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Sex-Based Differences in Cardiometabolic Biomarkers

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Abstract

Background—Few data are available comparing cardiovascular disease (CVD) biomarker profiles between women and men in the general population. We analyzed sex-based differences in multiple biomarkers reflecting distinct pathophysiological pathways, accounting for differences between women and men in CVD risk factors, body composition, and cardiac morphology.

Methods—A cross-sectional analysis was performed using data from the Dallas Heart Study, a multi-ethnic probability based study. Associations between sex and 30 distinct biomarkers representative of 6 pathophysiologic categories were evaluated using multivariable linear regression adjusting for age, race, traditional CVD risk factors, kidney function, insulin resistance, magnetic resonance imaging and Dual-Energy X-ray Absorptiometry measures of body composition and fat distribution, and left ventricular mass.

Results—After excluding participants with CVD, the study population included 3439 individuals, mean age 43 years, 56% women and 52% African American. Significant sex-based differences were seen in multiple categories of biomarkers, including lipids, adipokines, and biomarkers of inflammation, endothelial dysfunction, myocyte injury and stress, and kidney function. In fully adjusted models, women had higher levels of HDL-C and HDL particle concentration, leptin, D-dimer, homoarginine, and N-terminal pro B-type natriuretic peptide, and

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lower levels LDL-C, adiponectin, lipoprotein-associated phospholipase A2 mass and activity, monocyte chemoattractant protein-1, soluble endothelial cell adhesion molecule, symmetric dimethylarginine, asymmetric dimethylarginine, high sensitivity troponin T and cystatin C.

Conclusions—Biomarker profiles differ significantly between women and men in the general population. Sex differences were most apparent for biomarkers of adiposity, endothelial dysfunction, inflammatory cell recruitment, and cardiac stress and injury. Future studies are needed to characterize whether pathophysiological processes delineated by these biomarkers contribute to sex-based differences in the development and complications of CVD.

Keywords

Sex differences; Women; Cardiovascular disease; Biomarkers; Body composition; Menopause

Journal Subject Terms

biomarkers; cardiovascular disease; women; obesity; metabolism

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While cardiovascular disease (CVD) is the dominant cause of mortality in both women and men, manifestations of CVD differ between sexes.[1] Women have a lower incidence and later presentation of acute coronary syndromes (ACS), with fewer lipid-rich atheromata and a lower incidence of plaque rupture.[2] Women with heart failure present at older age and are more likely to have a non-ischemic etiology and preserved left ventricular function.[3] Interestingly, despite their relative protection from coronary artery disease (CAD) and adverse cardiac remodeling, women are equally vulnerable to peripheral arterial disease compared with men.[4] Although there is a lower incidence of atrial fibrillation (AF) among women than men, women with AF have higher mortality and stroke risk than age-matched men.[5, 6]

Pathophysiological mechanisms underlying these sex-based differences in CVD remain incompletely understood. Women and men seem to respond differently to cardiovascular stressors, as conditions such as diabetes mellitus, dyslipidemia, and smoking may play a larger role in the pathogenesis of CVD in women.[2, 7–10] Women are more prone to microvascular dysfunction, whereas men are more prone to macrovascular disease.[11] The pre-menopausal state is protective in women, suggesting that sex hormones, and the relative balance between estrogens and androgens, play at least some role in modulating sex-based differences in CVD.[1] Circulating biomarkers may provide additional insight into the mechanisms underlying sex-based differences in CVD. Sex differences are known to exist in multiple circulating biomarkers associated with cardiovascular risk.[12–14] For example, compared with men presenting with ACS, women with ACS have lower troponin levels and higher levels of natriuretic peptides and high-sensitivity C-reactive protein (hs-CRP).[12, 13] In studies from general population cohorts, women tend to have higher levels of adipokines and inflammatory biomarkers than men.[14, 15]

Few previous studies investigating sex-based differences in CVD biomarkers have been performed in general population cohorts free from CVD, and the scope of these studies has been limited to small numbers of biomarkers. Also, prior studies have not adequately

accounted for important differences in body composition and cardiac morphology that may confound comparisons of biomarkers between sexes. Therefore, we compared levels of a large panel of biomarkers representative of different pathophysiologic pathways contributing to CVD between women and men, incorporating extensive phenotyping to account for sex-based differences in body composition and cardiac morphology.

Methods

Study Population

The Dallas Heart Study (DHS) is a multi-ethnic probability-based population cohort study of Dallas County adults, with intentional oversampling of self-identified Blacks.[16] Phase 1 of the DHS was conducted between 2000 and 2002 and included 3 separate visits: Visit 1 – an initial home visit (n=6101) for collection of demographic data, medical history, blood pressure, and anthropometric data; Visit 2 (a subset of Visit 1) – a second home visit (n=3557) for collection of fasting blood and urine samples; and Visit 3 (a subset of Visit 2) – a final visit to the University of Texas Southwestern Medical Center (n=2971) for completion of detailed imaging studies. The current study represents a cross sectional analysis of individuals from DHS who participated in Visit 2 (the blood and urine collection visit). Participants with CVD at baseline, defined as self-reported history of MI, revascularization, heart failure, or stroke, were excluded (n=118), resulting in a final study population of 3439 individuals. Data from DHS phase 2, conducted from 2007 to 2009, are not included in this study. The study protocol was approved by the Institutional Review Board of the University of Texas Southwestern Medical Center, and all participants provided written informed consent.

Variable Definitions

Demographic data including age, sex, and race were obtained by participant self-report. Hypercholesterolemia was defined as fasting calculated low-density lipoprotein (LDL) cholesterol ≥160 mg/dl, nonfasting direct LDL ≥160 mg/dl, total cholesterol ≥240 mg/dl, or use of statin medication.[17] Diabetes was defined as fasting glucose ≥126 mg/dl, nonfasting glucose >200, or self-reported diabetes with concomitant use of antihyperglycemic medication.[18] Blood pressure was measured a total of 5 times during each visit, with the average of the last three readings representing the blood pressure for that visit.[19] Hypertension was defined as average systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mmHg, or use of antihypertensive medications.[19] Homeostasis Model Assessment of Insulin Resistance Index (HOMA-IR) was calculated as fasting insulin (mU/liter) x fasting glucose (mmol/liter)/22.5.[20] Estimated glomerular filtration rate (eGFR) was calculated using the abbreviated Modification of Diet and Renal Disease calculation, eGFR $[ml \cdot min^{-1} \cdot (1.73\ m^2)-1] = 186$ x (S_{cr} in mg/dl) x 1.154 x (age in years)$^{-0.203}$ x 0.742 (if female) x 1.21 (if African American), where S_{cr} is serum creatinine concentration.

Measures of Body Composition

Body mass index (BMI) was calculated as weight (kg)/height (meters)^2. Body surface area (BSA, cm^2) was calculated as 128.1 x weight (kg)$^{0.44}$ x height (cm)$^{0.60}$ for men and 147.4 x weight (kg)$^{0.47}$ x height (cm)$^{0.55}$ for women based on the Tikuisis equations.[21] Dual-Energy

X-ray Aborptiometry (DEXA) in array mode and Oasis software (Delphi W scanner, Hologic Inc., Bedford, MA) was used to quantify total fat mass and lean mass (fat-free mass) in kilograms, with separate calculations for 3 body compartments (head, trunk, and upper and lower extremities) as described previously.[19, 22] Lower body fat consisted of the total fat mass below 2 oblique lines set crossing the femoral necks, intersecting below the pubic symphysis and included gluteal-femoral fat.[22] Visceral and subcutaneous fat mass (kg) were quantified by 1.5-T magnetic resonance imaging (MRI; Intera, Philips Medical Systems, The Netherlands), using a single axial slice through the L2-L3 intervertebral level, based on an accurate method of fat mass prediction described previously.[23]

Cardiac Imaging

Left ventricular (LV) measurements were performed using 1.5 T cardiac MRI (Phillips Medical Systems, The Netherlands) in short axis, breath hold, electrocardiographic-gated cine, as described previously.[24] The summation of manually-traced endocardial and epicardial borders of slices extending from the apex to the LV base was used to calculate LV cavity volume, wall thickness, and mass.[19, 24]

Measurement of Circulating Biomarkers

Venous blood was collected in standard blood collection tubes containing citrate EDTA and samples were maintained at 4°C for 4 hours, centrifuged (1430g for 15 minutes) at 4°C, and plasma removed from these samples was frozen at –70 °C until assays were performed. Only fasting blood samples were used for the present study.

Thirty circulating biomarkers, representative of 6 different pathophysiologic categories (lipids, adipokines, and markers of inflammation, endothelial injury, myocyte injury and stress, and kidney function) were included in the analyses. These biomarkers were selected a priori based on biological plausibility, clinical relevance, and the availability of accurate assay methods. Details of assay methods and characteristics are included in Supplemental Table 1.

Menopausal Status

Menopausal status was defined using age, self-reported and measured variables including history of menopause, history of bilateral salpingo-oophorectomy with or without hysterectomy, last menstrual period, and follicle stimulating hormone levels (in a subset of 696 women). [25] Based on these definitions, there were 662 pre-menopausal women, 928 post-menopausal women, and 327 considered as unclear and therefore excluded from the analyses associating menopausal status with biomarkers (Supplemental Figure 1).

Statistical Analysis

All biomarkers are reported as continuous variables as medians (interquartile range), with the exception of high sensitivity cardiac troponin-T (hs-cTnT), which is reported as a categorical variable as the proportion ≥ the limit of blank of the assay (≥3 ng/L).[26] Linear regression analyses were performed to assess associations of sex with log-transformed biomarker concentrations. A series of sequential multivariable linear regression models were applied, as follows: Model 1 (n=3431) adjusting for age and race; Model 2 (n=2846)

adjusting for age, race, traditional risk factors (diabetes, hypertension, current smoking, statin use), HOMA-IR and eGFR; Model 3 (n=2500) adjusting for model 2 variables plus additional adjustment for body composition (lean mass, fat mass, body surface area, visceral fat, subcutaneous abdominal fat, lower body fat); Model 4 (n=2499) adjusting for model 3 variables plus additional adjustment for LV mass. Each biomarker was entered individually in each model. Beta coefficients for sex are reported for each biomarker in each model, with positive values signifying higher relative levels in women and negative values representing lower relative levels in women. The magnitude of the association of the biomarker with sex can be inferred from the absolute value of the β-coefficient.

We performed two exploratory analyses to investigate 1) the association of menopausal status with biomarker levels in women, and 2) the influence of menopausal status on the comparisons of biomarker levels between women and men. First, we performed an analysis limited to women only, comparing biomarker levels between pre- and post-menopausal women, using linear regression with serial adjustments performed according to the same four models as above. In these models, positive β-coefficients signify higher biomarker levels associated with post-menopausal status, and the magnitude of association with menopausal status is reflected by the absolute value of the β-coefficient. In the second analysis, we separated women into pre-menopausal and post-menopausal groups, and within each group matched each woman to a man of the same age. We then compared biomarker levels in pre-menopausal women vs age-matched men and separately compared post-menopausal women vs older age-matched men, excluding women with unclear menopausal status and post-menopausal women under age 40 years. We report only the fully adjusted β-coefficients (model 4), with positive β-coefficients representing higher biomarker levels in women than men within the specific subgroup.

All statistical analyses were performed using SAS version 9.2 (SAS Institute, Inc., Cary, North Carolina). For all statistical tests, 2-sided p values were adjusted for multiple testing using False Discovery Rate method, and p values <0.05 were considered significant.

Results

Baseline characteristics of the study population are shown in Table 1. Sex-based differences in the 30 biomarkers are shown in Table 2, with the results grouped according to 6 distinct biomarker categories: lipids, adipokines, inflammatory biomarkers, endothelial markers, biomarkers of myocyte injury and stress, and of kidney dysfunction.

Lipids

In unadjusted models, women had significantly higher HDL-C, HDL particle concentration (HDL-p), and Lipoprotein(a) (Lp(a)) and lower levels of LDL particle concentration (LDL-p) and triglycerides (TG) ($p<.0001$ for each). No significant difference was seen for total cholesterol, cholesterol efflux capacity, or LDL-C in unadjusted models. The differences in Lp(a), LDL-p, and TG were no longer significant after adjustment for body composition (model 3). After full adjustment, women had higher levels of HDL-C and HDL-p, and lower levels of LDL-C ($p\leq0.02$ for each).

Adipokines

Leptin and adiponectin were both significantly higher in women than men in unadjusted analyses (p<.0001 for each). Leptin remained significantly higher in women after full multivariable adjustment (p<.0001). However, the relationship between adiponectin and sex was reversed after adjusting for body composition (model 3), with lower levels in women after adjustment (p=0.04).

Inflammatory biomarkers

Women had higher unadjusted levels of D-dimer, hs-CRP, and osteoprotegerin (OPG) than men (all p<.0001) and lower levels of interleukin-18 (IL-18) and of lipoprotein phospholipase A2 (LP-PLA2) mass and activity (all p<.0001). There were no significant sex differences seen for soluble receptor for advanced glycation end products (sRAGE), soluble tumor necrosis factor receptor (sTNFR), and monocyte chemoattractant protein-1 (MCP-1) in unadjusted models. The sex differences seen for hs-CRP, OPG, and IL-18 were completely attenuated after accounting for body composition (model 3). After full adjustment, D-dimer remained higher in women and LP-PLA2 mass and activity remained lower in women (p<.0001 for each). While MCP-1 did not demonstrate a sex difference in unadjusted analyses, levels were lower in women after adjustment for body composition (p=0.03).

Endothelial Biomarkers

In unadjusted models, soluble endothelial cell selective adhesion molecule (sESAM), symmetrical dimethylarginine (SDMA), and homoarginine levels were lower in women (p=0.02, p<.0001, and p<.0001, respectively), with no significant difference seen for asymmetrical dimethylarginine (ADMA), soluble intercellular adhesion molecule (sICAM), and soluble vascular cell adhesion molecule (sVCAM). In fully adjusted models, sESAM and SDMA remained lower in women (p<.0001 for both). Lower adjusted levels of ADMA in women became evident only after accounting for body composition (p=0.0002), a difference that persisted after full multivariable adjustment (p<0.001). Homoarginine demonstrated a reversal of association after accounting for body composition, such that levels were significantly higher in women after adjustment for body composition and after full adjustment (p<0.001 for both). sICAM and sVCAM demonstrated no relationship with sex in fully adjusted models.

Biomarkers of Myocyte Injury and Stress

In unadjusted analysis, women had higher levels of N-terminal pro B-type natriuretic peptide (NT-proBNP; p<.0001), lower levels of soluble suppression of tumorigenicity 2 (sST2; p<0.01), and a lower probability of high-sensitivity cardiac troponin T (hs-cTnT) ≥3 ng/L (p<.0001). After full multivariable adjustment, women had significantly higher NT-proBNP (p<.0001) and a lower probability of hs-cTnT ≥3 ng/ml (p<.0001). The sex difference initially seen for sST2 was completely attenuated after accounting for body composition (model 3). There was no sex difference in growth differentiation factor-15 (GDF-15).

Kidney Biomarkers

Women had lower cystatin C in both unadjusted (p<.0001) and fully adjusted (p<.0001) models.

Analyses Accounting for Menopausal Status

A subset of biomarkers exhibited independent associations with menopausal status after full multivariable adjustment (Table 3). Multiple lipid biomarkers, including HDL-C, HDL-p, LDL-C, LDL-p, TG, and total cholesterol were higher among post-menopausal compared with pre-menopausal women (p<0.05 for each). ADMA was also positively associated with post-menopausal status in the fully adjusted model (p<0.05). The biomarkers that were lower in post-menopausal women included D-dimer, sVCAM and NT-proBNP (p<0.05 for each). In a sensitivity analysis in which postmenopausal women under age 40 years of age (n=19) were included with the postmenopausal group, results were similar for all markers except for sVCAM, which was no longer significantly different between the two groups after including the postmenopausal women under age 40.

In our analyses comparing pre-menopausal women with age-matched men and post-menopausal women with age-matched men, we observed that several of the sex differences in biomarkers in the overall cohort were specific to either the pre-menopausal or post-menopausal timeframes (Supplemental table 2). Markers with sex-associations specific to pre-menopausal women included LDL-C, total cholesterol, MCP-1, and ADMA, which were each lower in pre-menopausal women than age-matched men; and D-dimer and NT-proBNP, which were higher in pre-menopausal women (p≤0.01 for each). Sex differences in several other markers were specific to the post-menopausal group. HDL-C, HDL-p, and homoarginine displayed higher levels in post-menopausal women than age matched men (p<0.01 for each), with no differences seen in the comparisons of pre-menopausal women with younger men. Soluble ST2 and GDF-15 were lower only in post-menopausal women compared with age-matched men (p<0.05 for each), with no differences seen in either the pre-menopausal group or the overall comparisons. Other markers displayed consistent associations with sex in both menopausal status groups, including leptin, LP-PLA2 mass and activity, sESAM, SDMA, hs-cTnT, and cystatin C (Supplemental table 2).

Discussion

In a probability-based general population cohort free from known CVD, we observed multiple sex-specific differences in circulating cardiac biomarkers that reflect biological pathways known to be important in CVD. Moreover, we observed important effects of menopausal status, both on biomarker levels in women as well as the comparisons between women and men. In broad terms, women had higher levels of certain markers associated with adiposity and lower levels of markers associated with endothelial dysfunction, inflammatory cell recruitment, and vascular inflammation. Sex-based differences in cardiovascular biomarkers have undergone limited study to date, and to our knowledge this study represents the first large-scale comparison of circulating biomarkers associated with cardiometabolic risk between women and men without known CVD in a well-characterized, population-based cohort.

Biomarkers Independently Associated with Sex

Certain biomarkers revealed strong associations with sex independent of confounding factors, highlighting potential intrinsic differences in circulating biomarkers between women and men. Among the lipid biomarkers, the strongest associations with sex were seen with HDL-C and HDL particle concentration, which were both higher in women. Interestingly, cholesterol efflux capacity, a functional property of HDL and marker of reverse cholesterol transport,[27] did not share this association with female sex. Significant differences in adipokine levels were seen between women and men, but only leptin remained independently associated with female sex after accounting for differences in fat mass and distribution. Increased levels of leptin, which may indicate leptin resistance, have been associated with multiple adverse cardiac and metabolic parameters.[28, 29] Leptin levels are higher in women than men across all BMI levels.[30] Although larger subcutaneous fat mass was thought to underpin these differences,[30, 31] in our study, leptin remained higher in women even after accounting for differences in fat mass and distribution.

We observed no consistent pattern of association with sex within the category of inflammatory biomarkers. Rather, divergent associations with sex were seen for individual inflammatory biomarkers, suggesting sex-based differences in specific rather than global inflammatory pathways. Among the inflammatory markers, D-dimer was the only one positively associated with female sex under all modeling conditions. D-dimer is a product of fibrin degradation and a marker of systemic inflammation and thrombotic activity, and has been reported to be robustly associated with future venous thromboembolism in the general population.[32] In contrast, women had lower levels of LP-PLA2 mass and activity. LP-PLA2 is an enzyme secreted by inflammatory cells that circulates bound to LDL-cholesterol and other lipoproteins and may contribute to vascular inflammation and plaque instability through the generation of inflammatory lipid products.[33] LP-PLA2 has been shown to be lower in women compared with men[33] and higher LP-PLA2 mass and activity have been associated with increased risk of primary cardiovascular events[34] and coronary heart disease with no significant interaction between LP-PLA2 activity and sex with regards to CHD.[34] Therapies targeting the LP-PLA2 pathway have been the focus of extensive recent investigation.[35]

Women had lower levels of certain markers of endothelial dysfunction, including sESAM and SDMA, and higher levels of homoarginine, a marker associated with a protective endothelial phenotype.[36] ESAM is a unique cell adhesion molecule with expression limited to vascular endothelium and activated platelets.[18] While studies on sex difference in soluble ESAM are currently lacking, sESAM has been associated with measures of subclinical atherosclerosis such as coronary artery calcium and aortic wall thickness, as well as vascular stiffness."[18] It is thought to participate in atherosclerosis by its role in leukocyte recruitment into areas of damaged endothelium.[18] SDMA and ADMA are endogenous methylated byproducts of protein turnover shown to interfere with nitric oxide synthesis. In this study, SDMA and ADMA were lower in women after full adjustment. Homoarginine, in contrast, is a nitric oxide (NO) precursor, which increases NO availability and enhances endothelial function.[36] Prior studies have demonstrated lower baseline levels of homoarginine in women

compared with men[37] and inverse associations between homoarginine and markers of atherosclerosis, major adverse cardiovascular events, and all-cause mortality."[36, 38]

Our findings confirm multiple previous studies showing that women have higher levels of natriuretic peptides and lower levels of cardiac troponins.[25, 39] Natriuretic peptides, which are released under conditions of cardiomyocyte stretch, serve a counter-regulatory function by promoting natriuresis, diuresis, vasodilation, and inhibition of the renin-angiotensin - aldosterone and sympathetic nervous systems. They may also promote fat distribution away from visceral to more favorable subcutaneous depots.[40] Importantly, we found that NT-proBNP levels remained higher in women even after accounting for differences between sexes in body composition and LV mass. Cardiac troponins are specific markers of cardiac injury that are associated with LV hypertrophy and increased death and heart failure events in population-based cohorts.[41] Consistent with prior studies, we found strong associations between female sex and lower hs-cTnT levels. Interestingly, the lower levels of hs-cTnT were not explained by smaller heart size in women, as adjustment for LV mass did not attenuate the differences in hs-cTnT.

Cystatin C is secreted from all nucleated cells, circulates in bodily fluids, is freely filtered across the glomerular membrane, and serves as a marker of kidney function independent of age, sex, and muscle mass.[42] Higher levels of cystatin C have been associated with increased cardiovascular (CV) mortality, heart failure, and increased LV mass, concentricity, and wall thickness.[42] We found that cystatin C was significantly lower in women independent of CV risk factors, kidney function, body composition, and LV mass. However the exact role that cystatin C plays in the development of CVD remains unclear and warrants further investigation.

Effect of Body Composition and Fat Distribution on Sex-Based Differences in Biomarkers

Accounting for age, race, traditional risk factors, and LV mass in general did not have a major impact on the association of sex with biomarker levels. Among the covariables considered in our modeling strategy, measures of body composition and fat distribution had the largest impact on the beta coefficients for sex. Multiple biomarkers demonstrated attenuation or even complete reversal of sex associations after adjusting for these measures, suggesting that body composition may be a critical factor influencing different pathophysiological manifestations of CVD in women and men. Cardiometabolic consequences of adiposity differ depending on distribution within various body compartments.[22, 31] While visceral abdominal fat has been associated with different biomarkers of insulin resistance, dyslipidemia, and subclinical atherosclerosis, subcutaneous fat has demonstrated no association with atherosclerosis and inconsistent associations with dysmetabolic phenotypes.[22, 43] Women have less visceral fat and more lower body fat than men, and also appear to have an exaggerated inflammatory response to increased adiposity compared with men.[15] An important strength of our study is the ability to account for these differences in body composition, fat distribution, and adipokine levels between women and men.

Several markers were associated with significantly higher or lower levels in women at baseline, but this relationship was attenuated with adjustment for body composition. The

lipid marker Lp(a) and inflammatory markers hs-CRP and OPG were initially higher in women, but became insignificant when accounting for body composition. Associations of other biomarkers that were initially lower in women, including LDL particle concentration and triglycerides, and the inflammatory markers IL-18 and sST2, were completely attenuated after accounting for body composition. The vascular inflammatory marker MCP-1 and the methylarginine ADMA displayed significant inverse associations with female sex only after adjusting for body composition. Finally, two biomarkers, homoarginine and adiponectin, displayed reversal of sex association after adjusting for body composition. Our findings with adiponectin suggest that, in contrast with leptin (where sex differences persisted after multivariable adjustment), higher adiponectin levels seen in women are entirely attributable to a more favorable body composition profile.

Effect of Menopausal Status on Biomarker Levels in in Women

With menopause, women experience a fall in circulating estrogens with relative increase in androgen-to-estrogen ratio, redistribution of fat from lower body to abdominal compartments, shift towards more atherogenic lipid profiles, and increase in coronary disease and its risk factors.[44] While women are relatively protected from CVD compared with men in premenopausal years, this gap narrows as women cross through menopause.[7, 44] Given the significant influence of menopause, it is important to consider sex differences in biomarkers in the context of life cycle changes in women.

In our study menopause was associated with upward shifts in most lipid parameters. Moreover, higher levels of HDL in women than men were restricted to post-menopausal women, whereas lower LDL-C in women than men was restricted to the pre-menopausal group. D-dimer was associated with pre-menopausal status and the significantly higher levels seen in women than men were unique to pre-menopausal women, which may reflect a higher relative risk of thromboembolism in younger women than men.[45, 46] We also noted some differences in endothelial biomarkers when accounting for menopausal status. For example ADMA levels were higher in post-menopausal than pre-menopausal women, a finding that appeared to attenuate sex-based differences in this biomarker among older women. This finding highlights an increase in factors associated with endothelial dysfunction as women transition through menopause. Consistent with prior observations, we found that post-menopausal women have lower NT-proBNP levels than pre-menopausal women after full adjustment.[47] Moreover, the robust sex-based differences seen with NT-proBNP were entirely restricted to the pre-menopause group comparison. We have previously reported that androgens are inversely associated with NT-proBNP levels,[25] a finding that likely contributes to both the sex-based differences observed in pre-menopausal women vs younger men, and the change in natriuretic peptide levels in women after menopause. Loss of the natriuretic peptide “advantage” may be an important contributor to narrowing of differences in CVD rates between women and men after women transition through menopause. Interestingly, though sST2 and GDF-15 did not demonstrate significant sex associations in the overall cohort, we observed significantly lower levels in post-menopausal, but not pre-menopausal women compared with age-matched men.

Strengths and Limitations

Strengths of our study include the large sample size and sex and ethnic diversity of the DHS population, as well as the large numbers of biomarkers evaluated. The exclusion of participants with known CVD allows for greater generalizability to the otherwise healthy population and eliminates pre-existing CVD as a confounding variable for elevated biomarkers. Finally, the extensive phenotyping of the study population, including detailed anthropometric and cardiac imaging measurements, allows for better understanding of the role of body composition and heart size on sex differences in biomarkers.

A number of important limitations merit comment. First, the cross-sectional design of this study precludes assessment of the potential influence of sex-based differences in biomarkers on cardiac or metabolic phenoytpe expression or clinical outcomes. Additionally, sex hormone measurements in the DHS were limited to a subset of women, so we were unable to determine the influence of androgens and estrogens on sex differences in biomarkers. We acknowledge the exploratory nature of our study, and the potential for spurious findings due to the large numbers of statistical tests performed. However, the majority of the p values were highly significant even after accounting for multiple testing. Finally, we acknowledge that the findings are descriptive and can only be considered hypothesis generating regarding mechanisms of CVD.

Conclusions

Sex-specific differences were observed in multiple biomarkers reflecting pathways of cardiovascular risk. Women tend to exhibit higher circulating levels of adipokines and D-dimer and lower levels of biomarkers reflecting endothelial dysfunction, and inflammatory cell recruitment. Body composition and menopausal status had important influences on the observed sex-based differences in several biomarkers, highlighting the importance of these factors when interpreting differences in biomarkers and CVD between men and women.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Clinical Perspective

What is new?

- This is one of the largest and most comprehensive comparisons of biomarker profiles between men and women from the population, and provides insight into potential mechanisms contributing to sex differences in the pathogenesis of cardiovascular disease (CVD).
- Women had higher circulating levels of adipokines and D-dimer, and lower levels of biomarkers reflecting endothelial dysfunction and inflammatory cell recruitment. Body composition and menopausal status had important influences on the observed sex-based differences in multiple biomarkers.

What are the clinical implications?

- Sex is an important determinant of the circulating levels of multiple different biomarkers, including several biomarkers currently in wide clinical use, suggesting that sex-based cut points should be considered for several biomarkers.
- Differences in biomarker profiles between men and women highlight potentially important sex-based differences in the pathophysiological mechanisms contributing to CVD.
- Future studies should explore targeted approaches to CVD prevention that account for sex-based differences in disease pathogenesis.

Table 1

Baseline characteristics in Women and Men

COVARIATE	WOMEN n=1932	MEN n=1507	P-VALUE
Age, years	43 [36, 52]	43 [36, 51]	0.93
Black race	53.3%	49.3%	0.02
Hispanic ethnicity	17.7%	16.0%	0.21
Other race/ethnicity	1.6%	2.8%	0.01
Diabetes	11.6%	11.4%	0.87
Hypertension	34.7%	33.0%	0.28
Hypercholesterolemia	12.2%	13.9%	0.14
Current smoking	25.5%	33.8%	<.0001
Body mass index, kg/m^2	30.5 [25.6, 36.6]	28.5 [25.1, 32.3]	<.0001
Lean mass, kg	47.2 [41.9, 53.6]	63.9 [58.1, 71.2]	<.0001
Fat mass, kg	30.1 [23.3, 40.0]	20.7 [15.2, 27.1]	<.0001
Body surface area, m^2	1.9 [1.7, 2.1]	2.0 [1.9, 2.2]	<.0001
Visceral fat, kg	1.8 [1.3, 2.3]	2.5 [1.8, 3.2]	<.0001
Subcutaneous abdominal fat, kg	5.2 [3.6, 7.6]	3.3 [2.4, 4.6]	<.0001
Lower body fat, kg	11.1 [8.6, 14.6]	6.5 [4.7, 8.6]	<.0001
HOMA-IR, units	3.2 [1.7, 5.3]	2.8 [1.5, 4.9]	0.001
eGFR, ml/min/1.73 m^2	98.5 [84.3, 115]	96.6 [84.8, 111]	0.004
Left ventricular mass, grams	137 [118, 158]	186 [163, 211]	<.0001

Abbreviations: HOMA-IR, Homeostasis Model Assessment of Insulin Resistance Index; eGFR, estimated glomerular filtration rate

Table 2

Univariable and Multivariable Associations of Biomarkers with Sex

BIOMARKER	WOMEN	MEN	UNADJUSTED β-COEFFICIENT (P-VALUE)	MODEL 1 β-COEFFICIENT (P-VALUE)	MODEL 2 β-COEFFICIENT (P-VALUE)	MODEL 3 β-COEFFICIENT (P-VALUE)	MODEL 4 β-COEFFICIENT (P-VALUE)
LIPIDS							
HDL-C, mg/dL	51 [43, 60]	44 [37, 52]	0.14 (p<.0001)	0.14 (p<.0001)	0.15 (p<.0001)	0.06 (p=0.02)	0.06 (p=0.02)
HDL-p, μmol/L	34 [29, 38]	32 [28, 35]	0.07 (p<.0001)	0.07 (p<.0001)	0.07 (p<.0001)	0.05 (p=0.006)	0.05 (p=0.008)
Lp(a), nmol/L	56 [22, 119]	43 [15, 95]	0.26 (p<.0001)	0.22 (p<.0001)	0.21 (p<.0001)	0.003 (p=0.98)	0.002 (p=0.98)
LDL-C, mg/dL	102 [82, 124]	107 [84, 130]	−0.03 (p=0.05)	−0.03 (p=0.06)	−0.03 (p=0.04)	−0.09 (p=0.02)	−0.09 (p=0.01)
LDL-p, μmol/L	1157 [933, 1418]	1282 [1032, 1584]	−0.10 (p<.0001)	−0.10 (p<.0001)	−0.10 (p<.0001)	−0.03 (p=0.44)	−0.03 (p=0.39)
TG, mg/dL	90 [64, 131]	107 [72, 167]	−0.19 (p<.0001)	−0.18 (p<.0001)	−0.17 (p<.0001)	0.03 (p=0.56)	0.03 (p=0.61)
Total Cholesterol, mg/dL	176 [153, 201]	178 [154, 205]	−0.008 (p=0.40)	−0.007 (p=0.44)	−0.003 (p=0.79)	−0.03 (p=0.19)	−0.03 (p=0.16)
Cholesterol Efflux, units	0.99 [0.83, 1.2]	1.0 [0.84, 1.2]	−0.02 (p=0.16)	−0.02 (p=0.16)	−0.02 (p=0.22)	−0.02 (p=0.53)	−0.02 (p=0.53)
ADIPOKINES							
Leptin, ng/mL	23 [14, 37]	5.7 [2.7, 10]	1.5 (p<.0001)	1.5 (p<.0001)	1.4 (p<.0001)	0.84 (p<.0001)	0.84 (p<.0001)
Adiponectin, μg/mL	7.6 [5.0, 11]	5.6 [3.8, 8.1]	0.29 (p<.0001)	0.31 (p<.0001)	0.33 (p<.0001)	−0.12 (p=0.02)	−0.10 (p=0.04)
INFLAMMATION							
D-dimer, μg/mL	0.28 [0.18, 0.44]	0.16 [0.10, 0.26]	0.53 (p<.0001)	0.51 (p<.0001)	0.51 (p<.0001)	0.40 (p<.0001)	0.42 (p<.0001)
hs-CRP, mg/dL	3.9 [1.5, 8.8]	2.0 [0.90, 4.3]	0.53 (p<.0001)	0.51 (p<.0001)	0.50 (p<.0001)	−0.05 (p=0.68)	−0.05 (p=0.64)
OPG, pg/mL	1272 [933, 1738]	1096 [813, 1443]	0.19 (p<.0001)	0.18 (p<.0001)	0.19 (p<.0001)	0.08 (p=0.19)	0.09 (p=0.13)
LP-PLA2 mass, μg/L	176 [147, 208]	202 [168, 233]	−0.13 (p<.0001)	−0.12 (p<.0001)	−0.12 (p<.0001)	−0.15 (p<.0001)	−0.15 (p<.0001)
LP-PLA2 activity, μmol/min/L	132 [110, 156]	162 [135, 187]	−0.19 (p<.0001)	−0.18 (p<.0001)	−0.18 (p<.0001)	−0.11 (p<.0001)	−0.11 (p<.0001)
IL-18, pg/mL	499 [352, 733]	560 [378, 864]	−0.13 (p<.0001)	−0.12 (p<.0001)	−0.14 (p<.0001)	−0.15 (p=0.11)	−0.15 (p=0.10)
MCP-1, pg/mL	164 [121, 222]	171 [126, 228]	−0.03 (p=0.20)	−0.02 (p=0.27)	−0.03 (p=0.16)	−0.11 (p=0.03)	−0.11 (p=0.03)
sRAGE, ng/mL	1.3 [0.92, 1.9]	1.3 [0.89, 1.9]	−0.002 (p=0.89)	0.007 (p=0.61)	0.01 (p=0.54)	0.02 (p=0.64)	0.02 (p=0.62)
sTNFR, pg/mL	0.60 [0.41, 0.86]	0.60 [0.43, 0.85]	−0.008 (p=0.78)	−0.008 (p=0.77)	0.01 (p=0.76)	−0.13 (p=0.05)	−0.13 (p=0.05)
ENDOTHELIAL FUNCTION / DYSFUNCTION							
sESAM, ng/mL	34 [27, 42]	35 [27, 44]	−0.04 (p=0.02)	−0.04 (p=0.02)	−0.04 (p=0.03)	−0.20 (p<.0001)	−0.20 (p<.0001)
SDMA, μmol/L	0.39 [0.34, 0.45]	0.42 [0.37, 0.50]	−0.03 (p<.0001)	−0.03 (p<.0001)	−0.02 (p<.0001)	−0.05 (p<.0001)	−0.05 (p<.0001)
ADMA, μmol/L	0.48 [0.42, 0.56]	0.48 [0.42, 0.55]	0.0005 (p=0.86)	0.001 (p=0.82)	0.001 (p=0.76)	−0.03 (p=0.0002)	−0.02 (p=0.0006)

BIOMARKER	WOMEN	MEN	UNADJUSTED β-COEFFICIENT (P-VALUE)	MODEL 1 β-COEFFICIENT (P-VALUE)	MODEL 2 β-COEFFICIENT (P-VALUE)	MODEL 3 β-COEFFICIENT (P-VALUE)	MODEL 4 β-COEFFICIENT (P-VALUE)
Homoarginine, μmol/L	2.7 [2.1, 3.5]	2.9 [2.3, 3.6]	−0.08 (p<.0001)	−0.08 (p<.0001)	−0.10 (p<.0001)	0.13 (p=0.0004)	0.12 (p=0.0008)
sICAM, ng/mL	603 [452, 823]	604 [449, 837]	0.009 (p=0.67)	0.007 (p=0.75)	0.01 (p=0.57)	0.04 (p=0.44)	0.05 (p=0.41)
sVCAM, ng/mL	982 [710, 1364]	991 [740, 1411]	−0.01 (p=0.64)	−0.01 (p=0.61)	−0.02 (p=0.57)	−0.03 (p=0.63)	−0.03 (p=0.60)
MYOCYTE INJURY / STRESS							
NT-proBNP, pg/mL	39 [20, 75]	17 [7.5, 39]	0.76 (p<.0001)	0.76 (p<.0001)	0.80 (p<.0001)	0.42 (p<.0001)	0.51 (p<.0001)
hs-cTnT, % ≥3 ng/L	14%	42%	−0.43 (p<.0001)	−0.44 (p<.0001)	−0.45 (p<.0001)	−0.36 (p<.0001)	−0.32 (p<.0001)
sST2, μg/L	0.40 [0.40, 0.54]	0.40 [0.40, 0.64]	−0.06 (p=0.008)	−0.07 (p=0.0006)	−0.05 (p=0.04)	−0.05 (p=0.44)	−0.05 (p=0.44)
GDF-15, ng/L	0.66 [0.48, 0.90]	0.67 [0.50, 0.92]	−0.02 (p=0.44)	−0.03 (p=0.19)	−0.008 (p=0.75)	−0.05 (p=0.35)	−0.04 (p=0.43)
KIDNEY DYSFUNCTION / EXTRACARDIAC INVOLVEMENT							
Cystatin C, mg/L	0.79 [0.71, 0.89]	0.85 [0.77, 0.95]	−0.07 (p<.0001)	−0.07 (p<.0001)	−0.06 (p<.0001)	−0.17 (p<.0001)	−0.17 (p<.0001)

+β-coefficient: higher in women

−β-coefficient: lower in women

Significantly higher in women

Significantly lower in women

No significant difference between women and men

Variables adjusted for:

Model 1: age, race

Model 2: Model 1 + diabetes, hypertension, smoking, statin use, HOMA-IR, eGFR

Model 3: Model 2 + lean mass, fat mass, body surface area, visceral fat, subcutaneous fat, lower body fat

Model 4: Model 3 + left ventricular mass

Abbreviations: HDL-C, high density lipoprotein cholesterol; HDL-p, high density lipoprotein particle concentration; Lp(a), lipoprotein(a); LDL-C, low-density lipoprotein cholesterol; LDL-p, low-density lipoprotein particle concentration; TG, triglycerides; hs-CRP, high-sensitivity C-reactive protein; OPG, osteoprotegerin; LP-PLA2, lipoprotein phospholipase A2; IL-18, interleukin-18; MCP-1, monocyte chemoattractant protein-1; sRAGE, soluble receptor for advanced glycation end products; sTNFR, soluble tumor necrosis factor receptor; sESAM, soluble endothelial cell selective adhesion molecule; SDMA, symmetrical dimethylarginine methylarginine; ADMA, asymmetrical dimethylarginine; sICAM, soluble intercellular adhesion molecule; sVCAM, soluble vascular cell adhesion molecule; NT-proBNP, N-terminal of the prohormone brain natriuretic peptide; hs-cTnT, high-sensitivity cardiac troponin T; sST2, soluble suppression of tumorigenicity 2; GDF-15, growth differentiation factor-15; HOMA-IR, homeostatic model assessment-insulin resistance; eGFR, estimated glomerular filtration rate

P-values adjusted for multiple testing using false discovery rate method

Table 3

Biomarkers independently associated with menopausal status (post- vs. pre-menopausal women)

BIOMARKER	FULLY ADJUSTED β-COEFFICIENT	P-VALUE
HDL-C, mg/dL	0.05	0.03
HDL-p, μmol/L	0.09	<.0001
LDL-C, mg/dL	0.08	0.03
LDL-p, μmol/L	0.06	0.04
Triglycerides, mg/dL	0.16	0.0004
Total cholesterol, mg/dL	0.07	0.0003
D-dimer, μg/mL	−0.31	<.0001
ADMA, μmol/L	0.02	0.04
sVCAM, ng/mL	−0.15	0.009
NT-proBNP, pg/mL	−0.22	0.04

+β-coefficient: higher in post-menopausal women

−β-coefficient: lower in post-menopausal women

Biomarkers that were not associated with menopausal status included Lp(a), cholesterol efflux, leptin, adiponectin, hs-CRP, OPG, LP-PLA2 mass, IL-18, MCP-1, sRAGE, sTNFR, sESAM, SDMA, homoarginine, sICAM, hs-cTnT, sST2, GDF-15