we have assumed d and l for all major arteries to be 10% smaller than in our male model.

3.2. Cardiovascular model versions

In order to assess the impact that the change in each of the aforementioned inputs has on the distribution of blood volume and pressure throughout the cardiovascular system, we proceed by considering the following different versions of the model:

- *Idealized male model:* in this version, henceforth referred to as *male model* for simplicity, all the values of the model parameters are the same as those reported in (7);
- *HR model:* in this version, all model parameters are the same as for the male model except for HR, which is assumed to be 5% higher than what was reported in (7);
- *ELS model:* in this version, all model parameters are the same as for the male model except for ELS, which is assumed to be 15% higher than what was reported in (7);
- *ELD model:* in this version, all model parameters are the same as for the male model except for ELD, which is assumed to be 30% higher than what was reported in (7);
- *ERS model:* in this version, all model parameters are the same as for the male model except for ERS, which is assumed to be 15% higher than what was reported in (7);
- *Arterial diameter model:* in this version, all model parameters are the same as for the male model except for the diameters of the major arteries, which are assumed to be 10% smaller than what was reported in (7);
- *Arterial length model:* in this version, all model parameters are the same as for the male model except for the lengths of the major arteries, which are assumed to be 10% smaller than what reported in (7);
- *Idealized female model:* in this version, henceforth referred to as *female model* for simplicity, the changes in HR, arterial diameter and length, ELS, ELD and ERS listed above are implemented simultaneously.

By comparing the cardiovascular outputs (see Section 3.3) obtained for each model version listed above, we will be able to study the effect of changing the value of one parameter at a time versus changing them all together. The summary of the parameter values pertaining to each version are summarized in Table 3. Boldface fonts have been utilized to emphasize the values of those parameters that differ from the rest. The values of the model parameters not reported explicitly in Table 2 are assumed to be the same as those in (7).

3.3. Cardiovascular model output

For a given set of parameters, the outputs of the cardiovascular model are quantities computed from the solution of the system of nonlinear ordinary differential equations describing the model (see Appendix of (7)). In this study we will be focusing on three main types of outputs: volumetric outputs, cardiovascular indexes, and BCG waveform.

- *Volumetric outputs.* The model-predicted EDV and ESV are computed as the maximum and minimum values, respectively, of the simulated volume waveforms for the left and right ventricles. From these values, we compute SV = EDV − ESV, CO = HR × SV, and EF = SV/EDV.

TABLE 3 Details of the subjects recruited for the synchronous acquisition of ECG and BCG signals.

Subject ID	Sex	Age	Height (cm)	Weight (kg)
1M	Male	25	189.0	72.6
2M	Male	23	179.8	54.0
3M	Male	32	180.0	70.0
1F	Female	26	164.6	47.6
2F	Female	28	165.0	49.9
3F	Female	29	163.0	50.1

- *Cardiovascular indexes.* The model-predicted end-systolic pressure (ESP) is computed as the maximum pressure value simulated for the left and right ventricles. From these values and the volumetric outputs, we compute Ea = ESP / SV, Ees = ESP / ESV, VAC = Ea / Ees, and RV − PA = Ees/Ea.
- *BCG waveform.* The model-predicted volume waveforms at the various nodes of the model provide the time-dynamics of how blood volume redistributes throughout the cardiovascular system. Using basic physics principles (7, 10), this information can be used to calculate the force associated with the motion of the center of mass of the human body, which gives rise to the BCG waveform.

3.4. Model implementation and solution

The cardiovascular model has been implemented using OpenModelica (35), an open-source modelica-based modeling and simulation environment. The mathematical equations representing the system have been solved using a differential algebraic system solver, DASSL, with time step of 0.001 s and tolerance of 10^{-6}, as in (7). Exploiting the library PyFMI, a Python script has been written to call a functional mockup unit (FMU) generated by OpenModelica that can solve the cardiovascular model for specified input values. In order to ensure that the solution reaches a periodic behavior, the system is solved over a time interval of 8 cardiac cycles. The solution segment corresponding to the last cardiac cycle is then considered for analysis. Post-processing of results has also been implemented in Python.

3.5. Experimental data acquisition

Six subjects were recruited for this study. Sex, age, height, and weight are reported in **Table 3**. Data collection was performed in a controlled laboratory environment, where subjects were asked to lie on the suspended bed described previously in (7). ECG and BCG were synchronously recorded by a three-lead configuration and by a Kionix accelerometer with 1000 mV/g sensitivity placed on the suspended bed frame, respectively. An ADInstrument PowerLab 16/35 data acquisition system was used to collect the signals synchronously. ECG and BCG signals were both filtered via a 6th-order Butterworth bandpass filter to remove the high-frequency noise and the low-frequency respiration movement. ECG and BCG signals were filtered with a cut-off frequency of [0.7–40] and [1.25–15] Hz, respectively (15). R peaks in the ECG signal were used to segment the BCG signal at each cardiac cycle. For each subject, the mean BCG wave of the bundle of segmented waveforms was used as a pattern.

4. Results

The outputs of the cardiovascular model obtained for the various versions illustrated in Section **3.2** are compared in terms of volumetric outputs (Section **4.1**), cardiovascular indexes (Section **4.2**), and BCG waveforms (Section **4.3**). BCG waveforms estimated from the model are then compared with the BCG signals obtained experimentally (Section **4.3**). We recall that, starting from the idealized male model version based on the model parameters reported in (7), we vary specific parameter values individually (i.e. HR, ELS, ELD, ERS, arterial diameter and length), thereby yielding six additional model versions. All changes are simultaneously incorporated into a single version called idealized female model version. Since the specific values of the modified parameters reported in **Table 2** may vary from person to person, we also tested the cases in which such values were altered by ±5%.

4.1. Comparison of volumetric outputs

Figure 2 shows the EDV (shown as blue bars) and ESV (shown as red bars) for the LV (left panel) and the RV (right panel). The error bars indicate the change in outputs due to ±5% alteration in the parameter value that characterizes the corresponding model. The EDV and ESV obtained for the LV in the female model are 137.2 and 54.9 mL, respectively. These values are 11.7% and 10.1% lower than those obtained for the male model, where EDV and ESV result to be 155.4 and 66.1 mL, respectively. These volume values are within the ranges reported in clinical studies, which are reported alongside the model predictions in **Table 4** for ease of comparison. The barplots suggest that the increase in ELD and ELS contribute the most to this volumetric difference. These results seem reasonable since a higher ELD value corresponds to a reduction in ventricular relaxation that limits the filling phase, whereas a higher ELS value corresponds to a stronger contractility that reduces the volume of blood remaining in the LV after each contraction.

Similarly to the LV, the blood volumes predicted by the model in the female RV are lower than in males. The model-predicted EDV and ESV in the RV of the female model are 144.3 and 57.0 mL compared to 167.7 and 70.6 mL obtained for males. The smaller blood volumes predicted for both ventricles of the female model are consistent with the smaller ventricular size observed in women (18).

Figure 3 is organized in three rows, reporting the results for stroke volume (SV), cardiac output (CO) and ejection fraction (EF), and two columns, corresponding to the left and right ventricles (LV, RV). The model simulations predict lower SV for both the LV and the RV in the female model when compared to the male model. The percent difference is 7.7% in the LV and

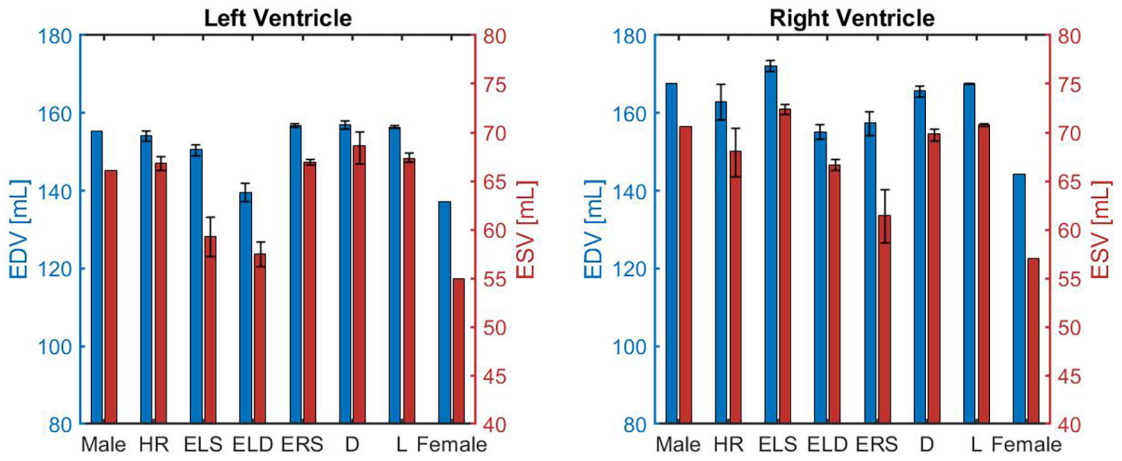

FIGURE 2
Comparison of left and right ventricular end-diastolic volume (EDV) and end-systolic volume (ESV) obtained for different model versions, namely the idealized male model (Male), HR model (HR), ELS model (ELS), ELD model (ELD), ERS model (ERS), Arterial diameter model (*D*), Arterial length model (*L*), and the idealized female model (Female) described in Section 3.2.

TABLE 4 Comparison between model predictions and clinical data for cardiovascular markers in males and females.

Parameter	Male		Female	
	Model prediction	Clinical data	Model prediction	Clinical data
Left ventricle				
EDV [mL]	155.4	168.4 ± 27.2	137.2	124.0 ± 27.1
ESV [mL]	66.1	78.6 ± 20.3	54.9	53.5 ± 11.9
SV [mL]	89.2	89.8 ± 15.3	82.3	69.3 ± 19.7
CO [L/min]	6.7	5.6 ± 1.4	6.4	4.9 ± 1.5
EF [%]	57.4	53.7 ± 6.5	60.0	57.2 ± 5.1
Ees [mm Hg/mL]	2.08	1.74	2.54	2.13
Ea [mm Hg/mL]	1.54	1.20	1.7	1.45
Ea/Ees ratio	0.74	0.69	0.67	0.68
Right ventricle				
EDV [mL]	167.7	157.9 ± 47.5	144.3	132.5 ± 46.7
ESV [mL]	70.6	80.9 ± 43.3	57.0	65.2 ± 44.0
SV [mL]	97.1	95.0 ± 26.0	87.3	74.0 ± 18.0
CO [L/min]	7.3	5.6 ± 1.4	6.9	4.4 ± 1.0
EF [%]	57.9	57.0 ± 8.0	60.5	60.0 ± 7.0
Ees [mm Hg/mL]	0.56	0.7 ± 0.2	0.68	0.8 ± 0.2
Ea [mm Hg/mL]	0.41	0.5 ± 0.2	0.44	0.6 ± 0.3
Ees/Ea ratio	1.38	1.4 ± 0.4	1.53	1.7 ± 0.9
BCG amplitude [10^5 Dyne]	1.93	2.26 ± 0.48	1.43	1.21 ± 0.07

The reference for the clinical data are the same as those reported in Table 1.
HR, heart rate; EDV, end-diastolic volume; ESV, end-systolic volume; SV, stroke volume; CO, cardiac output; EF, ejection fraction; Ees, end-systolic elastance; Ea, arterial elastance; Ed, diastolic elastance; BCG, ballistocardiogram.

10% in the RV. Interestingly, when compared to the male model, the SV is higher in the ELS model, due to increased contractility, and lower in the ELD model, due to higher diastolic stiffness. The latter effect seems to be predominant since, overall, the SV predicted for the female model is lower than in the male model. Interestingly, the increase in HR compensates for the SV reduction leading to a simulated CO for the female model that is only 4.5% smaller than in the male model for the LV and 5.5% for the RV. Furthermore, the model predicted EF for the female model is higher than the value obtained for the male model in both ventricles. The barplots show that this is mainly due to the increase in ELS and ERS, which represent stronger LV and RV ventricular contractions, respectively. These results are consistent with the trends exibited by the clinical data reported in Table 4.

4.2. Comparison of cardiovascular indexes

Cardiovascular indexes obtained with the different versions of the cardiovascular model are reported as barplots in Figure 4. The

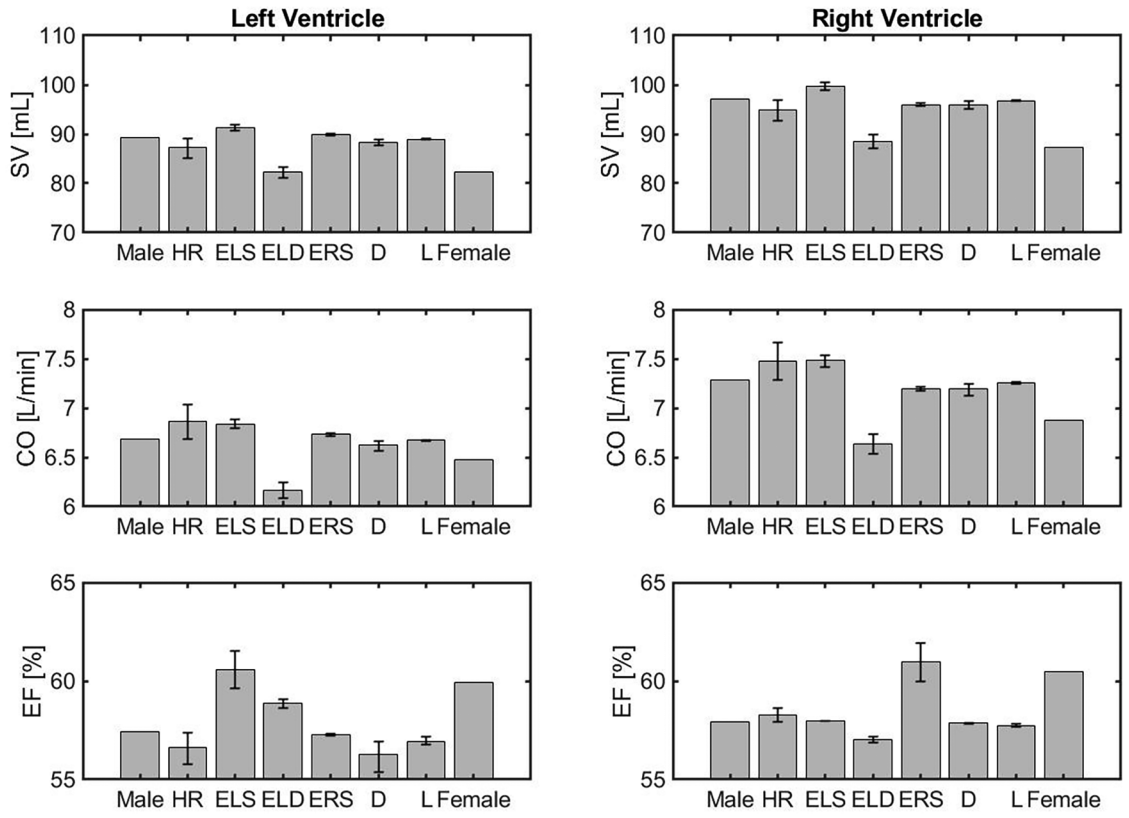

FIGURE 3
Comparison of volumetric outputs obtained for different model versions, namely the idealized male model (Male), HR model (HR), ELS model (ELS), ELD model (ELD), ERS model (ERS), Arterial diameter model (*D*), Arterial length model (*L*), and the idealized female model (Female) described in Section 3.2.

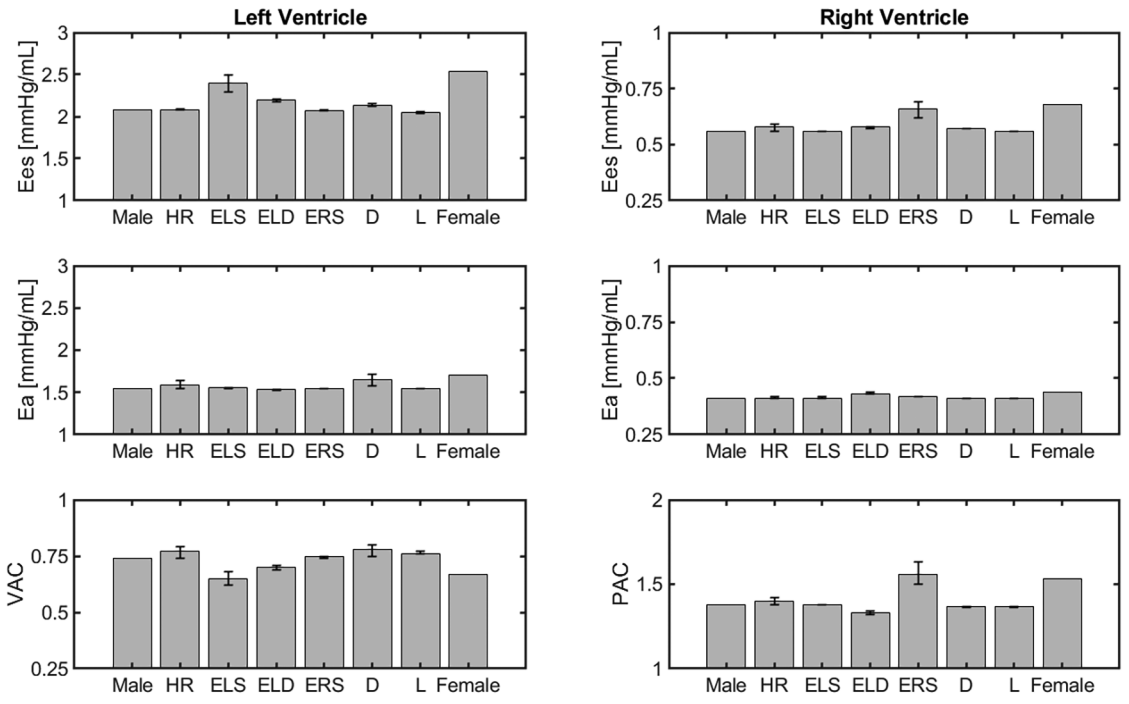

FIGURE 4
Comparison of cardiovascular indexes obtained for different model versions, namely the idealized male model, HR model, ELS, model, ELD model, ERS model, Arterial diameter model, Arterial length model, and the idealized female model described in Section 3.2.

panels in the figure are organized in 3 rows, reporting the results of Ees, Ea and their ratio for the left and right ventricles. The error bars indicate the change in outputs due to $\pm 5\%$ alteration in the parameter value that characterizes the corresponding model.

The model simulations predict higher Ees values in the female model compared to the male model. This is mainly due to an increase in ventricular contractility, represented by ELS in the LV and ERS in the RV. This is consistent with the clinical findings, as summarized in **Table 4**.

The model predicted Ea values for both the LV and RV are slightly higher in the female model than in the male model. As a result, the VAC ratios obtained for the female and male models are quite similar, equal to 0.67 and 0.74 respectively, and within the healthy human range [0.6–1.2] reported in the literature (25). The simulated RV-PA coupling (PAC) in the female and male models result to be 1.53 and 1.38, respectively, which are also within the reported optimal coupling range of [1.0–2.0] (28).

4.3. Comparison of predicted BCG signal

The BCG waveforms reconstructed virtually with the 8 different models presented in Section **3.2** are displayed in **Figure 5**. Specifically, *idealized female* and *idealized male* model simulations are highlighted in red and black solid lines, respectively, whereas the other cases are reported in dashed lines. Model simulations predict that an increase in ELS (red dashed line with triangles) enhances and anticipates the peak of the systolic phase in the BCG waveform. This result is consistent with the findings reported in (9), where the associations between changes in ventricular contractility and changes in BCG amplitude and timing were established on a preclinical swine model using induced myocardial infarction. Simulations also predict that a reduction in arterial diameter (blue dashed line with diamonds) decreases and anticipates the peak of the systolic phase in the BCG waveform. Moreover, the effect of the arterial diameter reduction is reflected in the post-systolic phase of the BCG, where the shape of the waveform differs from the male model with the presence of double peaks between 0.3 and 0.5 s. This result is consistent with the findings of Inan et al. where authors hypothesize that the second peak in the BCG waveform could be related to the mechanical resonance of the vasculature (31). Indeed, similar simulation results in the post-systolic phase can be observed when the arterial length is reduced (purple dashed line with squares). This behavior in the BCG waveform might be traced back to the fact that smaller and shorter tubes induce faster wave propagation. On the RV side, an increase in the value of ERS (violet dashed line in **Figure 6**) does not seem to influence the BCG waveform. This interesting finding suggests that the BCG signal may not be strongly influenced by RV function. When the BCG waveform obtained for the various model versions is compared with that obtained for the *idealized female* model, it appears that the reduction of arterial diameter is the dominant feature affecting sex-related BCG changes. Indeed, female BCG predicted amplitude has been found to be 25.9% smaller than in the proposed *idealized male* model. This important outcome is in line with the results of (31, 36).

Figure 6 shows the comparison between model-predicted and experimentally-measured BCG waveforms. In the left panel, the BCG waveform for the idealized male model (black solid curve) is compared with the experimental waveforms of three male subjects (dashed curves). Similarly, in the right panel the BCG waveform for the idealized female model (red solid curve) is compared with the experimental waveforms of three female

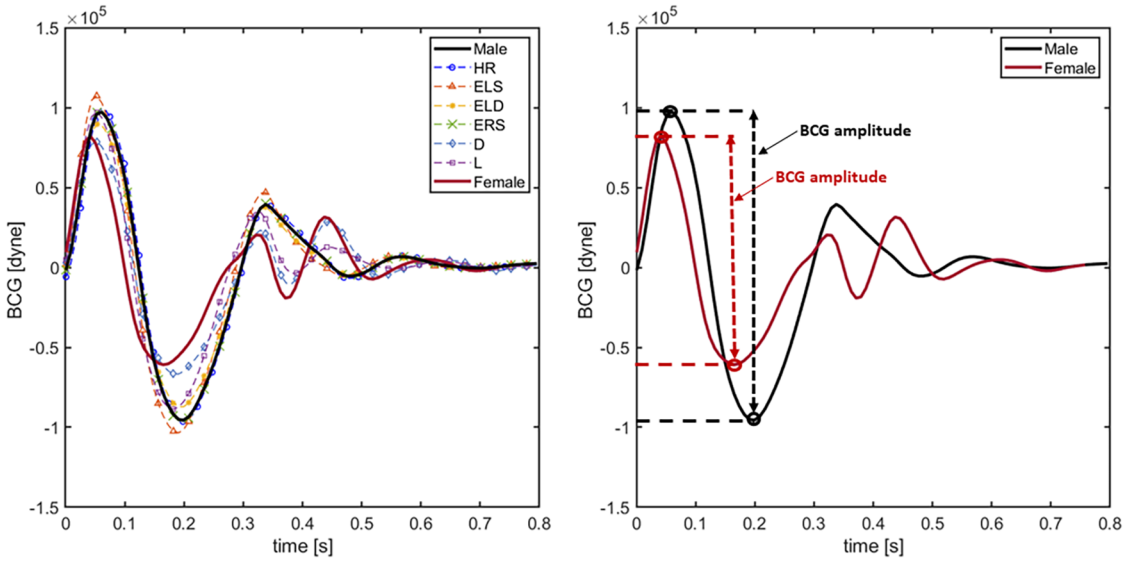

FIGURE 5
BCG waveforms obtained simulating the 8 different models presented in Section **3.1**. In particular solid black line indicates the result employing the original *idealized male* model. Solid red line is obtained using the *idealized female* model. Blue, orange, yellow, violet, green and cyan dashed lines represent the BCG waveforms using the HR, ELS, ERS, arterial diameter (*D*) and arterial length (*L*) models, respectively.

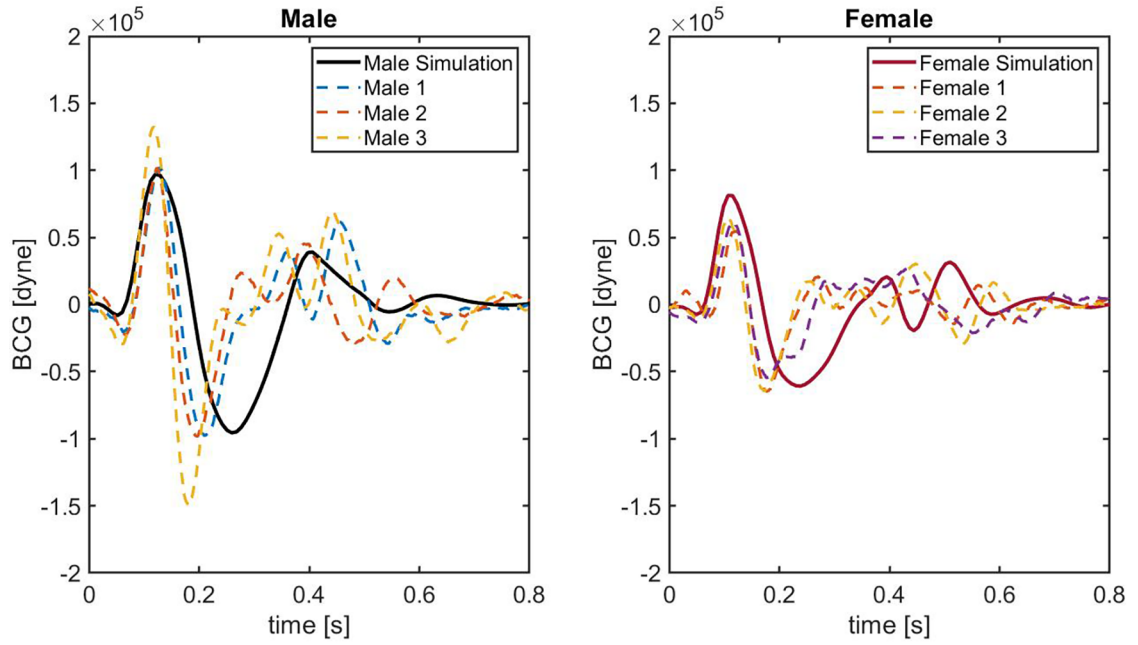

FIGURE 6
BCG waveforms obtained on 6 different healthy subjects. Solid black line indicates the result employing the original *idealized male* model. Solid red line is obtained using the *idealized female* model. Colored dashed lines represent the BCG waveforms acquired on each subject.

subjects (dashed curves). Notably, in the systolic phase between 0.0 and 0.3 s, the agreement between the model-predicted BCG for male and female and the measured BCG curves is quite satisfactory. The similarity in the systolic peaks is clearly detectable, both in terms of timing and amplitude. While comparable in terms of amplitude, shape of the BCG waveform in the post-systolic phase reported experimentally is more complex than the one predicted by the model. This result is not surprising, since capturing the features of the BCG in the post-systolic phase remains challenging both experimentally and theoretically (7, 10), and it motivates further research in this direction.

5. Conclusions and future perspective

This study provides novel insights on the effect of sex-related anatomical and functional differences on blood circulation. Theoretical predictions based on a mechanism-driven cardiovascular model were used to interpret clinical and experimental data and describe how different factors contribute to determine the healthy baseline conditions in women and men. This is a fundamental step towards a deeper understanding of sex differences in cardiovascular disease. Our results indicate that the balance between LV contractile function and arterial impedance is achieved differently in females and males, with higher LV contractility in females being met by smaller arterial diameters. This difference in cardiovascular balance is captured by the BCG waveform, where the reduced arterial diameters lead to smaller amplitudes and earlier timing of the BCG peaks. These insights help deepen our understanding of sex-related baseline differences in cardiovascular function and could enable better tailoring of therapeutic and monitoring approaches to cardiovascular disease in women and men. Thanks to its noninvasiveness and strict relationship with blood volume distribution, sex-informed BCG sensing could be used to monitor cardiovascular changes in many different situations, spanning from optimizing physical exercise (37, 38) to detecting risks during pregnancy (39, 40).

Noninvasive cardiovascular monitoring based on BCG waveforms can provide advantages with respect to accessibility and cost-effectiveness when compared to other techniques such as echocardiography, making BCG-based devices suitable for routine monitoring in home and ambulatory settings. Routine monitoring could potentially lead to early diagnosis of cardiovascular complications, facilitating timely interventions and reducing the burden of cardiovascular diseases. While BCG waveforms hold great promise, a critical challenge for widespread clinical use is the standardization of BCG waveform acquisition. To-date, several devices and methods have been used to measure the BCG in various settings, and these are not always comparable. Therefore, the great challenge is to standardize BCG measurement to ensure that data collected from different sources can be consistent and comparable.

The results presented in this work should be contextualized within the limitations of the methods utilized to obtain them. The model simulations have been compared with published values of cardiac volumes and related parameters. These values, however, should be considered more as general indicators than as exact numbers since they may vary depending on the particular subjects under consideration. The cardiovascular model considered here does not include a detailed description of the atria, which could affect the ability to capture post-systolic events

in the distribution of blood volumes and, consequently, in the BCG waveform. Furthermore, the comparison between experimentally-measured and model-predicted BCG waveforms could benefit from a larger pool of subjects. A larger cohort spanning across ages would better facilitate insights into how aging may affect cardiovascular function in women and men. These are very important directions of research that could be explored by extending the approach presented here.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors upon request.

Ethics statement

The studies involving humans were approved by Institutional review board of University of Missouri, Columbia MO, USA. 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

All authors have contributed to the conception and design of the study, contributed to manuscript revision, and finalized the draft. GG and MZ have contributed to drafting the manuscript. GG, MZ, MS, MP, DH, LD, SA, CE, and VH have contributed to the BCG-sensing technique and the interpretation of the results. GG, MZ, and LS have contributed to the implementation of the mathematical model and its numerical simulations. GG and MZ have contributed to all aspects of the project. All authors contributed to the article and approved the submitted version.

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Conflict of interest

GG would like to disclose that she received remuneration from Foresite Healthcare LLC for serving as a consultant. MS would like to disclose that she received remuneration from Foresite Healthcare LLC for serving on the advisory board. These relationships do not conflict with the work in this article and are pursuant to the University of Missouri's policy on outside activities.

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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Association of sex with post-arrest care and outcomes after out-of-hospital cardiac arrest of initial shockable rhythm: a nationwide cohort study

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Background: Research has described differences in the provision of prehospital treatment for women who experience out-of-hospital cardiac arrest. However, studies have reported conflicting results regarding survival outcomes or in-hospital interventions between sexes. Thus, this study aimed to investigate the association of sex with survival outcomes and in-hospital treatments in Japan.
Methods: We retrospectively analyzed data from the Japanese Association for Acute Medicine–Out-of-Hospital Cardiac Arrest Registry. Patients aged ≥18 years who presented with a shockable rhythm at the scene between June 2014 and December 2020 were included in our analysis. Outcome measures were 30-day survival and in-hospital interventions. We compared the outcomes between the sexes using multivariable logistic regression.
Results: In total, 5,926 patients (4,270 men; 1,026 women) with out-of-hospital cardiac arrest were eligible for our analysis. The proportions of patients with 30-day survival outcomes were 39.5% (1685/4,270) and 37.4% (384/1,026) in the male and female groups, respectively (crude odds ratio, 0.92; 95% confidence interval, 0.80–1.06). Although there were no significant differences, survival outcomes tended to be better in women than in men in the multiple regression analysis (adjusted odds ratio: 1.38; 95% confidence interval: 0.82–2.33). Furthermore, there was no significant difference between the sexes in terms of patients who received extracorporeal cardiopulmonary resuscitation (adjusted odds ratio: 0.81; 95% confidence interval: 0.49–1.33) or targeted temperature management (adjusted odds ratio: 0.99; 95% confidence interval: 0.68–1.46).
Conclusions: After adjusting for prognostic factors, there were no differences in survival rates and in-hospital interventions between men and women.

KEYWORDS

sex, shockable rhythm, out-of-hospital cardiac arrest, in-hospital treatment, survival outcome

Abbreviations

AED, automated external defibrillator; AOR, adjusted odds ratios; CI, confidence intervals; ECPR, extracorporeal cardiopulmonary resuscitation; EMS, emergency medical services; IABP, intra-aortic balloon pump; ILCOR, international Liaison Committee on Resuscitation; OHCA, out-of-hospital cardiac arrest; OR, odds ratio; PCI, percutaneous coronary intervention; PEA, pulseless electrical activity; ROC, receiver operating characteristic; TTM, targeted temperature management; VF, ventricular fibrillation; VT, ventricular tachycardia.

1 Introduction

Out-of-hospital cardiac arrest (OHCA) is a major public health concern worldwide with high occurrence and mortality rates (1–3). In Japan, there are approximately 70,000 cases of OHCA annually (4, 5). The incidence of emergency medical services (EMS)-treated OHCA in the United States is estimated to be 356,461, with nearly 90% of the cases being fatal (6). The overall prognosis and neurological outcomes are relatively poor following OHCA, and survival to hospital discharge after EMS-treated cardiac arrest is approximately 10% (1–6). The American Heart Association-International Liaison Committee on Resuscitation advocates the "chain of survival" model, which emphasizes the need for timely access to medical care and early intervention (7). Moreover, the guidelines address sex-related inequalities, with female victims less likely to receive bystander cardiopulmonary resuscitation (CPR), which is a major issue (7).

In recent years, there has been growing interest in examining the differences in the prognosis of various diseases between men and women (8–13). Research has found sex-related disparities in pathophysiology, clinical symptoms, and outcomes, as well as in medical care received (8–13). Cardiovascular disease is the most extensively analyzed topic of sex-related differences. Studies have shown that there is a variation in clinical outcomes after percutaneous coronary intervention (PCI) for acute myocardial infarction between sexes (14). Women appear to have a greater risk of heart failure following PCI for acute myocardial infarction than men, despite having the same technical success rate (15). Studies also suggest that women are less likely to receive mechanical cardiac support or undergo diagnostic procedures such as coronary angiography or PCI (16). Researchers have conducted multiple studies on possible sex differences associated with OHCA prognosis; however, their findings have been inconsistent (17, 18). Whether sex-based variations affect the prognosis of OHCA is unclear.

The treatment of OHCA in hospitals has seen a surge in specialized interventions, such as extracorporeal cardiopulmonary resuscitation (ECPR), targeted temperature management (TTM), and PCI, making them standard protocols globally (19, 20). Despite these advances, research on sex-related differences in in-hospital treatment and post-resuscitation care is lacking. Studies conducted in Japan indicate that women aged 18–64 years are less likely to receive CPR in public, and men are more likely to receive aggressive prehospital treatment (21, 22). Therefore, we hypothesized that women in Japan receive fewer in-hospital treatments or have poorer survival outcomes than men. This study aimed to explore sex-associated differences in in-hospital treatment and survival outcomes in Japan using the JAAM-OHCA registry, a multicenter prospective database.

2 Methods

2.1 Study design and setting

We retrospectively analyzed data from the JAAM-OHCA registry, a Japanese multicenter nationwide prospective database that includes prehospital and in-hospital information and outcomes of patients with OHCA transported to the emergency departments of the participating institutions. The ongoing registry was started in June 2014 and currently has no anticipated end date. It includes 95 institutions: 71 university hospitals and/or critical care medical centers and 24 community hospitals providing emergency care (23). The registry includes all patients with OHCA who required resuscitation by EMS and were transported to the participating institutions. The detailed methodology of the JAAM-OHCA registry has been described previously (24). Briefly, EMS collected prehospital data according to the international Utstein style (25), in-hospital data were collected by the medical staff of each institution in accordance with a standardized format using an Internet-based system, and the prehospital and in-hospital information was integrated by the JAAM-OHCA registry committee. The causes of arrest were classified as cardiac or noncardiac. The presumed cardiac cause category was determined by exclusion (i.e., the diagnosis was made when there was no evidence of a noncardiac cause) based on the Utstein style guidelines. Cardiac or noncardiac origin was clinically determined by the physician in charge.

2.2 Patient selection

This study enrolled the following patients: those aged ≥18 years who sustained cardiac arrest in a prehospital setting, patients for whom resuscitation was attempted, and those who were then transported to the participating institutions in Japan from 1 June 2014 to 31 December 2020. Cases of noncardiac origin were excluded because their outcomes differed (4). Cases of non-shockable rhythm at the scene were also excluded because shockable rhythms provide a suitable comparator for judging the success of systems nationally and internationally, as previous adult Utstein templates focused on witnessed ventricular fibrillation (VF) arrests (25). The ethics committee of each participating institution approved the study protocol.

2.3 EMS organization in Japan

The details of the EMS system in Japan have been previously described (2–4). In brief, the EMS system in Japan is managed by local fire stations and provides emergency services 24/7. Emergency life-saving technicians (ELSTs) are highly trained prehospital emergency care personnel who work in teams of three professionals per ambulance. ELSTs are authorized to perform various medical procedures, including the use of an intravenous line, an advanced airway, and a semi-automated external defibrillator (AED). Specially trained ELSTs are also permitted to perform tracheal intubation and administer intravenous adrenaline. CPR is performed according to the Japanese CPR guidelines, and living wills or do-not-resuscitate orders are not widely accepted. EMS personnel are not authorized to terminate resuscitation in the field, and patients with OHCA without rigor mortis, incineration, decomposition,

decapitation, or dependent cyanosis are transported to the hospital for further treatment.

2.4 Data collection and outcome measures

The following data were obtained from the JAAM-OHCA registry: sex, age, cause of arrest, arrest witnessed by bystanders, bystander-initiated CPR, first documented rhythm, resuscitation time course, actual treatments in prehospital and hospital settings (e.g., adrenaline, TTM, PCI, and ECPR), and outcome data. In cases of shock delivery by bystanders using a public-access AED, the patient's first recorded rhythm was considered pulseless ventricular tachycardia (VT) or VF. The outcome measure was 1-month survival or 1-month survival with favorable neurological outcomes. Neurological outcomes were evaluated using the cerebral performance category (CPC) scale. A favorable neurological outcome was defined as CPC 1 or 2 (2–4). Furthermore, sex disparities related to in-hospital interventions (adrenaline, antiarrhythmic drugs (amiodarone, lidocaine, nifekalant, magnesium), coronary artery angiography [CAG], PCI, intra-aortic balloon pump [IABP], ECPR, and TTM) were analyzed.

2.5 Statistical analysis

Categorical variables are presented as counts with proportions, and the χ^2 test was used to evaluate differences between the two groups. Continuous variables are presented as medians with interquartile ranges, and the Mann–Whitney *U*-test was used to evaluate differences between the two groups.

Furthermore, multiple logistic regression analysis was used to assess factors associated with survival outcomes or in-hospital interventions, and adjusted odds ratios (AORs) and 95% confidence intervals (CIs) were calculated. As potential confounders, factors that were biologically essential and considered to be associated with clinical outcomes were included in the multivariate analyses (2–4, 24, 26, 27). The variables included age (grouped as 18–19, 20–29, 30–39, 40–49, 50–59, 60–69, 70–79, 80–89, and ≥90 years), witness status (yes/no), origin (coronary disease/others/unknown), daytime (9:00 am–4:59 pm) (yes/no), weekend/holiday (yes/no), use of an AED (yes/no), bystander chest compression (yes/no), advanced airway management by EMS (yes/no), administration of adrenaline by EMS (yes/no), return of spontaneous circulation (ROSC) status (after hospital arrival, at hospital arrival, no ROSC), in-hospital first documented rhythm [VF or pulseless VT/pulseless electrical activity (PEA) or asystole/presence of pulse], time from call to hospital arrival, and year of onset. For another model for survival outcome analysis, additional variables such as antiarrhythmic drugs (yes/no), CAG (yes/no), IABP (yes/no), ECPR (yes/no), and TTM (yes/no) were added. A subgroup analysis of in-hospital treatments was also performed in terms of in-hospital treatments by narrowing based on treatment received (in particular, patients with ST-elevation on 12-lead electrocardiogram [ECG] after ROSC received CAG or PCI; VF/pulseless VT patients with first documented rhythm at hospital arrival received ECPR, adrenaline, or antiarrhythmic drugs; and patients with ROSC after/at hospital arrival received TTM or IABP).

All statistical analyses were performed using STATA version 16 (StataCorp LP, College Station, TX, USA). All tests were two tailed, and *p*-values <0.05 were considered statistically significant.

2.6 Ethics approval

This manuscript complies with the STROBE statement for the reporting of cohort and cross-sectional studies (28). The study design was approved by the Ethics Committee of the Osaka University Graduate School of Medicine (approval number: 21304-3). The requirement for written informed consent was waived due to the retrospective nature of the study. Personal identifiers were not included in the JAAM-OHCA records.

3 Results

A flow chart of the patient selection process is shown in **Figure 1**. During the study period, 68,110 OHCA cases were documented in the JAAM-OHCA registry. Among them, 5,296 adult patients (4,270 men and 1,026 women) with shockable rhythms at the scene were eligible for analysis.

Table 1 shows patient characteristics according to sex. Women were older and less likely to have coronary disease than men. A higher proportion of OHCA cases was observed in men than in women. The proportion of women who received shock from both a public AED and bystander-initiated chest compression or had an ROSC status was similar to that of men. Men were more likely to receive prehospital adrenaline administration and sustain VF/pulseless VT rhythm at hospital arrival than women. The time from call to hospital arrival was longer in men than in women.

Sex disparities in outcomes are shown in **Table 2**. Overall, 37.4% of women (384 of 1,026) and 39.5% of men (1,685 of 4,270) had 1-month survival (crude odds ratio [OR], 0.92; 95% CI: 0.80–1.06). In the multivariate logistic regression analyses, the outcome tended to be better in women than in men (AOR: 1.30; 95% CI: 0.81–2.09 after adjusting for only prehospital factors) (AOR: 1.38; 95% CI: 0.82–2.33 after adjusting for both prehospital and in-hospital factors), although it was not significantly different. In cases of 1-month survival with favorable neurological outcomes, the results were similar.

Sex disparities in in-hospital treatment are shown in **Table 3**. With a focus on highly specialized interventions, 13.0% of women (133 of 1,026) and 22.0% of men (939 of 4,270) received ECPR (crude OR: 0.53; 95% CI: 0.43–0.64). In contrast, there was no significant difference between the sexes in terms of those who received ECPR (AOR, 0.81; 95% CI, 0.49–1.33) in the multivariate logistic regression analyses. Similarly, the number of male patients who received TTM was 1,533 (35.9%), whereas the number of female patients who received TTM was 302 (29.4%) (crude OR: 0.74; 95% CI: 0.64–0.86; AOR: 0.99; 95% CI: 0.68–1.46).

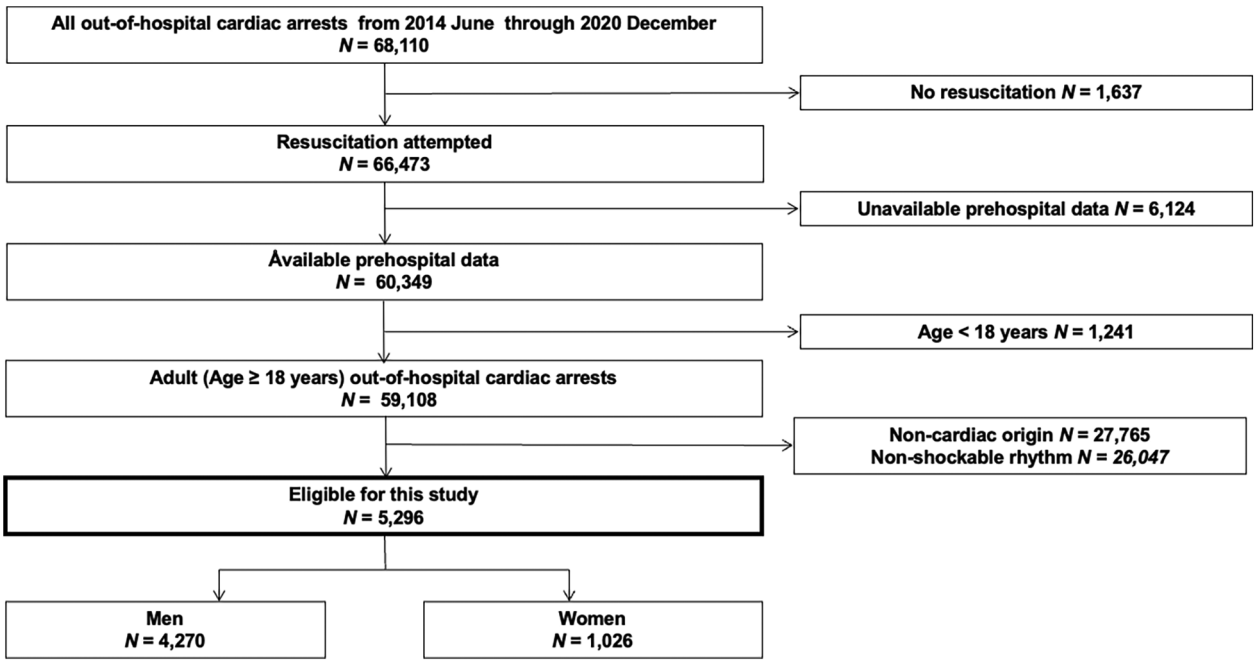

FIGURE 1
Flow chart of the patient inclusion process used in this study.

In the subgroup analysis focused on ST-segment elevation in 12-lead ECG after ROSC, in cases with VF/pulseless VT as the first documented rhythm at hospital arrival and in ROSC after/at hospital arrival, women were almost equally likely to receive in-hospital treatment when compared with men (Table 4).

TABLE 1 Characteristics of adults with out-of-hospital cardiac arrests of initial shockable rhythm in Japan.

		Total	Men	Women	*p*-Value
		N = 5,296	*N* = 4,270	*N* = 1,026	
Age, median (IQR), years	Median (IQR)	65 (53–75)	64 (53–73)	72 (56–82)	<0.001
Age group, *n* (%), years	18–64	2,515 (47.5%)	2,163 (50.7%)	352 (34.3%)	<0.001
	65–74	1,389 (26.2%)	1,174 (27.5%)	215 (21.0%)	
	75-	1,392 (26.3%)	933 (21.9%)	459 (44.7%)	
Witnessed, *n* (%)		3,997 (75.5%)	3,250 (76.1%)	747 (72.8%)	0.027
Cause, *n* (%)	Coronary Disease	1,979 (37.4%)	1,716 (40.2%)	263 (25.6%)	<0.001
	Others	1,475 (27.9%)	1,118 (26.2%)	357 (34.8%)	
	Unknown	1,842 (34.8%)	1,436 (33.6%)	406 (39.6%)	
Weekend, *n* (%)		1,785 (33.7%)	1,440 (33.7%)	345 (33.6%)	0.95
Daytime, *n* (%)		2,419 (45.7%)	1,960 (45.9%)	459 (44.7%)	0.50
Shock by a public-access AED, *n* (%)		1,017 (19.2%)	818 (19.2%)	199 (19.4%)	0.86
Bystander-initiated chest compression, *n* (%)		3,055 (57.7%)	2,463 (57.7%)	592 (57.7%)	0.99
Prehospital advanced airway management, *n* (%)		2,417 (45.6%)	1,958 (45.9%)	459 (44.7%)	0.52
Prehospital Adrenaline administration, *n* (%)		1,685 (31.8%)	1,387 (32.5%)	298 (29.0%)	0.034
Call to hospital arrival, median (IQR), min		37 (29–48)	37 (30–49)	36 (27–45)	0.018
Prehospital ROSC, *n* (%)		1,865 (35.2%)	1,492 (34.9%)	373 (36.4%)	0.39
ROSC status, *n* (%)	ROSC after hospital arrival	1,921 (36.3%)	1,578 (37.0%)	343 (33.4%)	0.11
	ROSC at hospital arrival	1,586 (29.9%)	1,263 (29.6%)	323 (31.5%)	
	No ROSC	1,789 (33.8%)	1,429 (33.5%)	360 (35.1%)	
First documented rhythm at hospital arrival, *n* (%)	VF/pulseless VT	1,625 (30.7%)	1,360 (31.9%)	265 (25.8%)	<0.001
	PEA/Asystole	2,183 (41.2%)	1,729 (40.5%)	454 (44.2%)	
	Presence of pulse	1,488 (28.1%)	1,181 (27.7%)	307 (29.9%)	

AED, automated external defibrillator; IQR, interquartile range; PEA, pulseless electrical activity; ROSC, return of spontaneous circulation; VF, ventricular fibrillation; VT, ventricular tachycardia.

TABLE 2 Survival outcomes among adults with out-of-hospital cardiac arrests of initial shockable rhythm in Japan.

	Total	Men	Women
	N = 5,296	*N* = 4,270	*N* = 1,026
Admitted to ICU/ward, *n* (%)	3,525 (66.6%)	2,883 (67.5%)	642 (62.6%)
	Crude OR	Reference	0.80 (0.70–0.93)
	Adjusted OR (model 1)[a]	Reference	0.96 (0.53–1.74)
	Adjusted OR (model 2)[b]	Reference	0.67 (0.30–1.54)
1-month survival, *n* (%)	2,070 (39.1%)	1,686 (39.5%)	384 (37.4%)
	Crude OR	Reference	0.92 (0.80–1.06)
	Adjusted OR (model 1)[a]	Reference	1.30 (0.81–2.09)
	Adjusted OR (model 2)[b]	Reference	1.38 (0.82–2.33)
CPC 1 or 2 at 1 month after OHCA	1,507 (28.5%)	1,229 (28.8%)	278 (27.1%)
	Crude OR	Reference	0.92 (0.79–1.07)
	Adjusted OR (model 1)[a]	Reference	1.52 (0.90–2.57)
	Adjusted OR (model 2)[b]	Reference	1.65 (0.95–2.85)

AED, automated external defibrillator; CAG, coronary artery angiography; CI, confidence interval; CPC, cerebral performance category; ECPR, extracorporeal cardiopulmonary resuscitation; EMS, emergency medical services; IABP, intra-aortic balloon pump; ICU, intensive care unit; OHCA, out-of-hospital cardiac arrest; OR, odds ratio; PEA, pulseless electrical activity; ROC, receiver operating characteristic; ROSC, return of spontaneous circulation; TTM, targeted temperature management; VF, ventricular fibrillation; VT, ventricular tachycardia.

[a]Model 1 included age (grouped into 10-year intervals), witness status, origin (coronary disease/others/unknown), daytime (9:00 am–4:59 pm) (yes/no), weekend/holiday (yes/no), use of an AED (yes/no), bystander chest compression (yes/no), advanced airway management by EMS (yes/no), adrenaline by EMS (yes/no), ROSC status (after hospital arrival, at hospital arrival, no ROSC), in-hospital first documented rhythm (VF or pulseless VT/PEA or asystole/presence of pulse), call to hospital arrival and year of onset. The area under the receiver operating characteristic (ROC) curve was 0.9234.

[b]Model 2 included age (grouped into 10-year intervals), witness status, origin (coronary disease/others/unknown), daytime (9:00 am–4:59 pm) (yes/no), weekend/holiday (yes/no), use of an AED (yes/no), bystander chest compression (yes/no), advanced airway management by EMS (yes/no), adrenaline by EMS (yes/no), ROSC status (after hospital arrival, at hospital arrival, no ROSC), in-hospital first documented rhythm (VF or pulseless VT/PEA or asystole/presence of pulse), antiarrhythmic drug (yes/no), CAG (yes/no), IABP (yes/no), ECPR (yes/no), TTM (yes/no), call to hospital arrival and year of onset. The area under the ROC curve = 0.9395.

4 Discussion

In this study, we evaluated the association between sex and survival outcomes of patients with OHCA with shockable rhythm at the scene or during in-hospital interventions using the JAAM-OHCA nationwide registry. There were no significant differences between women and men in terms of survival outcomes or hospital interventions, and this result was consistent in the subgroup analysis.

Several studies have examined the potential effect of sex on outcomes associated with OHCA and have reported varying results (17, 18, 29, 30). Some studies found no disparity between male and female survival rates following OHCA, whereas others found a survival advantage in males over females (30). According to a recent meta-analysis (31), women continue to exhibit substantially lower discharge survival rates and poorer neurological prognoses than men. Our findings, however, did not align with this conclusion, showing no statistically significant difference in survival outcomes between sexes. As noted in the meta-analysis, differences in medical care received after admission may play a role in the observed differences between male and female survival rates and should be considered when interpreting results from

TABLE 3 In-hospital treatments among adults with out-of-hospital cardiac arrests of initial shockable rhythm in Japan.

	Total	Men	Women
	N = 5,296	*N* = 4,270	*N* = 1,026
Adrenaline	3,473 (65.6%)	2,818 (66.0%)	655 (63.8%)
	Crude OR	Reference	0.91 (0.79–1.05)
	Adjusted OR	Reference	1.17 (0.69–1.98)
Antiarrhythmic drug[a]	1,791 (33.8%)	1,502 (35.2%)	289 (28.2%)
	Crude OR	Reference	0.72 (0.62–0.84)
	Adjusted OR	Reference	1.11 (0.75–1.66)
Coronary angiography	2,727 (51.5%)	2,288 (53.6%)	439 (42.8%)
	Crude OR	Reference	0.65 (0.56–0.74)
	Adjusted OR	Reference	1.04 (0.69–1.57)
Percutaneous coronary intervention	1,419 (26.8%)	1,255 (29.4%)	164 (16.0%)
	Crude OR	Reference	0.46 (0.38–0.55)
	Adjusted OR	Reference	0.89 (0.52–1.50)
Intra-aortic balloon pumping	1,344 (25.4%)	1,151 (27.0%)	193 (18.8%)
	Crude OR	Reference	0.63 (0.53–0.74)
	Adjusted OR	Reference	1.33 (0.88–2.02)
Extracorporeal Cardiopulmonary Resuscitation	1,072 (20.2%)	939 (22.0%)	133 (13.0%)
	Crude OR	Reference	0.53 (0.43–0.64)
	Adjusted OR	Reference	0.81 (0.49–1.33)
Targeted temperature management	1,835 (34.6%)	1,533 (35.9%)	302 (29.4%)
	Crude OR	Reference	0.74 (0.64–0.86)
	Adjusted OR	Reference	0.99 (0.68–1.46)

CI, confidence interval; OR, odds ratio.

[a]Amiodarone, lidocaine, nifekalant, magnesium.

TABLE 4 Subgroup analysis.

	Total	Men	Women
	N = 5,296	*N* = 4,270	*N* = 1,026
ST-elevation (12-lead ECG after ROSC)			
Coronary angiography	1,220 (82.9%)	1,051 (83.6%)	169 (78.6%)
	Crude OR	Reference	0.72 (0.50–1.03)
	Adjusted OR	Reference	1.56 (0.52–4.69)
Percutaneous coronary intervention	880 (59.8%)	776 (61.7%)	104 (48.4%)
	Crude OR	Reference	0.58 (0.43–0.78)
	Adjusted OR	Reference	0.88 (0.35–2.21)
VF/pulseless VT (First documented rhythm at hospital arrival)			
Extracorporeal Cardiopulmonary Resuscitation	690 (42.5%)	604 (44.4%)	86 (32.5%)
	Crude OR	Reference	0.60 (0.46–0.79)
	Adjusted OR	Reference	1.05 (0.57–1.97)
Adrenaline	1,338 (82.3%)	1,123 (82.6%)	215 (81.1%)
	Crude OR	Reference	0.91 (0.65–1.27)
	Adjusted OR	Reference	1.03 (0.46–2.30)
Antiarrhythmic drug[a]	1,052 (64.7%)	883 (64.9%)	169 (63.8%)
	Crude OR	Reference	0.95 (0.72–1.25)
	Adjusted OR	Reference	1.56 (0.85–2.87)
ROSC after hospital arrival/ROSC at hospital arrival			
Targeted temperature management	1,715 (48.9%)	1,432 (50.4%)	283 (42.5%)
	Crude OR	Reference	0.73 (0.61–0.86)
	Adjusted OR	Reference	0.88 (0.58–1.33)
Intra-aortic balloon pumping	1,180 (33.6%)	1,010 (35.6%)	170 (25.5%)
	Crude OR	Reference	0.62 (0.51–0.75)
	Adjusted OR	Reference	1.25 (0.78–2.01)

CI, confidence interval; ECG, electrocardiography; OR, odds ratio; ROSC, return of spontaneous circulation; VF, ventricular fibrillation; VT, ventricular tachycardia.

[a]Amiodarone, lidocaine, nifekalant, magnesium.

individual studies that do not account for this in-hospital factor adjustment. Furthermore, previous research conducted on sex-related differences in OHCA has shown significant variance in terms of the study participants' baseline characteristics and design, as a considerable proportion of patients with OHCA present with non-VF rhythm (31). This heterogeneity is a major bias in determining the sex-related differences described above. Hence, the outcomes of studies on sex-related disparity may not be as credible when combined or generalized because of selection and information bias. To simplify the diverse study design and baseline data, our study concentrated on sex differences in patients with OHCA with a shockable rhythm at the scene.

Our study showed that women tended to have better survival rates after adjusting for cardiac arrest. This OR reversal was also found in previous OHCA studies (32–34). Various factors, such as age, witnessed arrest, and bystander CPR, are associated with favorable outcomes in male patients with OHCA. Men also commonly experience an arrest of cardiac etiology and shockable rhythm as their initial cardiac rhythm, whereas women are more likely to have noncardiac etiologies (31). Consistent with this, the crude OR for survival outcome was also lower in women than in men in our study. However, after adjusting for prognostic factors, this analysis identified that survival outcomes tended to be better in women than in men. Therefore, we speculate that prehospital baseline factors and in-hospital care significantly affect OHCA survival rates between sexes, and previous studies may not have considered these factors, resulting in inconsistent findings. Contrary to our findings, a previous study conducted in the United States between 2003 and 2012 demonstrated that women had a higher risk of adjusted in-hospital mortality than men, particularly when diagnosed with VT/VF arrests (18). This could be due to the absence of prehospital information, interventions, or early in-hospital care in their research.

Reports have indicated varying rates of prevalence in channelopathies among different sexes (35). For instance, congenital long-QT syndrome has a higher predilection for women compared to men, putting women at a greater risk for Torsades de pointes and sudden cardiac death (36). Conversely, Brugada syndrome primarily affects adult men, who face a significantly elevated risk of arrhythmic, sudden cardiac death compared to women (37). This aspect holds significance for cardiomyopathies as well (38). Additionally, disparities in clinical characteristics may stem from societal and environmental factors that disadvantage women. According to previous reports, men tend to receive more post-admission interventions such as PCI, CAG, and TTM than women (39–41). Factors such as education level, religious beliefs, and economic level also contribute to women being more inclined to issue "do-not-resuscitate" instructions during OHCA, opting for more conservative treatment (42). One reason for this sex-related disparity might be that these treatments are invasive or expensive (43). Post-resuscitation care may also vary based on factors such as families' requests and professional concerns (17, 44). In contrast, our findings showed no significant difference in in-hospital treatment between the sexes. Furthermore, Japan's insurance system ensures that early cessation of expensive care for socioeconomic reasons is somewhat lower than that in other countries (45).

In our study, we showed that patients with OHCA with shockable rhythm who should be provided with highly specialized interventions have no sex-related disparity in survival outcomes or in-hospital treatment. Most previous studies have adjusted only for prehospital factors in patients with OHCA; thus, studies adjusted for in-hospital treatments such as care for post-cardiac arrest status are limited (31). Therefore, these results are considered appropriate. Our findings provide insights into sex-related disparities in patients with OHCA.

This study had some limitations. First, the Utstein style registry does not contain information on medications, medical history, and daily activities of each patient before cardiac arrest, which could affect the decision to pursue aggressive treatment options. Second, the registry does not provide detailed information on factors such as cardiovascular risk, symptom onset, and time to cardiac arrest, which might have skewed the results. Other potential factors, such as education level and sex inequality, may also play a role. Another limitation is the applicability of the study; as it is a single-country study, it would be intriguing to observe whether these findings hold true in a multinational study involving diverse patient populations and healthcare systems. Further investigations are required to confirm these findings and assess their generalizability.

In conclusion, using a prospective, nationwide, multicenter, OHCA registry in Japan, we focused on sex-related differences in patients with OHCA with shockable rhythm at the scene. After adjusting for prognostic factors, we found no difference in the survival rates or hospital interventions between men and women.

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 author.

Ethics statement

The studies involving humans were approved by the Ethics Committee of the Osaka University Graduate School of Medicine (approval number: 21304-3). 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

SH: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Writing – original draft. TI: Conceptualization, Formal Analysis, Funding

acquisition, Investigation, Project administration, Writing – original draft, Writing – review & editing. SN: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft. LZ: Data curation, Software, Validation, Writing – original draft. KK: Data curation, Software, Validation, Writing – original draft. TK: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Writing – original draft. HO: Supervision, Writing – review & editing. JO: 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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Sex-specific association of low-renin hypertension with metabolic and musculoskeletal health in Korean older adults

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Introduction: Low-renin hypertension (LRH) accounts for approximately one-third of patients with hypertension and are more prevalent in women and the older adult population. Previous study has found a link between the renin-angiotensin-aldosterone system (RAAS) and sex hormones. However, there are insufficient data on the relationship between LRH and metabolic or musculoskeletal outcomes in older adults.

Methods and materials: Among the 343 participants from a population-based cohort study conducted between May 2018 and August 2019, a total of 256 (86 men older than 50 years and 170 postmenopausal women) were included. The presence of LRH was defined as plasma renin activity (PRA) <1 ng/mL/h and systolic blood pressure (BP) ≥130 or diastolic BP ≥80 mmHg based on the 2017 ACC/AHA guidelines. Individuals with missing data, and those who had used medications that could affect PRA within the past six months were excluded. Bone mineral density (BMD), trabecular bone score (TBS), and appendicular lean mass (ALM) index were assessed using dual-energy X-ray absorptiometry; degraded TBS was defined as partially degraded to degraded levels (≤1.350). Muscle function was assessed according to the Asian Working Group for Sarcopenia guidelines. PRA was measured using radioimmunoassay.

Results: The median age was 66 [61–72] years, and the body mass index (BMI) was 24.7 [23.0–26.4] kg/m^2. Individuals with LRH, accounting for 34.8%, had lower diabetes mellitus; more dyslipidemia; and poorer muscle function, BMD, and TBS than those in the non-LRH group. In addition, PRA was positively correlated with C-peptide, HOMA-IR, TBS, and ALM index. After adjusting for covariates including age and BMI, LRH was negatively associated with femur neck T-score (adjusted $\beta = -0.30$, 95% CI [−0.55 to −0.05], $p = 0.021$) and the presence of LRH was significantly associated with degraded TBS in women (adjusted odds ratio = 3.00, 95% CI [1.36–6.58], $p = 0.006$).

Conclusion: Our findings suggest that LRH can influence clinical features and metabolic risk in older adults. Notably, LRH in postmenopausal women was linked to lower femur neck T-scores and degraded TBS, indicating sex-specific effects of LRH on bone health. Larger prospective studies are required

to elucidate how changes in the RAAS affect metabolic and musculoskeletal outcomes in older adults.

KEYWORDS

low-renin hypertension, plasma renin activity, primary aldosteronism, bone mineral density, trabecular bone score, sex difference

1 Introduction

The renin-angiotensin-aldosterone system (RAAS) is a circulatory system that is crucial for regulating fluid balance as well as sodium and potassium homeostasis, and its dysregulation may lead to arterial hypertension (1). Classification based on renin levels can help differentiate the pathophysiological causes of hypertension (2). Approximately one-third of patients with hypertension have essential hypertension and low plasma renin activity (PRA), also known as low-renin hypertension (LRH) (3). LRH is more common in women, older adults, and individuals of African descent (4, 5). LRH is a multifactorial disease category that includes patients with a high-sodium diet, primary aldosteronism (PA), hypercortisolism, congenital adrenal hyperplasia, Gordon syndrome, Liddle syndrome, and medications, such as nonsteroidal anti-inflammatory drugs and cyclooxygenase-2 inhibitors (6). Although the final mechanism for LRH development is the activation of mineralocorticoid receptors, which is also linked to the pathogenesis of PA (7), the clinical significance of LRH remains unclear.

The relationship between the RAAS and sex hormones has been widely investigated. Estradiol increases angiotensinogen, angiotensin-converting enzyme 2 (ACE2), and angiotensin type 2 (AT2) receptor expression, while decreasing renin, ACE, and AT1 receptor expression (8, 9). It also acts as an immunomodulator of RAAS (10). Estradiol deficiency after menopause induces low-grade inflammation, which may contribute to the proinflammatory activity of the RAAS and increase oxidative stress (11). Little is known about the effects of androgens on RAAS; however, studies have demonstrated that testosterone activates renin, ACE, and AT1 receptors and inhibits AT2 receptors (12).

Several studies have reported an association among impaired metabolism, musculoskeletal health, and RAAS (13–15). Activation of RAAS may play a central role in the development of obesity; elevated angiotensin II levels are also associated with hypertension, dyslipidemia, and insulin resistance (16). Moreover, bone tissue expresses receptors for RAAS components, such as AT1, AT2, or mineralocorticoid, suggesting that activating the local RAAS response could increase bone turnover, and consequently, bone density (17). Accordingly, LRH is likely to have different clinical features depending on the sex. However, whether LRH is associated with metabolic and musculoskeletal health, particularly among older adults, remains unclear.

Therefore, this study aimed to investigate (1) the differences in clinical features depending on the presence of LRH, (2) the association between RAAS components and metabolic and musculoskeletal parameters, and (3) the sex-specific association of LRH with metabolic and musculoskeletal health in the older adults.

2 Materials and methods

2.1 Study participants

Study participants were recruited from 1,894 individuals who completed the third follow-up survey (from May 2011 to October 2017) among those already enrolled in the Korean Genome and Epidemiology Study on Atherosclerosis Risk of Rural Areas in the Korean General Population (KoGES-ARIRANG), a population-based cohort study to assess the prevalence, incidence, and risk factors of chronic disorders such as hypertension, diabetes, metabolic syndrome, and cardiovascular disease (18, 19). As shown in Figure 1, among the 348 participants enrolled in the fatty liver cohort, those with missing data, including bone mineral density (BMD) or trabecular bone score (TBS), and those who used medications affecting plasma renin levels within the past six months (nonsteroidal anti-inflammatory drugs and beta-blockers) were excluded. A total of 256 participants (86 men and 170 women) were included for analyses. All included participants were men aged >50 years or post-menopausal women. This study was approved by the Institutional Review Board (IRB), and all study participants provided written informed consent before participation (IRB numbers CR317131, CR318003, and CR322353).

2.2 Measurements

Venous blood samples were drawn from the study participants after fasting for >12 h or overnight. Serum aliquots were stored at −80°C freezer until thawed for analysis within one week after blood extraction. A calibrated Roche Cobas® 8,000 modular analyzer consisting of c702a and e801 modules was used to perform routine biochemical tests using the manufacturer's reagents and calibrators (Roche, Mannheim, Germany). Enzymatic colorimetric techniques were used to assess the triglycerides, total cholesterol, and high-density lipoprotein (HDL) cholesterol levels. Colorimetric techniques were used to measure the aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine, albumin, blood urea nitrogen (BUN), protein, and total bilirubin levels. The hexokinase method was used to measure the fasting blood glucose levels. An electrochemiluminescence immunoassay was used to measure insulin and C-peptide levels. Molybdate UV and 3 NM-BAPTA techniques were used to quantify calcium and phosphorus, respectively. The formula [fasting insulin (U/mL) × fasting glucose (mg/dL)]/405 was used to calculate the homeostatic model assessment index (HOMA-IR). According to the manufacturer's instructions, the serum concentrations of fibroblast growth factor 21 (FGF21), FGF19, growth differentiation factor 15 (GDF15), adiponectin, leptin, retinol binding protein 4 (RBP4), interleukin 6 (IL6), transforming growth factor beta 1 (TGF-β1), and

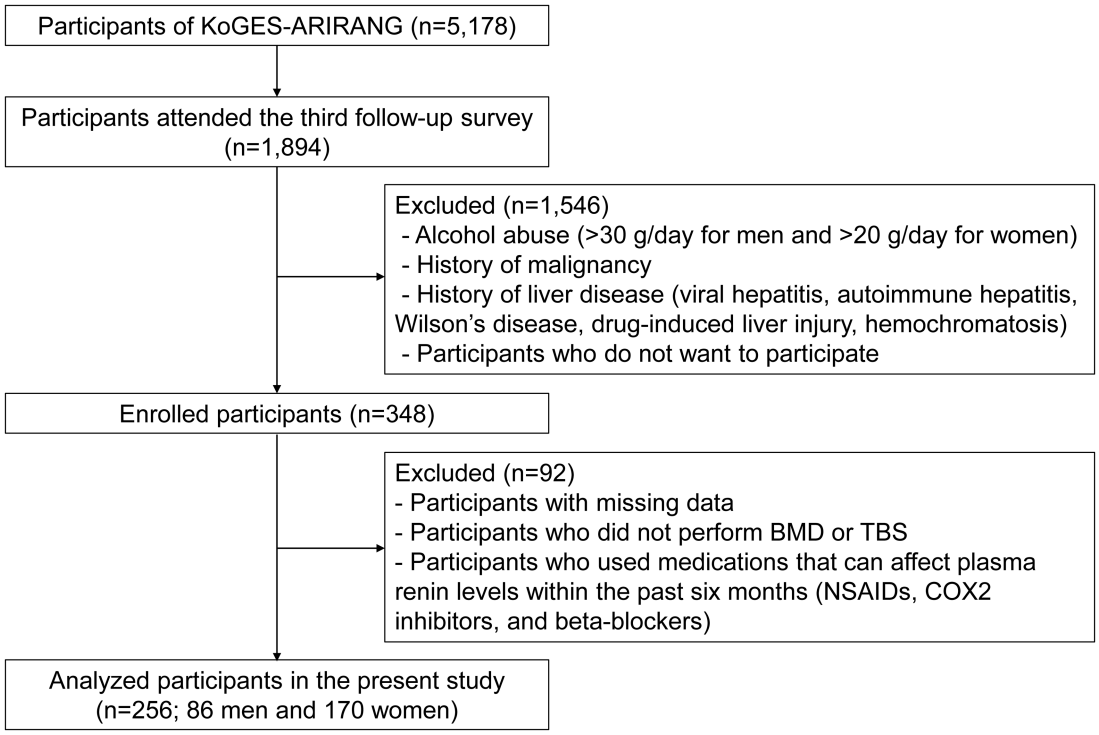

FIGURE 1
Study flow. KoGES-ARIRANG, the Korean Genome and Epidemiology Study on Atherosclerosis Risk of Rural Areas in the Korean General Population; BMD, bone mineral density; TBS, trabecular bone score; NSAIDs, nonsteroidal anti-inflammatory drugs; COX-2 inhibitors, cyclooxygenase-2 inhibitors.

myostatin were quantified using the human Quantikine ELISA kits (R&D Systems, Minneapolis, MN, United States) and decorin level was measured using the Raybio human DCN ELISA kit (RayBiotech, Norcross, GA, United States). All mean intra-assay and interassay coefficients of variation (CV) were < 10%.

PRA and aldosterone concentrations were measured using radioimmunoassay (RIA) (Immunotech, Czech Republic), and angiotensin II levels were measured in the sitting position using an ELISA kit (Elabscience, Houston, Texas, United States). The intraassay CV for PRA, aldosterone, and angiotensin II were 11.3, 11.9, and < 10%, respectively, and the inter-assay CVs were 20.9, 10.2%, and < 10%, respectively.

The presence of LRH was defined as a PRA < 1 ng/mL/h and the presence of hypertension, which was defined systolic blood pressure (BP) ≥ 130, or diastolic BP ≥ 80 mmHg based on the 2017 ACC/AHA guidelines or taking antihypertensive medication (20). The non-LRH group included participants with normal-to-high renin (PRA ≥ 1 ng/mL/h) hypertension as well as those without hypertension. BMD and TBS were measured using dual-energy X-ray absorptiometry (DXA); degraded TBS was defined as partially degraded to degraded levels (≤1.350) (21). The Short Physical Performance Battery (SPPB), usual gait speed, timed-up-and-go test, 5-timed chair stand test were assessed according to the Asian Working Group for Sarcopenia guidelines (22).

2.3 Statistical analyses

Data are presented as mean ± standard deviation (SD), median [interquartile range (IQR)], or numbers (percentages) in Table 1 and Supplementary Table S1. Independent *t*-test, Wilcoxon rank-sum test, or Pearson's chi-square test were performed, as appropriate, to compare the clinical characteristics between the LRH and non-LRH groups. The correlations between the RAAS components and metabolic and musculoskeletal parameters were assessed using the Spearman correlation coefficient (r). Univariate and multivariate regression models were used to investigate the association between the presence of LRH and femur neck T-scores as well as muscle function tests. Univariate and multiple linear regression analyses were performed to determine whether the presence of LRH influenced the degraded TBS. We used STATA 17.0 (Stata Corp LP, College Station, TX, United States) in all analyses. All statistical tests were two-sided, and the significance level was set at $p < 0.05$.

3 Results

3.1 Clinical characteristics of the study participants

Among the 256 participants, 170 (66.4%) were women, 120 (46.9%) had hypertension, and 89 (34.8%) had LRH (Figure 2). The median age of study population was 66 [61–72] years, and the body mass index (BMI) was 24.7 [23.0–26.4] kg/m^2. Men accounts for 24.7 and 38.3% in the LRH and non-LRH groups, respectively, and the difference was significant ($p = 0.028$). Individuals with LRH were prescribed more antihypertensive medications, had less diabetes mellitus (DM) and more dyslipidemia than non-LRH group (antihypertensive medication, 58.4% vs. 40.1%, $p = 0.005$; DM, 11.2%

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TABLE 1 Baseline characteristics.

	LRH (*n* = 89)	Non-LRH (*n* = 167)	Total (*n* = 256)	*p*-value
Age (y)	67 (62–72)	65 (61–72)	66 (61–72)	0.284
Sex [men, *n* (%)]	22 (24.7%)	64 (38.3%)	86 (33.6%)	0.028*
HTN med (*n*, %)	52 (58.4%)	67 (40.1%)	119 (46.5%)	0.005*
DM (n, %)	10 (11.2%)	42 (25.1%)	52 (20.3%)	0.008*
Dyslipidemia (*n*, %)	47 (52.8%)	61 (36.5%)	108 (42.2%)	0.012*
Osteoporosis (*n*, %)	24 (27.0%)	30 (18.0%)	54 (21.1%)	0.093
PRA (ng/mL)	0.6 (0.3–0.7)	1.2 (0.6–2.9)	0.8 (0.5–1.6)	<0.001*
Aldosterone (pg/mL)	15.6 (12.8–19.1)	14.8 (11.9–18.9)	15.2 (12.0–19.0)	0.348
Angiotensin II (pg/mL)	66.6 (42.7–115.2)	75.7 (58.3–105.6)	74.4 (53.7–108.2)	0.189
AST (U/L)	22 (20–26)	22 (19–26)	22 (19–26)	0.732
ALT (U/L)	18 (14–22)	18 (13–23)	18 (13–23)	0.806
Cr (mg/dL)	0.7 (0.6–0.8)	0.8 (0.6–0.9)	0.7 (0.6–0.9)	0.386
Metabolic stress-related biomarkers				
RBP4 (μg/mL)	29.7 (26.0–36.2)	31.2 (27.1–35.9)	30.7 (26.8–36.0)	0.450
IL6 (pg/mL)	1.65 (1.19–2.41)	1.44 (1.04–2.31)	1.53 (1.08–2.35)	0.107
Myostatin (pg/mL)	2.70 (2.08–3.16)	2.59 (2.00–3.38)	2.61 (2.03–3.31)	0.965
Tgfb1 (pg/mL)	24.0 (20.5–28.9)	24.3 (19.5–30.0)	24.2 (20.0–29.4)	0.734
Decorin (pg/mL)	6.9 (5.9–8.2)	6.9 (5.9–7.8)	6.9 (5.9–8.0)	0.348
GDF15 (pg/mL)	859 (708–1,027)	849 (656–1,197)	854 (671–1,151)	0.784
FGF19 (pg/mL)	169 (109–289)	172 (110–311)	170 (110–301)	0.889
FGF21 (pg/mL)	235 (133–318)	183 (119–301)	191 (127–317)	0.156
Metabolic parameters				
BMI (kg/m^2)	24.4 (23.3–26.3)	24.9 (23.0–26.6)	24.7 (23.0–26.4)	0.783
Total fat (%)	38.5 (32.9–41.7)	36.8 (30.3–40.6)	37.3 (30.8–41.3)	0.082
Glucose (mg/dL)	98 (92–105)	100 (93–110)	99 (93–110)	0.200
Triglyceride (mg/dL)	114 (89–167)	129 (90–212)	125 (90–192)	0.196
Total cholesterol (mg/dL)	179 ± 35	176 ± 35	177 ± 35	0.502
HDL cholesterol (mg/dL)	54 (48–61)	51 (42–62)	52 (42–62)	0.158
C-peptide (ng/mL)	1.9 (1.6–2.8)	2.2 (1.5–3.2)	2.1 (1.5–3.1)	0.186
Insulin (uIU/mL)	6.8 (4.7–11.6)	7.3 (5.1–12.0)	7.1 (5.1–11.9)	0.363
HOMA-IR	1.6 (1.0–2.9)	1.8 (1.2–3.2)	1.7 (1.2–3.1)	0.203
HOMA-β	74 (52–111)	69 (49–105)	72 (50–107)	0.537
Adiponectin (ng/mL)	7.6 (4.2–11.9)	6.1 (3.5–9.9)	6.4 (3.7–10.8)	0.123
Leptin (ng/mL)	9.6 (4.4–14.1)	7.3 (3.8–12.9)	8.1 (3.9–13.5)	0.072
Musculoskeletal parameters				
Ca (mg/dL)	9.5 ± 0.3	9.6 ± 0.3	9.5 ± 0.3	0.090
P (mg/dL)	3.7 ± 0.4	3.8 ± 0.5	3.8 ± 0.4	0.693
ALP (IU/L)	66 (58–80)	70 (56–81)	68 (57–81)	0.365
ALM index (kg/m^2)	5.6 (5.1–6.4)	5.9 (5.2–6.7)	5.7 (5.2–6.6)	0.147
Handgrip strength (kg)	23.6 (19.5–29.4)	26.8 (21.8–34.5)	25.7 (20.6–33.4)	0.009*
Gait speed (m/s)	1.04 (0.96–1.16)	1.05 (0.95–1.16)	1.05 (0.95–1.16)	0.967
Chair stand test (sec)	10.7 (8.3–13.6)	9.4 (8.3–11.5)	10.0 (8.3–12.5)	0.031*
TUG time (sec)	8.8 (8.3–10.5)	8.8 (7.9–9.8)	8.8 (8.1–9.9)	0.080
SPPB (score)	11 (11–12)	12 (11–12)	12 (11–12)	0.004*

(Continued)

TABLE 1 (Continued)

	LRH (n = 89)	Non-LRH (n = 167)	Total (n = 256)	p-value
TBS	1.33 (1.28–1.39)	1.37 (1.31–1.41)	1.36 (1.30–1.41)	0.024*
Lumbar spine T-score	−1.0 (−2.1 to 0.2)	−1.0 (−1.9 to −0.1)	−1.0 (−2.0 to 0.0)	0.868
Femur neck T-score	−1.7 (−2.2 to −0.7)	−1.3 (−1.9 to −0.5)	−1.4 (−2.0 to −0.6)	0.020*
Total hip T-score	−0.7 (−1.3 to −0.1)	−0.5 (−1.1 to 0.3)	−0.7 (−1.1 to 0.2)	0.095
Fracture history (n, %)	18 (20.7%)	27 (16.9%)	45 (17.6%)	0.458
Nontraumatic fracture history (n, %)	7 (8.1%)	10 (6.2%)	17 (6.6%)	0.585

Comparisons between groups using the Independent two sample t-test, Wilcoxon rank sum test or Pearson's chi-square tests. * indicates a significant difference $p < 0.05$. LRH, low renin hypertension; y, years; HTN med, antihypertensive medication; DM, diabetes mellitus; PRA, plasma renin activity; AST, aspartate aminotransferase; ALT, alanine aminotransferase; Cr, creatinine; RBP4, retinol binding protein 4; IL6, interleukin-6; Tgfb1, transforming growth factor beta 1, GDF15, growth and differentiation factor 15; FGF19, fibroblast growth factor 19; FGF21, fibroblast growth factor 21; BMI, body mass index; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; HOMA-β, Homeostatic Model Assessment for beta cell function; Ca, calcium; P, phosphorus; ALP, alkaline phosphatase; ALM index, appendicular lean mass index; TUG time, timed up and go time; SPPB, short physical performance battery; TBS, trabecular bone score.

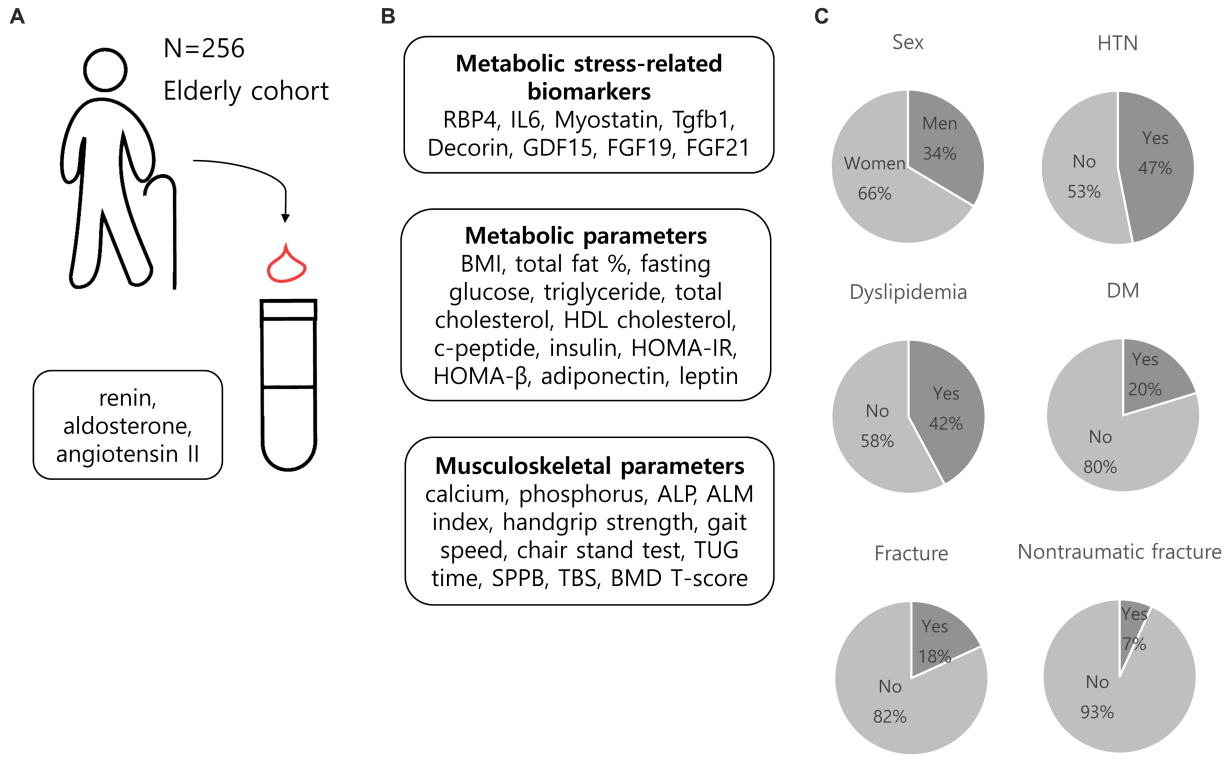

FIGURE 2
Overview of the cohort of the current study. **(A)** We measured renin, aldosterone, and angiotensin II levels in 256 older adults participants. **(B)** Metabolic stress-related parameters, metabolic parameters, and musculoskeletal parameters were also evaluated. **(C)** Graphical representation of baseline characteristics of the cohort. RBP4, retinol binding protein 4; IL6, interleukin-6; Tgfb1, transforming growth factor beta 1, GDF15, growth and differentiation factor 15; FGF19, fibroblast growth factor 19; FGF21, fibroblast growth factor 21; BMI, body mass index; HDL cholesterol, high-density lipoprotein cholesterol; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; HOMA-β, Homeostatic Model Assessment for beta cell function; ALP, alkaline phosphatase; ALM index, appendicular lean mass index; TUG time, timed up and go time; SPPB, short physical performance battery; TBS, trabecular bone score; BMD, bone mineral density; HTN, hypertension; DM, diabetes mellitus.

vs. 25.2%, $p = 0.008$; dyslipidemia, 52.8% vs. 36.5%, $p = 0.012$) (Table 1). When stratified according to sex, no significant difference was observed in the prevalence of DM in the presence of LRH (Supplementary Table S1). Participants with LRH had poorer handgrip strength, chair stand time, SPPB, TBS, and femur neck T-score than those in the non-LRH group (handgrip strength, 23.6 [19.5–29.4] vs. 26.8 [21.8–34.5], $p = 0.009$; chair stand time, 10.7 [8.3–13.6] vs. 9.4 [8.3–11.6], $p = 0.031$; SPPB, 11 (11–12) vs. 12 (11–12), $p = 0.004$; TBS, 1.33 [1.28–1.39] vs. 1.37 [1.31–1.41], $p = 0.024$; femur neck T-score, −1.7 [−2.2 to −0.7] vs. −1.3 [−1.9 to −0.5], $p = 0.020$). TBS and femur neck T-score were significantly lower in the LRH than in non-LRH group (TBS, 1.33 [1.28–1.39] for LRH group vs. 1.37 [1.31–1.41] for non-LRH group, $p = 0.024$; femur neck T-score, −1.7 [−2.2 to −0.7] for LRH group vs. −1.3 [−1.9 to −0.5] for non-LRH group, $p = 0.020$) (Table 1). Women with LRH had more dyslipidemia and lower TBS and femur neck T-score compared with women

without LRH; meanwhile, there were no significant differences in T-score and TBS between men with and without LRH (Supplementary Table S1).

3.2 Correlation between components of RAAS and each metabolic stress-related, metabolic, and musculoskeletal parameter in older adults

The correlation between the RAAS components and each parameter was investigated to determine whether RAAS affects the metabolic and musculoskeletal health of older adults (Figure 3).

PRA was positively associated with glucose, C-peptide, insulin, and HOMA-IR levels, which are parameters related to insulin secretion or resistance, whereas there was a negative correlation between PRA and total fat or total cholesterol. Angiotensin II levels were positively correlated with serum calcium levels and negatively correlated with the appendicular lean mass index (ALM index). In contrast, aldosterone levels showed no significant association with most parameters.

In a multivariable regression analysis adjusted for age, sex, BMI, use of antihypertensive medication, presence of DM or dyslipidemia, and serum creatinine level, the associations that remained significant were between PRA and fasting glucose levels (adjusted $\beta = 1.133$ [0.530–1.735], $p < 0.001$), PRA and HOMA-IR (adjusted $\beta = 0.137$ [0.040–0.233], $p = 0.006$), as well as angiotensin II and serum calcium level (adjusted $\beta = 0.0003$ [0.0001–0.0007], $p = 0.020$).

3.3 Association between the presence of LRH and musculoskeletal health among older adults

As shown in Table 2, femur neck T-score showed a significant negatively associated with the presence of LRH in women (unadjusted $\beta = -0.31$, 95% CI [−0.57, −0.04], $p = 0.022$). In multiple regression analysis, the femur neck T-score also showed a significant association with LRH, independently of age, BMI, the presence of diabetes or dyslipidemia, the use of antihypertensive medication, and serum creatinine level in women (adjusted $\beta = -0.29$, 95% CI [−0.54 to −0.04], $p = 0.025$) (Table 2). However, lumbar spine T-score, total hip T-score, and TBS did not seem to show a significant association with the presence of LRH in univariable regression analysis in women (lumbar spine T-score, unadjusted $\beta = 0.03$, 95% CI [−0.03 to 0.40], $p = 0.884$; total hip T-score, unadjusted $\beta = -0.23$, 95% CI [−0.50 to 0.04], $p = 0.091$; TBS, unadjusted $\beta = -0.02$, 95% CI [−0.04 to 0.00], $p = 0.064$).

Considering the possibility that a significant association could not be shown due to the small size of TBS, logistic regression analysis was performed by classifying as degraded TBS (≤ 1.350) or non-degraded TBS (> 1.350). Univariate logistic regression analysis revealed a significant correlation between the presence of LRH and a degraded TBS in women. Moreover, even after adjusting for age, BMI, the presence of diabetes or dyslipidemia, the use of antihypertensive medication, and serum creatinine level, the association between the presence of LRH and degraded TBS in women remained significant (adjusted odds ratio = 3.22, 95% CI [1.46–7.11], $p = 0.004$) (Table 3).

In men, the presence of LRH did not show any correlation with BMD T-scores or TBS.

There was no significant relationship between the presence of LRH and muscle function (handgrip strength, chair stand test, and SPPB), as well as muscle mass when analyzed using univariate and multivariate regression analyses.

4 Discussion

Our findings suggest that sex differences in metabolic and musculoskeletal health among older adults were found according to the presence of LRH. Unlike in older adults men, LRH was associated with lower femur neck T-score and degraded TBS in postmenopausal women, indicating a higher risk of osteoporosis and future fracture.

LRH encompasses a wide spectrum of disorders, but common causes of LRH encountered in clinical practice include PA, a high-sodium diet, and some medications known to disturb RAAS (6). LRH and PA share a common mechanism of mineralocorticoid activation, which results in a higher risk of cardiovascular disease and mortality (23, 24). PA has an unrecognized prevalence rate of 6%–17% even in normotensive individuals (25). Similar to PA, LRH is associated with a higher incidence of cardiovascular diseases than essential hypertension (23, 24). In addition to PA, several studies have reported an association between high sodium intake and adverse cardiovascular mortality and morbidity (26). This association may be attributed to increased extracellular sodium concentrations, potentially leading to adverse effects on vascular reactivity and growth as well as the stimulation of myocardial fibrosis (27). A decrease in RAAS activity with age is also associated with a reduction in renin levels, leading to a lower PRA (28). Consequently, LRH is more prevalent and significant in older adults (4, 5).

Previous studies on the effects of LRH on metabolic components such as diabetes and dyslipidemia have been conducted; however, this subject remains controversial. A study of 275 adolescents and adults without classic PA reported that PRA was negatively correlated with age, BMI, percent body fat, waist-to-hip ratio, and low-density lipoprotein (LDL) cholesterol (29). The negative correlation between PRA and percent body fat is also consistent with our results. However, according to Monticone et al. (3) there were no significant differences in metabolic parameters, including blood glucose, triglyceride, and LDL cholesterol levels between LRH and normal-high renin groups. This difference may be attributed to the age discrepancy of the participants and the PA inclusion between the two studies. In our study, the positive association between PRA and glucose and the lower prevalence of diabetes in participants with LRH might be explained by previous literature showing that diabetic patients have higher PRA levels than normal controls (30). Furthermore, there was a difference in muscle function between the LRH and non-LRH groups; however, this difference was not statistically significant in the multivariate regression analysis. This could potentially be due to the impact of bone deterioration and the inclusion of individuals with probable PA; osteoporosis is associated with decreased muscle function (31). Muscle weakness can occur in patients with PA, particularly when the plasma concentration of potassium is less than 2.5 meq/L (32).

Previous studies have reported the relationship between LRH and bone health. Tylavsky et al. reported a positive correlation between PRA and distal BMD in premenopausal women with high sodium

		Renin	Aldosterone	Angiotensin II
A	*Metabolic stress-related parameters*			
	RBP4	0.216***	0.150*	-0.066
	IL6	0.032	0.072	0.032
	Myostatin	-0.015	-0.135*	-0.163**
	Tgfb1	0.029	0.134*	-0.037
	Decorin	-0.056	0.002	-0.059
	GDF15	0.220***	0.042	-0.012
	FGF19	-0.011	0.032	0.042
	FGF21	0.057	0.119	0.049
B	*Metabolic parameters*			
	BMI	0.044	-0.044	-0.031
	Total fat %	-0.229***	0.073	0.116
	Fasting glucose	0.190**	-0.055	0.064
	Triglyceride	0.075	0.120	0.075
	Total cholesterol	-0.227***	0.043	0.041
	HDL cholesterol	-0.093	0.011	0.031
	C-peptide	0.247***	0.043	-0.053
	Insulin	0.183**	-0.007	-0.041
	HOMA-IR	0.209***	-0.011	-0.024
	HOMA-β	0.027	0.031	-0.097
	Adiponectin	-0.043	0.059	-0.001
	Leptin	-0.146*	0.042	0.038
C	*Musculoskeletal parameters*			
	Calcium	0.161*	0.108	0.135*
	Phosphorus	-0.005	0.099	0.049
	ALP	-0.040	0.003	0.061
	ALM index	0.201**	-0.050	-0.164**
	Handgrip strength	0.151*	-0.026	-0.088
	Gait speed	-0.047	0.083	-0.080
	Chair stand test	-0.040	-0.103	0.058
	TUG time	0.026	-0.042	-0.011
	SPPB	0.060	0.079	-0.087
	TBS	0.160*	0.015	-0.062
	LS T-score	0.069	0.024	-0.148*
	FN T-score	0.088	0.063	-0.123*
	TH T-score	0.084	0.062	-0.117

* p<0.05, ** p<0.01, *** p<0.001

FIGURE 3
Associations of renin, aldosterone, and angiotensin II with metabolic stress-related parameters, metabolic, or musculoskeletal parameters in total population. **(A)** Metabolic stress-related parameters. **(B)** Metabolic parameters. **(C)** Musculoskeletal parameters. Significant correlations between each parameter were evaluated by spearman correlation analysis. An orange square indicates a positive association, and a blue square indicates a negative association. Abbreviations: RBP4, retinol binding protein 4; IL6, interleukin-6; Tgfb1, transforming growth factor beta 1; GDF15, growth and differentiation factor 15; FGF19, fibroblast growth factor 19; FGF21, fibroblast growth factor 21; BMI, body mass index; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; HOMA-β, Homeostatic Model Assessment for beta cell function; ALP, alkaline phosphatase; ALM index, appendicular lean mass index; TUG time, timed up and go time; SPPB, short physical performance battery; TBS, trabecular bone score; LS, lumbar spine; FN, femur neck; TH, total hip. * for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$.

intake, indicating the negative metabolic effects of LRH on the bone (14). In addition, Kuipers et al. (15) demonstrated a significant association between PRA and BMD or bone turnover markers in the general population, with a positive correlation between PRA and trabecular volumetric BMD measured by quantitative computed tomography, and a negative correlation between PRA and osteocalcin levels in 373 African ancestry family members. However, these two studies included relatively young age groups. In contrast, our research

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TABLE 2 Results of univariable and multivariable regression analysis of femur neck T-score.

(A) Men				
Femur neck T-score	Unadjusted		Adjusted	
	β coefficient (95% CI)	*p*-value	β coefficient (95% CI)	*p*-value
The presence of LRH	0.20 (−0.29 to 0.69)	0.413	0.22 (−0.23 to 0.66)	0.335
Age (years)	−0.04 (−0.07 to −0.01)	0.006*	−0.04 (−0.07 to −0.01)	0.007*
BMI (kg/m^2)	0.14 (0.07 to 0.21)	<0.001*	0.14 (0.07 to 0.21)	<0.001*
HTN med	−0.09 (−0.52 to 0.34)	0.675	−0.21 (−0.61 to 0.18)	0.289
The presence of DM or dyslipidemia	0.02 (−0.41 to 0.45)	0.912	0.06 (−0.34 to 0.45)	0.772
Cr (mg/dL)	0.00 (−1.32 to 1.33)	0.996	0.02 (−1.16 to 1.21)	0.970

(B) Women				
Femur neck T-score	Unadjusted		Adjusted	
	β coefficient (95% CI)	*p*-value	β coefficient (95% CI)	*p*-value
The presence of LRH	−0.31 (−0.57 to −0.04)	0.022*	−0.29 (−0.54 to −0.04)	0.025*
Age (years)	−0.05 (−0.07 to −0.03)	<0.001*	−0.05 (−0.07 to −0.03)	<0.001*
BMI (kg/m^2)	0.08 (0.04 to 0.13)	<0.001*	0.07 (0.03 to 0.11)	0.002*
HTN med	0.00 (−0.26 to 0.27)	0.983	0.18 (−0.08 to 0.44)	0.173
The presence of DM or dyslipidemia	−0.10 (−0.47 to 0.28)	0.612	0.04 (−0.21 to 0.29)	0.755
Cr (mg/dL)	−0.39 (−1.58 to 0.81)	0.522	0.08 (−1.02 to 1.17)	0.887

Univariable and multivariable analyses using linear regression model adjusted for age, BMI, the use of hypertension medication, the presence of DM or dyslipidemia, and serum creatinine level. *indicates significant association $p < 0.05$. 95% CI, 95% confidence interval; LRH, low renin hypertension; BMI, body mass index; HTN med, antihypertensive medication; DM, diabetes mellitus; Cr, serum creatinine.

TABLE 3 Results of univariable and multivariable logistic regression analysis of degraded TBS (≤1.350).

(A) Men				
Degraded TBS	Unadjusted		Adjusted	
	β coefficient (95% CI)	*p*-value	β coefficient (95% CI)	*p*-value
The presence of LRH	0.17 (0.02–1.38)	0.097	0.17 (0.20–1.40)	0.100
Age (years)	1.05 (0.97–1.14)	0.238	1.05 (0.96–1.15)	0.260
BMI (kg/m^2)	0.97 (0.80–1.19)	0.797	0.95 (0.77–1.17)	0.635
HTN med	2.30 (0.71–7.42)	0.162	2.35 (0.68–8.10)	0.176
The presence of DM or dyslipidemia	1.05 (0.34–3.21)	0.931	0.78 (0.23–2.61)	0.684
Cr (mg/dL)	1.03 (0.03–32.13)	0.986	0.84 (0.03–23.26)	0.919

(B) Women				
Degraded TBS	Unadjusted		Adjusted	
	β coefficient (95% CI)	*p*-value	β coefficient (95% CI)	*p*-value
The presence of LRH	2.39 (1.21–4.73)	0.012*	3.22 (1.46–7.11)	0.004*
Age (years)	1.07 (1.01–1.12)	0.013*	1.08 (1.02–1.15)	0.007*
BMI (kg/m^2)	1.01 (0.90–1.14)	0.814	1.05 (0.93–1.20)	0.410
HTN med	0.81 (0.43–1.54)	0.519	0.40 (0.18–0.88)	0.023*
The presence of DM or dyslipidemia	0.64 (0.27–1.56)	0.329	0.94 (0.46–1.92)	0.874
Cr (mg/dL)	2.71 (0.13–55.09)	0.517	1.21 (0.04–36.22)	0.912

Univariable and multivariable analyses using logistic regression model adjusted for age, BMI, the use of hypertension medication, the presence of DM or dyslipidemia, and serum creatinine level. *indicates significant association $p < 0.05$. TBS, trabecular bone score; 95% CI, 95% confidence interval; LRH, low renin hypertension; BMI, body mass index; HTN med, antihypertensive medication; DM, diabetes mellitus; Cr, serum creatinine.

was conducted in an older adult population where LRH is more common but seldom studied and showed sex differences.

Interestingly, in our study, LRH was associated with lower femur neck T-score and degraded TBS in postmenopausal women. TBS is a measure that assesses changes in the gray-scale intensity of pixels in DXA images of the lumbar spine, which indirectly reflects the microarchitecture of the trabecular bone, and it has the potential to enhance fracture prediction compared to using DXA alone (21). The mechanism underlying the association between LRH and bone quantity or quality has not yet been elucidated, but the following three mechanisms are possible. First, it is possible that a large number of PA were included, as mentioned earlier. In our study, probable PA, defined as PRA < 1 ng/mL/h and ARR of 20 or higher, was significantly higher in LRH group than in non-LRH group (70 [78.7%] in the LRH group vs. 48 [28.7%] in the non-LRH group, $p < 0.001$). PA may deteriorate bone health through (1) secondary hyperparathyroidism due to increased urinary calcium (33); (2) a direct effect on bone health through the distribution of mineralocorticoid receptors in human osteoclasts, osteoblasts, osteocytes, and parathyroid tissue (34, 35); and (3) reduced bone formation and increased apoptosis of osteoblasts and osteocytes caused by inflammation due to oxidative stress (36, 37). PA is a well-known risk factor for fractures (38), and a significant number of PA may have influenced the study results. Second, some participants consuming a high-sodium diet may also have affected our findings. High sodium intake can cause LRH by inhibiting renin activity and aldosterone release (39). In addition, increased sodium intake may increase urine calcium excretion, which can accelerate bone remodeling and loss (40). A previous meta-analysis showed a positive association between sodium intake and osteoporosis risk (41). Third, increased local angiotensin II activity in patients with LRH may affect bone deterioration (32). In patients with LRH, the activity of angiotensin II can rise locally within certain tissues, such as the vascular endothelium, kidneys, and adrenal glands, even if angiotensin II levels are normal (32). Although no significant difference in angiotensin II level was observed between the LRH and non-LRH groups, there was a significant association between angiotensin II and lumbar spine T-score, as well as femur neck T-scores in this study. Angiotensin II may influence bone cells by binding to AT1 receptors on osteoblasts and triggering the release of mediators that activate osteoclasts, which are thought to modulate blood flow in the bone marrow capillaries and contribute to osteoclastic bone resorption (42, 43). Moreover, angiotensin II potentially affects calcium metabolism by elevating parathyroid hormone and decreasing ionized calcium levels (44, 45).

The reasons for sex differences in the relationship between LRH and BMD or bone quality may include the following: first, women with relatively large sex hormonal variations might be more vulnerable to the harmful impacts of excessive aldosterone compared to men. Kim et al. demonstrated that only women with PA showed a lower TBS than those with nonfunctioning adenomas (46). Second, RBP4, which is significantly associated with PRA in women, may influence sex differences. In particular, the association between PRA and RBP4 levels may explain why BMD and PRA are associated only in women. RBP4 is secreted by adipocyte, and *in vivo* studies have demonstrated that chondrocytic RBP4 is involved in bone growth (47). However, in clinical studies, the role of RBP4 in bone health appears to vary with age and sex. Some studies reported a positive association between RBP4 and BMD in postmenopausal women with osteopenia or osteoporosis (48, 49). In contrast, another study in young men did not show any association between RBP4 and BMD (50). Furthermore, serum GDF15 level, which may affect bone metabolism or muscle homeostasis in old women via inflammatory responses (51), seemed to have positive linear correlation with the presence of LRH in the current study. Further research is essential to understand the role of RBP4 and GDF15 levels on musculoskeletal health in the older adults with LRH.

To our knowledge, this is the first clinical study to report a significant relationship between PRA and bone quality as well as BMD in older women, but not in older men. Additionally, while sex differences in hypertension and cardiovascular disease due to differential activation of the sympathetic nervous system, RAAS, and immune system have been previously acknowledged (52, 53), this is the first report to indicate a sex difference in the relationship between the presence of LRH and bone health in older participants. However, this study has several limitations. First, it was difficult to infer causality owing to the cross-sectional research design. Second, the sample size was small, which might in part have affected the insignificant results, especially in men, which may limit the generalizability and statistical power of the current results. Therefore, future research with a larger and more diverse samples will be required to enhance the applicability of our findings. Third, although liquid chromatography-mass spectrometry is more accurate and reliable than RIA for the measurement of PRA (54), we measured PRA using RIA because of its cost and availability. Fourth, hypercortisolism may have been included in the low renin-low aldosterone patient group (6), which may have affected the study results. Also, we did not investigate the form of could not precisely exclude secondary hypertension. Fifth, unfortunately, the specific medication profiles for all hypertensive patients were not retrieved, with only partial data available for some individuals. Finally, owing to the lack of data on 25-hydroxyvitamin D, parathyroid hormone levels, serum electrolyte levels, bone turnover markers, calcium/vitamin D supplementation, urinary sodium excretion, or sodium intake, the mechanisms by which LRH adversely affect bone density or quality cannot be well explained. However, an age-related decrease in the RAAS has been found in normal participants, regardless of sodium repletion (55).

In summary, our research suggests that clinical characteristics and metabolic risk in older adults could be influenced by the presence of LRH. LRH in postmenopausal women was associated with a lower femur neck T-score and degraded TBS, highlighting the sex-specific impact of LRH on bone health in old women. A more comprehensive, larger, prospective study is needed to clarify how RAAS affects metabolic and musculoskeletal outcomes in the older adult population.

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 this clinical study was permitted by the Institutional Review Board (IRB) of Yonsei University Wonju College of Medicine (IRB numbers CR317131,

CR318003, and CR322353). 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

JC, MK, K-SP, S-BK, and JL conceptualized and designed the study. SL, JC, MK, and JL have conducted the study. SL, JC, MK, K-SP, S-BK, and JL have collected and interpreted data. SL and JL have drafted the manuscript and have revised and finalized the manuscript. 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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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2024.1250945/full#supplementary-material

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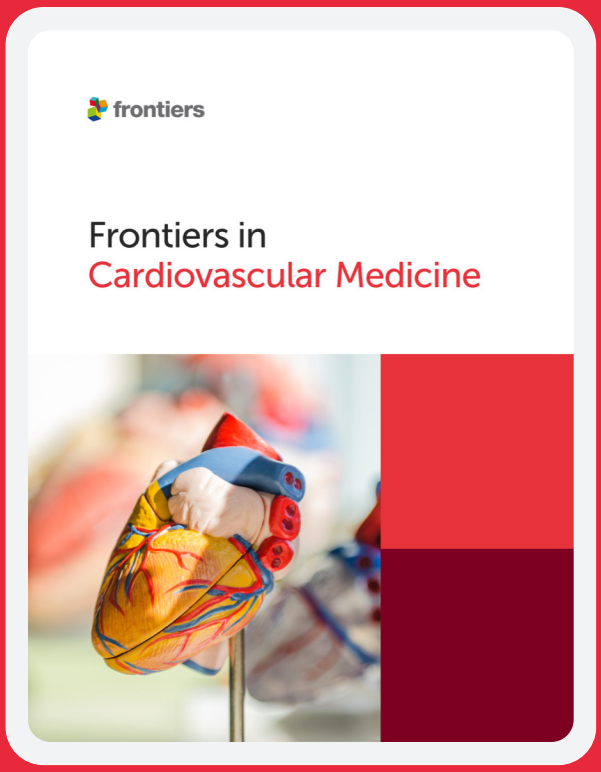

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