Cardiovascular Diabetology

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Chain effect of lifecourse reproductive characteristics and body fat and muscle on cardiovascular disease in women: a Mendelian randomization study

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Abstract

Background Delineating the causal chain effects of reproductive traits and fat- and muscle-related traits on cardiovascular disease (CVD) is essential for optimizing precision prevention and control of cardiovascular health in women.

Methods In this study, we applied the two-sample Mendelian randomization (MR) analyses and two-step MR framework to investigate the causal chain effects and the mediating effect pathways among reproductive factors and fat- and muscle-related traits on CVD outcomes in women, applying the genome-wide association study summary statistics of 16 women's reproductive traits across puberty and pre-pregnancy, pregnancy and postpartum, and menopausal transition stages, 16 women's fat- and muscle-related traits, and five CVD outcomes of coronary artery disease (CAD), myocardial infarction (MI), heart failure, atrial fibrillation, and ischemic stroke (IS) from over one million individuals of European descent.

Results The MR analyses revealed the associations of genetically predicted nine reproductive traits (i.e., age at menarche [odds ratio (OR) for CAD: 0.92], age at first sexual intercourse [AFS; 0.71], age at first birth [AFB; 0.89], hypertensive disorders of pregnancy [HDP; 1.21], pre-eclampsia [PE; 1.34], preterm birth [PTB; 1.09], sex hormone-binding globulin [SHBG; 0.73], bioavailable testosterone [BT; 1.17], and number of stillbirths [OR for IS: 2.14]) and 13 fat- and muscle-related traits with at least one of five CVD outcomes. Two-step MR identified 30 causal pathways where AFS, AFB, HDP, PE, PTB, SHBG, and BT mediated the effects of body composition on five CVD outcomes, and nine pathways where waist-to-hip ratio, trunk-trunk fat ratio, abdominal subcutaneous adipose tissue, and gluteofemoral adipose tissue mediated the effects of reproductive traits on CAD and MI.

Conclusions Lifecourse reproductive characteristics and fat- and muscle-related traits manifested reciprocal mediating effects on CVD, informing targeted strategies for bridging cardiovascular health inequalities in women.

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Keywords Cardiovascular disease, Lifecourse reproductive characteristics, Body fat, Muscle, Mendelian randomization

Introduction

Women's reproductive signatures across lifecourse can be divided into three stages, from menarche to menopause, including puberty and pre-pregnancy, pregnancy and postpartum, and menopausal transition [1]. Previous observational studies have associated multiple reproductive characteristics across the three stages, including polycystic ovary syndrome (PCOS), pregnancy complications such as hypertensive disorders of pregnancy (HDP), pre-eclampsia (PE), and gestational diabetes mellitus (GDM), and the onset of menopause, with the risk of cardiovascular disease (CVD) in late life [1–3]. On the other hand, women's different reproductive stages present varied manifestations in body composition, mainly fat and muscle [4–6]. For instance, girls acquire more fat mass and less fat-free mass than boys during puberty [4]. Women experience significant weight gain, especially increased body fat, during midlife [5] whereas there is reduced lean mass during the menopausal transition [6]. These changes in body composition challenge metabolic resilience and predispose women to subsequent CVD [7–9]. Although the associations of body fat and muscle function (e.g., sarcopenia) with CVDs have been well demonstrated in observational studies [9, 10], the associations between precisely measured local body composition traits and CVDs remain notably scarce. More importantly, refined evidence on the bidirectional associations between reproductive factors across the women's lifecourse stages and body composition and their causal chain of effect on CVD is limited, which is essential for optimizing precision prevention and control of CVD in women.

Recent Mendelian randomization (MR) studies have suggested associations of the genetically predicted adiposity indicators, measured by physical examination or body composition scanning with dual-energy X-ray absorptiometry, with age at menarche (AAM), PCOS, reproductive conditions, and pregnancy-related disorders, suggesting a broad relationship between obesity and women's reproductive health across different stages [11–14]. Compared with conventional observational studies, MR approaches utilize genetic variants as instrumental variables (IVs) to yield accurate causal relationships, which avoid reverse causality and are less likely to be affected by confounders [15]. In particular, the two-sample MR can investigate multiple exposures of interest in one study by using genome-wide association study (GWAS) summary statistics [16], and therefore facilitate the integration of the milestone reproductive characteristics throughout women's entire lifecourse and body composition markers to infer their impact on late life CVD risk. Further, the two-step MR provides a robust framework [17], which can be applied to establish the sophisticated mediating pathways from reproductive characteristics and body composition to CVD.

To this end, we performed this two-step, two-sample MR study to systematically explore the causal associations of 16 representative characteristics across three reproductive stages and 16 body fat- and muscle-related traits on five CVD outcomes. We further dissected the bidirectional associations between reproductive factors and body composition and specifically delineated their mutual mediating effect on CVD risk, to inform preventive and interventive targets for improving cardiovascular health in women.

Methods

Study design

Schematic overview of the study design is shown in Fig. 1. Firstly, we estimated the genetic correlations and causal associations of 16 reproductive traits across stages of puberty and pre-pregnancy, pregnancy and postpartum, and menopausal transition and 16 women-specific body composition traits including nine body fat-related traits and seven muscle-related traits on five CVD outcomes, by using linkage disequilibrium score regression (LDSC) and univariable MR (UVMR), respectively. Secondly, we tested the bidirectional associations between each reproductive and body composition trait using UVMR. Thirdly, we investigated the direct effect of each reproductive or body composition trait on CVD outcomes using multivariable MR (MVMR) and quantified their individual mediating effects using two-step MR. We reported this study following the STROBE-MR guidelines [18]. All the GWAS summary statistics used in this study are publicly available and can be downloaded from the original GWAS publications or the GWAS consortium websites. Ethical approval and informed consent for participants were provided in the original GWAS publications.

Data source

Based on the literature review, we selected 16 reproductive traits from 418,758 women of European descent across three stages from menarche to menopause to represent women's reproductive milestones (Fig. 1). We used women-specific GWAS summary statistics of body composition traits in up to 434,794 women of European descent, including nine body fat- and seven muscle- or sarcopenia-related traits. Five CVD phenotypes included coronary artery disease (CAD), myocardial infarction (MI), heart failure (HF), atrial fibrillation (AF), and

ischemic stroke (IS) from over one million individuals of European descent were selected as the outcome. Detailed information on GWAS datasets and definitions for each phenotype are shown in Supplementary Methods, Table 1, and Table S1.

Statistical analysis

LDSC analysis

We conducted the LDSC to estimate the genetic correlations between reproductive traits, body composition traits, and CVD outcomes [19].

UVMR and MVMR analysis

We performed the UVMR to estimate the bidirectional causal association between reproductive traits and body composition and their respective associations with CVD outcomes. We applied the MVMR to assess the direct effect of each reproductive or body composition trait on CVD outcomes. The IVs used in all MR analyses satisfied three core assumptions: (1) the IVs must be vigorously associated with the exposure in UVMR and at least one of the multiple exposures in MVMR; (2) the IVs must not be associated with confounders of the associations between instruments of each exposure and each outcome; and (3) the effect of IVs on the outcome must go through the exposure [20]. Detailed information on instrument selection can be found in Supplementary Methods. For the primary UVMR analysis, we used the random-effects inverse-variance weighted (IVW) method to obtain precise estimations when assuming all the IVs are valid [16] and four or more IVs are available in the trait [21]. Otherwise, a fixed-effect IVW method was used for traits with two or three IVs. In MVMR analyses, the MVMR-IVW method was used as the main analysis [22], and we combined IVs from each reproductive trait and each body composition following the same clumping criteria as the UVMR analysis. All the IVs used for each trait can be found in Table S2.

Mediation MR analysis

We used the two-step MR method to estimate the mediating effect of each reproductive or body composition trait in the association between each other and CVD [17]. The first step was to estimate the causal effect of the exposure on the mediator (β_1) using UVMR; the second step was to estimate the causal effect of the mediator on the CVD outcome (β_2) using MVMR with adjustment for the exposure, based on an established UVMR causal relationship between the mediator and the outcome. The criteria for determining the mediating pathways are listed in the Supplementary Methods. The mediation proportion of each mediator in the association between the reproductive or body composition trait and CVD was calculated as the mediating effect (the product of β_1 and β_2) divided by the total effect of the exposure on CVD. The delta method was used to calculate the 95% confidence interval (CI) of mediation proportion [23]. The negative mediation proportion was truncated at 0% because this is the minimum limit of the mediating effect.

Sensitivity analysis

We performed several sensitivity analyses (i.e., weighted median, weighted mode, MR-Egger, and MR pleiotropy residual sum and outlier [MR-PRESSO]) under different assumptions to validate the robustness of the IVW results. The MR-lap, MR-Egger intercept test, F-statistics, and Cochran's Q test were performed and calculated to reduce the influence of weak instrument bias and winner's curse on the causal effect estimation and the pleiotropic effect for IVs [24–26].

All MR analyses were performed using R software (version 4.3.1). In this study, the false discovery rate (FDR) adjustments included 80 tests for 16 reproductive traits and five CVD outcomes and another 80 tests for 16 body fat and muscle traits and five CVD outcomes. For the bidirectional associations between 16 reproductive traits and 16 body fat and muscle traits, 256 tests were included for both directions, respectively. In the sensitivity MR analyses (i.e., weighted median, weighted mode, MR-Egger, and MR-PRESSO), the FDR adjustments included 70 and 75 tests for 14 reproductive traits or 15 body fat and muscle traits and five CVD outcomes, respectively, since there were insufficient IV numbers in preterm birth (PTB), infertility, and abdominal subcutaneous adipose tissue (ASAT). Furthermore, for the bidirectional associations, the FDR adjustments included 224 tests for 14 reproductive traits and 16 body fat and muscle traits and 240 tests for 15 body fat and muscle traits and 16 reproductive traits. After the multiple testing correction, an FDR-adjusted $P<0.05$ was considered statistically significant in all analyses. The causal effects for case–control or continuous GWAS outcomes were reported as odds ratios (ORs) or β coefficients with 95% CI and FDR-adjusted P values. Detailed information on statistical analysis is shown in Supplementary Methods.

Results

Associations between reproductive traits and cardiovascular diseases

The LDSC indicated extensive genetic correlations between reproductive traits, body composition traits, and CVD outcomes (Figure S1 and Tables S3–S5). The UVMR supported that in the puberty and pre-pregnancy stage, genetically predicted later AAM was associated with lower risks of CAD (OR 0.92, 95% CI 0.89–0.96), MI (0.91, 0.87 to 0.96), and HF (0.88, 0.84 to 0.92; Fig. 2A and Table S6). Consistent inverse associations were observed between age at first sexual intercourse (AFS) and all

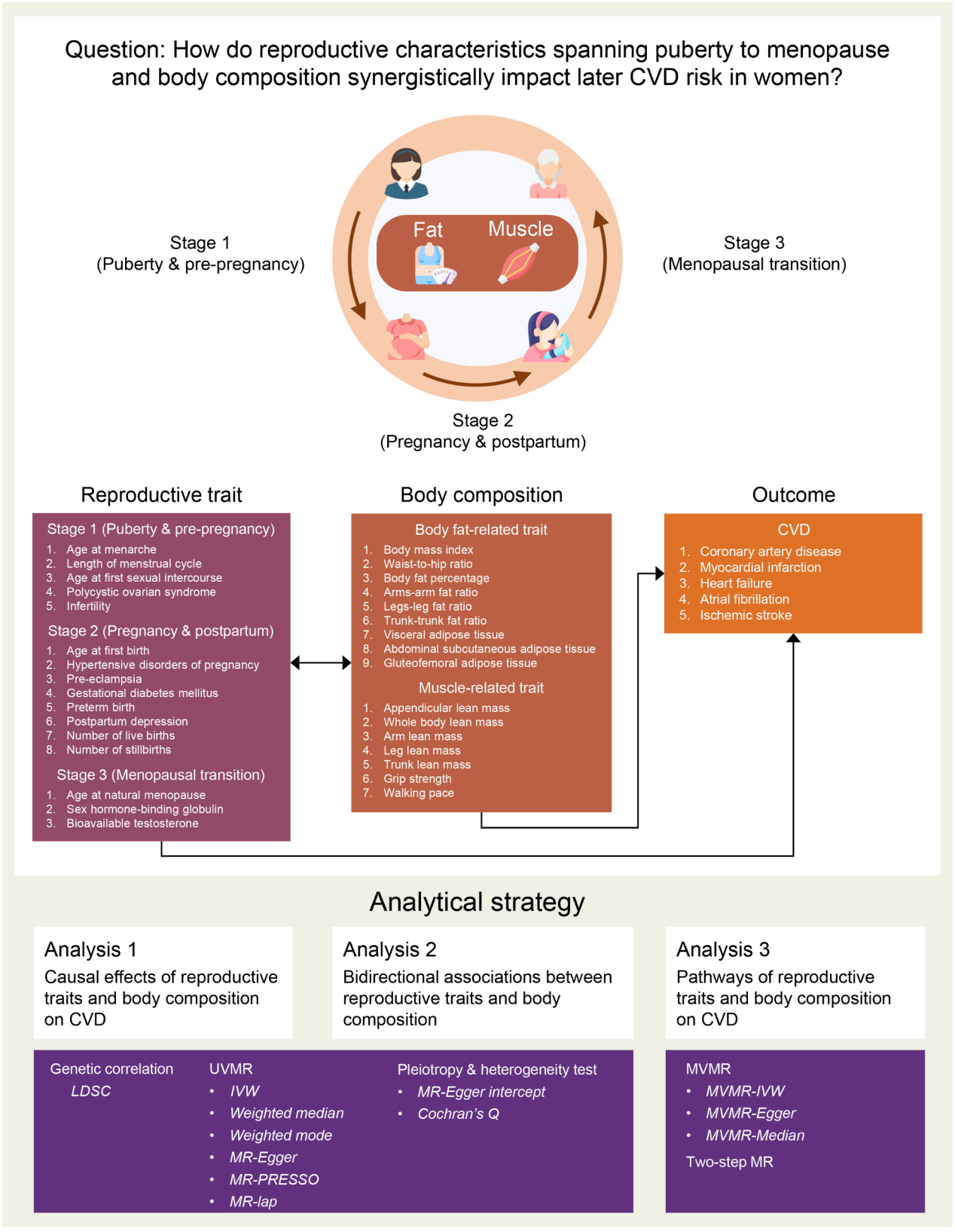

Fig. 1 (See legend on next page.)

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Fig. 1 Schematic overview of the study design. This MR study consisted of three analytical steps. In step 1, we estimated the genetic correlations and causal associations of reproductive traits across stages and women-specific body composition traits on CVD outcomes using LDSC and UVMR. In step 2, we tested the bidirectional associations between reproductive and body composition traits using UVMR. In step 3, we investigated the mediating pathway of each reproductive or body composition trait on CVD outcomes using MVMR under the two-step MR framework. *CVD* cardiovascular disease; *IVW* inverse-variance weighted; *LDSC* linkage disequilibrium score regression; *MR* Mendelian randomization; *MR-PRESSO* Mendelian randomization pleiotropy residual sum and outlier; *MVMR* multivariable Mendelian randomization; *UVMR* univariable Mendelian randomization

CVD outcomes. In the pregnancy and postpartum stage, HDP was positively associated with CAD, MI, HF, and AF. Similarly, PE, PTB, and higher number of stillbirths (NSB) were positively associated with CAD, MI, or IS. By contrast, age at first birth (AFB) was inversely associated with CAD, MI, HF, and AF. In the menopausal transition stage, genetically predicted higher bioavailable testosterone (BT) levels were associated with higher risks of CAD (OR 1.17, 95% CI 1.08–1.27) and MI (1.18, 1.07 to 1.30); while higher sex hormone-binding globulin (SHBG) levels were associated with lower risks of CAD, MI, and IS. These IVW estimates were confirmed by at least one sensitivity analysis, except for the results of AFS on AF and PTB on CAD (Table S6). Cochran's Q and MR-Egger intercept tests suggested heterogeneity of the IVs and pleiotropic effects of AFB with AF and SHBG with CAD, MI, and HF (Table S7).

Associations between body composition traits and cardiovascular diseases

The UVMR supported positive associations of body mass index (BMI) with all CVD outcomes, body fat percentage (BF%) with MI, HF, and AF, arms-arm fat ratio (Arm FR) with CAD, MI, and HF, and ASAT with CAD and MI and inverse associations of legs-leg fat ratio (Leg FR) with HF and AF and gluteofemoral adipose tissue (GFAT) with CAD and MI (Fig. 2B and Table S8). Inconsistent associations were observed: genetically predicted waist-to-hip ratio (WHR) increased the risk of CAD, MI, and IS but reduced the risk of AF, whereas trunk-trunk fat ratio (Trunk FR) reduced the risk of CAD and MI but increased the risk of AF. For muscle-related traits, each one-standard deviation increases in genetically predicted appendicular lean mass (ALM) was associated with lower risks of CAD, MI, and IS but a higher risk of AF (Fig. 2B and Table S8). Lean mass of the arm, leg, trunk, and whole body had consistent positive associations with HF and AF. Sensitivity analyses supported these IVW estimates (Tables S8 and S9).

Bidirectional associations between reproductive traits and body composition traits

The UVMR analysis revealed 18 pairs of bidirectional associations between reproductive traits (i.e., AAM, length of menstrual cycle, AFS, AFB, PE, SHBG, and BT) and body fat- or muscle-related traits (i.e., BMI, WHR, Arm FR, Trunk FR, ASAT, GFAT, and ALM) (Figure S2, Tables S10 and S11). A total of 24 unidirectional associations were observed for the effects of seven reproductive traits (i.e., AAM, infertility, AFB, PE, PTB, SHBG, and BT) on 13 body composition traits (all 16 traits excluding Arm FR, ALM, and grip strength). In contrast, 34 unidirectional associations were observed for the effects of 14 body composition traits (all 16 traits excluding ASAT and walking pace) on 14 reproductive traits (all 16 traits excluding AAM and NSB). The average F-statistics ranged from 23.16 to 104.10 indicating that the MR estimates were not influenced by weak instrument bias (Tables S10 and S11). Sensitivity analyses supported the primary results (Tables S12 and S13). The MRlap analysis showed no differences between observed effect sizes and corrected effect sizes, except for AFB on AF, demonstrating that the sample overlap of the GWAS summary dataset for most exposures and outcomes was unlikely to affect the MR estimates (Tables S14 and S15).

Mediating pathways from reproductive traits and body composition traits to cardiovascular diseases

Following the mediating pathway screening criteria, we identified nine mediating pathways from reproductive traits to CVD via body fat (Fig. 3, Tables S16 and S17). GFAT mediated 54.63% of the total effect of PTB on CAD, followed by WHR and Trunk FR. Other mediating pathways presented in the associations of AAM and sex hormone levels with CAD and MI, mediated by Trunk FR and ASAT. On the other hand, we identified 30 pathways from body composition to CVD via reproductive traits (Fig. 4 and Table S18). Seven reproductive traits (i.e., AFS, AFB, HDP, PE, PTB, SHBG, and BT) mediated 26.60–2.91% of the total effects of body composition (i.e., BMI, WHR, BF%, Arm FR, Trunk FR, GFAT, and ALM) on five CVD outcomes, respectively.

Discussion

In this study, we identified extensive genetic correlations and causal associations between reproductive traits, body fat and muscle, and five CVD outcomes. The MR analysis elucidated the effects of nine reproductive traits, spanning puberty and pre-pregnancy (i.e., AAM and AFS), pregnancy and postpartum (i.e., AFB, HDP, PE, PTB, and NSB), and menopausal transition (i.e., SHBG and BT) stages across women's lifecourse, and 13 body fat- or muscle-related traits on CAD, MI, HF, AF, and IS. In addition, 58 unidirectional and 18 pairs of bidirectional

Table 1 Overview of the GWAS summary statistics used in the study

Phenotype	Sample size (case/control)	Unit	Data source	Year of publication	PMID/link
Reproductive traits					
Age at menarche (AAM)	252,514	Year	ReproGen Consortium and UKB	2017	28436984
Length of menstrual cycle	32,847	1-SD	UKB	2018	nealelab.is/uk-biobank/
Age at first sexual intercourse (AFS)	214,547	Year	UKB	2021	34211149
Polycystic ovarian syndrome (PCOS)	25,295 (4,890/20,405)	Event	Rotterdam study, UK PCOS study, EGCUT cohort, deCODE cohort, Chicago PCOS cohort, and Boston PCOS cohort	2018	30566500
Infertility	126,342 (14,759/111,583)	Event	FinnGen (R10)	2023	36653562
Age at first birth (AFB)	418,758	Year	36 cohorts (included UKB)	2021	34211149
Hypertensive disorders of pregnancy (HDP)	221,492 (9,535/211,957)	Event	FinnGen (R10)	2023	36653562
Pre-eclampsia (PE)	219,334 (7,377/211,957)	Event			
Gestational diabetes mellitus (GDM)	230,310 (14,718/215,592)	Event			
Preterm birth (PTB)	233,290 (15,419/217,871)	Event	EGG Consortium	2023	37012456
Postpartum depression (PPD)	70,765 (17,339/53,426)	Event	18 cohorts (included UKB)	2023	37849304
Number of live births (NLB)	193,953	1-SD	UKB	2018	nealelab.is/uk-biobank/
Number of stillbirths (NSB)	60,453	1-SD			
Age at natural menopause (ANM)	201,323	Year	1000 Genomes imputed studies, BCAC, and UKB	2021	34349265
Sex hormone-binding globulin (SHBG)	189,473	1-SD	UKB	2020	32042192
Bioavailable testosterone (BT)	188,507	1-SD			
Body fat- and muscle-related traits					
Body mass index (BMI)	434,794	1-SD	GIANT Consortium and UKB	2019	30239722
Waist-to-hip ratio (WHR)	381,152	1-SD			
Body fat percentage (BF%)	190,991	1-SD	UKB	2018	nealelab.is/uk-biobank/
Arms-arm fat ratio (Arm FR)	191,802	1-SD	UKB	2019	30664634
Legs-leg fat ratio (Leg FR)	191,864	1-SD			
Trunk-trunk fat ratio (Trunk FR)	191,772	1-SD			
Visceral adipose tissue (VAT)	20,038	1-SD	UKB	2022	35773277
Abdominal subcutaneous adipose tissue (ASAT)	20,038	1-SD			
Gluteofemoral adipose tissue (GFAT)	20,038	1-SD			
Appendicular lean mass (ALM)	244,730	1-SD	UKB	2020	33097823
Whole body lean mass (WBLM)	190,993	1-SD	UKB	2018	nealelab.is/uk-biobank/
Arm lean mass (Arm LM)	190,934	1-SD			
Leg lean mass (Leg LM)	190,972	1-SD			
Trunk lean mass (Trunk LM)	190,833	1-SD			
Grip strength (GS)	193,305	1-SD			
Walking pace (WP)	193,007	Categorical variable 1 to 3			
CVD					
Coronary artery disease (CAD)	1,165,690 (181,522/984,168)	Event	10 studies (included UKB)	2022	36474045

Table 1 (continued)

Phenotype	Sample size (case/control)	Unit	Data source	Year of publication	PMID/link
Myocardial infarction (MI)	639,221 (61,505/577,716)	Event	CARDIoGRAMplusC4D Consortium and UKB	2021	33532862
Heart failure (HF)	977,323 (47,309/930,014)	Event	HERMES Consortium (included UKB)	2020	31919418
Atrial fibrillation (AF)	1,030,836 (60,620/970,216)	Event	Nord-Trøndelag Health Study, deCODE, Michigan Genomics Initiative, DiscovEHR, AFGen Consortium, and UKB	2018	30061737
Ischemic stroke (IS)	440,328 (34,217/406,111)	Event	MEGASTROKE Consortium	2018	29531354

Only women-specific GWAS summary statistics of body composition traits were used in this study

WHR, VAT, ASAT, and GFAT were adjusted for BMI

AFGen atrial fibrillation genetics; *BCAC* Breast Cancer Association Consortium; *CARDIoGRAMplusC4D* coronary artery disease genome wide replication and meta-analysis plus the coronary artery disease genetics; *EGG* early growth genetics; *GIANT* genetic investigation of anthropometric traits; *HERMES* heart failure molecular epidemiology for therapeutic targets; *SD* standard deviation; *UKB* UK Biobank

associations were observed between 15 reproductive traits (all traits excluding NSB) and all 16 body composition traits. We specifically outlined the causal chain of the effects on CVD: in 30 pathways, reproductive traits (i.e., AFS, AFB, HDP, PE, PTB, SHBG, and BT) mediated the effects of body composition on five CVD outcomes, mainly CAD and MI; in nine pathways, body fat (i.e., WHR, Trunk FR, ASAT, and GFAT) mediated the effects of reproductive traits on CAD and MI. Our findings, from a lifecycle lens, underscored the extensive influence of the reproductive hall markers throughout women's lifecourse and body composition characteristics on CVD risk and their mutual mediating effects herein, informing holistic and precise preventive strategies to improve cardiovascular health in women.

Current evidence suggests that reproductive characteristics across key stages of the women's lifecourse may be overlooked as risk factors that contribute to later cardiovascular dysfunction and lead to additional health inequalities in women [1–3]. Our findings were partly in line with previous MR results that greater AAM, AFS, AFB, and SHBG were protective factors for at least one of the five CVD outcomes, and HDP, PE, and BT were risk factors for CAD, MI, or IS [27–33]. Of note, we indicated novel MR associations including the positive associations of HDP, PE, PTB, and BT with HF, AF, MI, and CAD, as well as inverse associations of SHBG with MI. These relationships were consistent with observations from large cohorts that early menarche, early AFB, HDP, PE, PTB, and sex hormone levels in women were associated with multiple cardiometabolic diseases in late life. [1, 2, 34, 35]

During puberty and pregnancy, an early AAM and adverse pregnancy outcomes (i.e., HDP, PE, and PTB) share common biological mechanisms with CVD, which may be triggered by metabolic derangements and endothelial dysfunction [2, 36]. Interestingly, we observed a more pronounced association pattern of AAM and PE with CAD and MI, but less significant associations with AF. CAD and MI are primarily caused by coronary artery embolism, whereas AF mainly results from structural changes induced by ischemia and inflammation [37]. Therefore, different etiological mechanisms of different CVD subtypes may account for the different susceptibilities to the effects of the same exposure. For example, the pathophysiology of AAM and PE, including metabolic dysregulation, endothelial dysfunction, oxidative stress, and endoplasm reticulum stress [36], is closely related to thrombosis and atherosclerosis rooted in CAD and MI. On the other hand, women with early AFS and AFB may be more susceptible to adverse environments, such as low educational attainment, disadvantaged socioeconomic status, unhealthy lifestyles, and poor family and partner relationships, which are responsible for higher CVD risk [28]. Although the F-statistics indicated that the IVs of AFS and AFB are strong, our genetic conclusions on these reproductive behaviors might be partial. Future gene-environment interaction studies are warranted to deepen the understanding of the impact of reproductive behaviors on cardiovascular health in women. During the menopausal transition period, high androgen levels in post-menopausal women may induce platelet aggregation, vasoconstriction, and fat and lipid accumulation, which increase CVD risk [35]. Taken together, our MR findings deepen the understanding of the reproductive roots in women's cardiovascular health.

Furthermore, we distinguished the causal effect patterns of body fat and muscle on CVD outcomes in women, wherein BMI, BF%, Arm FR, ASAT, and body

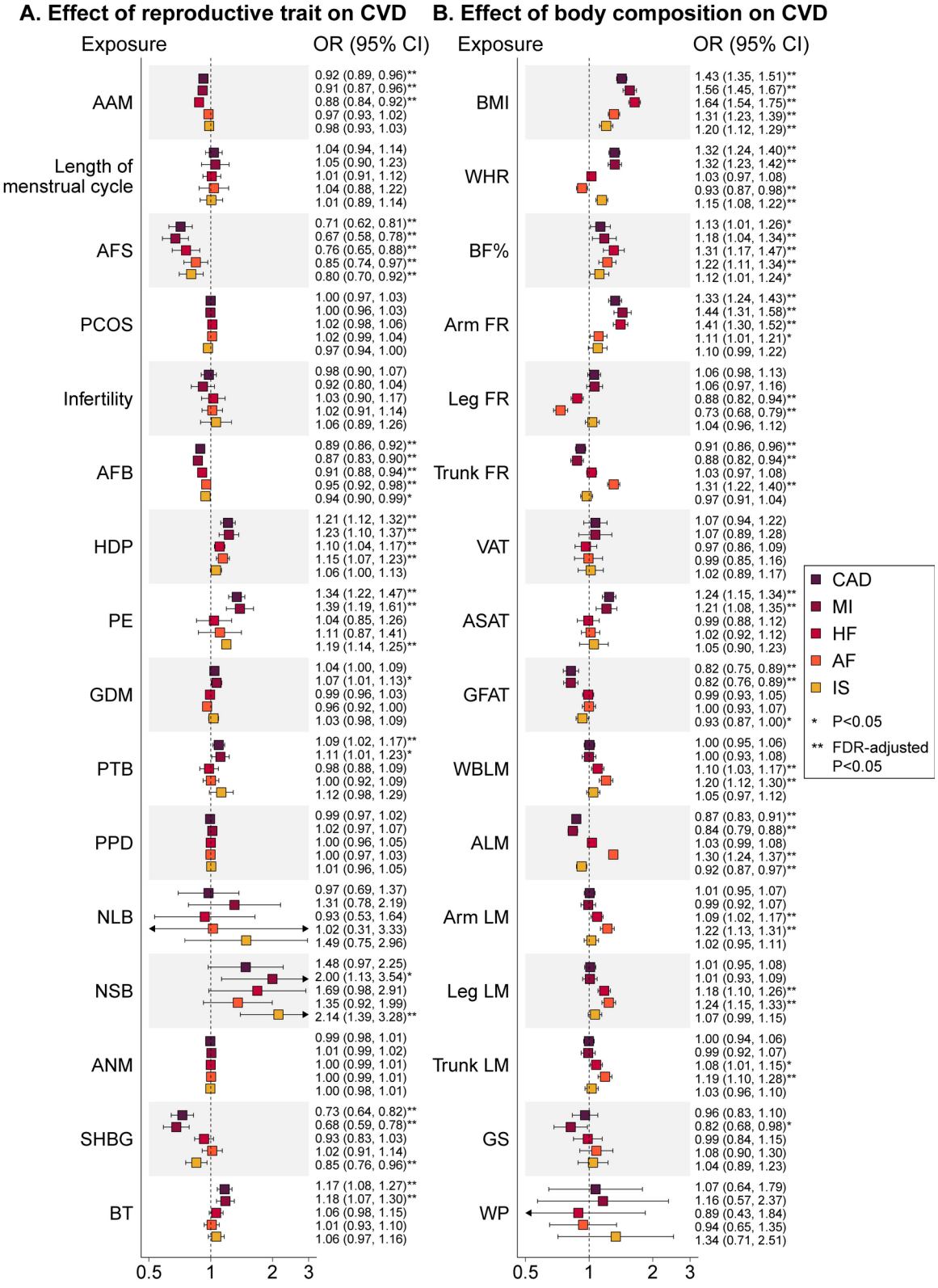

Fig. 2 (See legend on next page.)

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Fig. 2 UVMR estimates for the causal associations of reproductive traits and body composition traits with CVD outcomes. **A** Effects of reproductive traits on CVD outcomes. **B** Effects of body composition traits on CVD outcomes. Squares and error bars indicate the effect sizes and 95% CIs. "*" indicates the unadjusted $P < 0.05$. "**" indicates the FDR-adjusted $P < 0.05$. *AAM* age at menarche; *AF* atrial fibrillation; *AFB* age at first birth; *AFS* age at first sexual intercourse; *ALM* appendicular lean mass; *ANM* age at natural menopause; *Arm FR* arms-arm fat ratio; *Arm LM* right arm lean mass; *ASAT* abdominal subcutaneous adipose tissue; *BF%* body fat percentage; *BMI* body mass index; *BT* bioavailable testosterone; *CAD* coronary artery disease; *CI* confidence interval; *CVD* cardiovascular disease; *FDR* false discovery rate; *GDM* gestational diabetes mellitus; *GFAT* gluteofemoral adipose tissue; *GS* grip strength; *HDP* hypertensive disorders of pregnancy; *HF* heart failure; *IS* ischemic stroke; *Leg FR* legs-leg fat ratio; *Leg LM* right leg lean mass; *MI* myocardial infarction; *NLB* number of live births; *NSB* number of stillbirths; *OR* odds ratio; *PCOS* polycystic ovary syndrome; *PE* pre-eclampsia; *PPD* postpartum depression; *PTB* preterm birth; *SHBG* sex hormone-binding globulin; *Trunk FR* trunk-trunk fat ratio; *Trunk LM* trunk lean mass; *VAT* visceral adipose tissue; *WBLM* whole body lean mass; *WHR* waist-to-hip ratio; *WP* walking pace

lean mass showed risk effect; Leg FR and GFAT showed beneficial effect; and WHR, Trunk FR, and ALM showed inconsistent effects on different CVD outcomes, which aligns in part with previous evidence [38–43]. Intriguingly, our findings that lean mass increased the risk of AF and HF were in line with previous observations, [44, 45] which is plausible because greater lean mass may cause enlarged left heart, increased circulating blood volume, and elevated blood pressure [44, 45].

Our results of the nuanced bidirectional causal relationships of reproductive behaviors, functional indicators, and hormones with body fat-related traits imply the shared genetic basis between reproductive characteristics and body composition. Based on the identified unidirectional relationships between reproductive characteristics and body composition, we demonstrated that AAM, PTB, and sex hormones affect CAD and MI through mediating pathways by altering fat mass. In contrast, remarkably more mediating pathways were identified where reproductive traits across three stages (i.e., AFS, AFB, HDP, PE, PTB, and sex hormones) mediated the effects of obesity, fat distribution, and body lean mass on CAD, MI, IS, HF, or AF. Although the intricate mechanisms are complicated [1], our findings from an angle of mediating effect pathway imply one kind of synergistic impact of lifecourse reproductive factors and body composition on women's cardiovascular health. In terms of contributing to CVD, our results presented more possibilities for reproductive health as the mediators in the pathways of body fat or muscle on the risk of CVD outcomes, but not vice versa, which is consistent with compelling evidence of the more extensive and lifelong influence of adiposity on reproductive and cardiometabolic health [1, 5, 46]. Our findings suggested the influence of genetically predicted associations between body fat and muscle and reproductive factors, such as PCOS, pregnancy complications, and sex hormones. They have adverse impacts on late life cardiovascular health through complex mediation pathways. These findings may provide novel insights and evidence to clinicians and healthcare professionals for targeted interventions for individuals with genetic predisposition, thereby accurately improving their cardiovascular health in late life.

This study employed comprehensive MR methods based on the latest large-scale GWAS data of reproductive milestones in women's lifecourse and women-specific

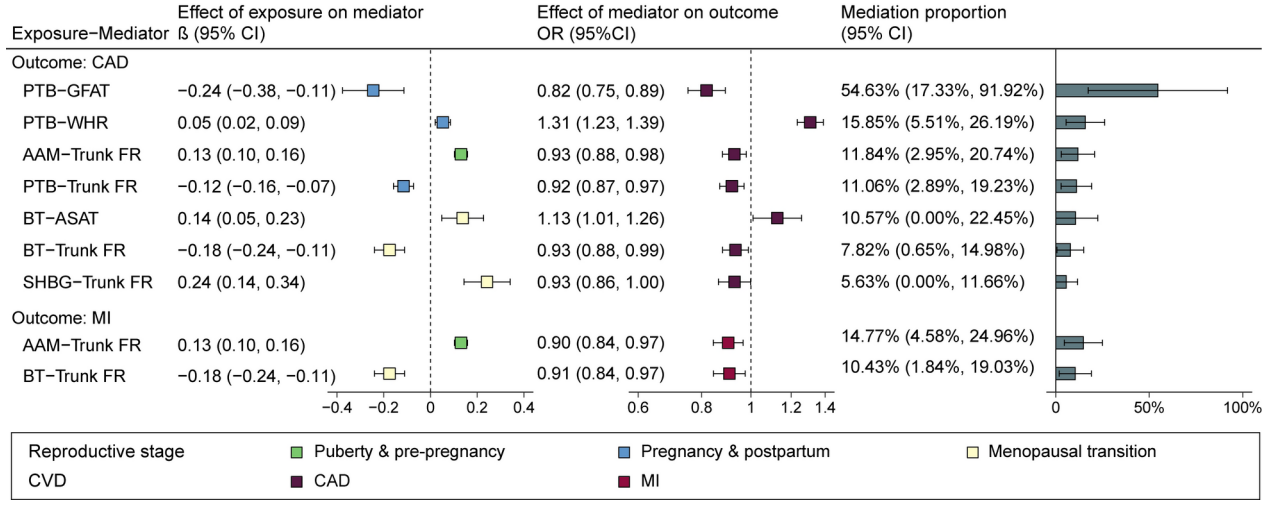

Fig. 3 Mediating roles of body composition traits in the causal associations between reproductive traits and CVD outcomes. Effects of reproductive traits on CAD or MI through body composition traits. *AAM* age at menarche; *ASAT* abdominal subcutaneous adipose tissue; *BT* bioavailable testosterone; *CAD* coronary artery disease; *CI* confidence interval; *GFAT* gluteofemoral adipose tissue; *MI* myocardial infarction; *OR* odds ratio; *PTB* preterm birth; *SHBG* sex hormone-binding globulin; *Trunk FR* trunk-trunk fat ratio; *WHR* waist-to-hip ratio

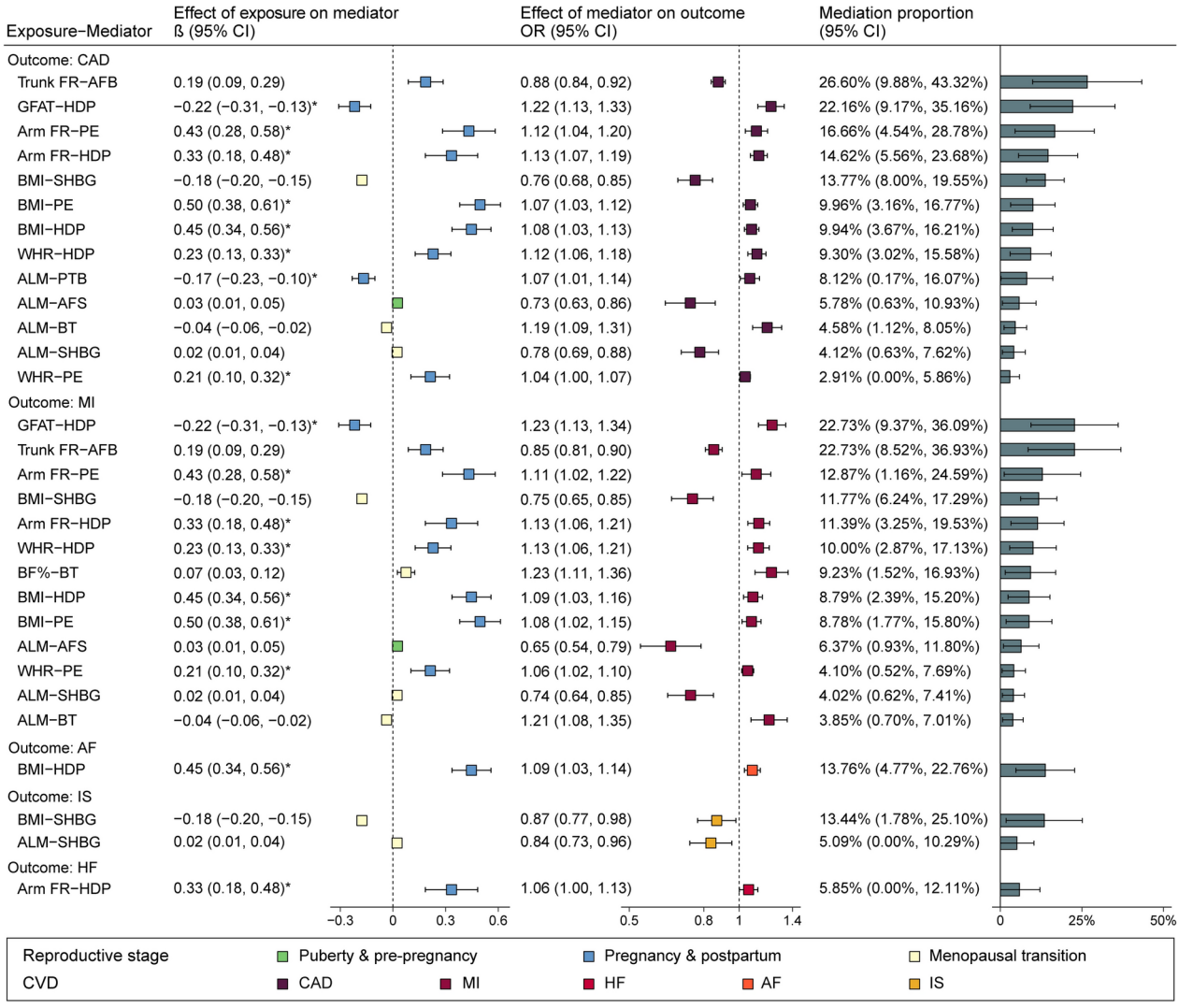

Fig. 4 Mediating roles of reproductive traits in the causal associations between body composition traits and CVD outcomes. Effects of body composition traits on CAD, MI, HF, AF, or IS through reproductive traits. The asterisk "*" indicates the β coefficient for the effect of exposures on the binary outcomes (i.e., HDP, PE, and PTB). The corresponding OR value for the effect of exposures on binary traits can be calculated using the exponential model of β coefficient. *AF* atrial fibrillation; *AFB* age at first birth; *AFS* age at first sexual intercourse; *ALM* appendicular lean mass; *Arm FR* arms-arm fat ratio; *BF%* body fat percentage; *BMI* body mass index; *BT* bioavailable testosterone; *CAD* coronary artery disease; *CI* confidence interval; *GFAT* gluteofemoral adipose tissue; *HDP* hypertensive disorders of pregnancy; *HF* heart failure; *IS* ischemic stroke; *MI* myocardial infarction; *OR* odds ratio; *PE* pre-eclampsia; *PTB* preterm birth; *SHBG* sex hormone-binding globulin; *Trunk FR* trunk-trunk fat ratio; *WHR* waist-to-hip ratio

body composition profile covering the whole body and specific compart fat- and muscle-related traits, to inform their multifaceted effects on five CVD outcomes. The rigorous statistical strategies such as the multi-phase mediator identification criteria and the solid causal association inferences derived from the primary analysis supported by various sensitivity analyses improved the accuracy and robustness of the findings. Several limitations also need attention. First, although we have conducted comprehensive sensitivity analyses to validate the robustness of the primary MR estimates and to support that our findings were less likely to be influenced by the bias due to potential assumption violations (e.g., weak instruments and horizontal pleiotropy), there remains the possibility of bias stemming from untestable assumption violations. Besides, we did not incorporate triangulation to validate our MR estimates. Therefore, the causal relationships should be interpreted with caution. Second, under the present two-step, two-sample MR framework, the interaction and homogeneity assumption between exposure and mediator cannot be fully addressed, which may bias the MR estimates [17]. However, we have utilized GWAS data from consistent populations (e.g., sex, age, ethnicity), and applied multiple sensitivity analyses (e.g., MR-Egger, weighted median, and weighted mode) to validate the primary MR results when some assumptions might

be partly violated to ensure the robustness of the findings [47]. Third, our findings were estimated based on the GWAS data from individuals of European descent. Caution should be taken when interpreting or generalizing these conclusions to other ethnic/racial populations. Fourth, several important phenotypes with unavailable GWASs were not included in this study. For example, estrogen withdrawal and hormone imbalance after menopause are closely related to women's cardiovascular health [48]. Therefore, our findings should be interpreted in conjunction with observational findings.

Conclusions

In conclusion, this study sheds light on refined relationships and mediating pathways between lifecourse reproductive characteristics, body composition, and CVD risk in women. Our findings imply a close interplay between reproductive health and body fat and muscle in the pathogenesis of CVD, informing specific preventive and interventive targets throughout lifecourse reproductive stages for mitigating cardiovascular burden and bridging cardiovascular health inequalities in women.

Supplementary Information

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Supplementary Material 1.

Supplementary Material 2.

Acknowledgments

The Figure 1 has been designed by using resources from Flaticon.com.

Author contributions

D.L. and T.W. contributed to the conception and design of the study. D.L. performed the statistical analysis and drafted the manuscript. T.W. critically revised the manuscript and checked the statistical analysis. T.W., Y.B., W.W., and G.N. obtained the funding. All authors contributed to the acquisition or interpretation of data, proofreading of the manuscript for important intellectual content and the final approval of the version to be published. T.W. is the guarantor of this work and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Availability of data and materials

All the GWAS summary statistics used in this study are publicly available and can be downloaded from the original GWAS publications or the GWAS consortium websites.

Declarations

Ethics approval and consent to participate

The ethical approval and informed consent for participants were provided in the original GWASs. The ethics review for this study is not required since only the summary level data was used.

Competing interests

All authors declare no competing interests.

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