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Sex Hormones and Change in N-Terminal Pro-B-Type Natriuretic Peptide Levels: The Multi-Ethnic Study of Atherosclerosis

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Context: Sex hormones (SH) are thought to influence sex differences in cardiovascular disease (CVD). N-Terminal pro-B-type Natriuretic Peptide (NT-proBNP), a predictor of CVD, is higher in women than men, which may relate to SH.
Objective: To evaluate whether SH *[Total testosterone (T), bioavailable T, free T, estradiol, dehydroepiandrosterone (DHEA), SH binding globulin (SHBG)]* are associated with cross-sectional and longitudinal NT-proBNP levels.
Design: Cohort study (MESA)
Participants: Cross-sectional sample included 2,371 postmenopausal women and 2,688 men free of CVD, of which 2,041 women and 2,348 men were included in longitudinal sample.
Main outcome measures: NT-proBNP at baseline (2000-2002) and ≥1 repeat NT-proBNP (through 2010-12). Analyses adjusted for CVD risk factors.
Results: Women had higher NT-proBNP than men (median 79.9 vs 38.5pg/ml). Cross-sectionally, higher bioavailable T, free T, DHEA, and lower SHBG levels were independently associated with lower NT-proBNP among both women and men (all $p<0.05$). Higher total T in

women and estradiol in men were also associated with lower NT-proBNP (both p<0.05). Longitudinally, in women, higher total T, bioavailable T, free T, DHEA, and lower estradiol and SHBG were associated with greater 10-year increase in NT-proBNP (all p<0.05). In men, higher free T and estradiol were associated with greater NT-proBNP increase (both p<0.05).
Conclusions: A more androgenic SH pattern was inversely associated with NT-proBNP cross-sectionally and may contribute to sex differences in NT-proBNP. Longitudinally, a more androgenic SH pattern was associated with greater increase in NT-proBNP in women, which may reflect a mechanism for increased CVD risk in women after menopause.

A more androgenic sex hormone pattern was found to be inversely associated with NT-proBNP levels cross-sectionally, while also associated with greater longitudinal change in NT-proBNP levels over time.

Introduction

Significant differences exist in the prevalence and manifestation of atherosclerotic cardiovascular disease (CVD) and with heart failure (HF) between men and women.(1,2) Premenopausal women have a lower risk of CVD and HF compared to men; however, this risk increases after menopause.(3-5) Sex hormones, particularly androgens, are associated with CVD risk factors and events and have been postulated to mediate the observed sex differences in CVD.(6-10)

B-type natriuretic peptides (BNP) are secreted from cardiomyocytes in response to myocardial wall stress.(11) BNP plays an important role in cardiovascular remodeling and volume homeostasis. It exerts numerous cardioprotective effects by promoting vasodilation, natriuresis, and ventricular relaxation, and by antagonizing fibrosis and the effects of the renin-angiotensin-aldosterone system.(11) While the physiological role of BNP is cardioprotective, pathologically elevated N-Terminal pro-B-type Natriuretic Peptide (NT-proBNP) levels are used clinically to indicate left ventricular hypertrophy, dysfunction, and myocardial ischemia.(12-15) Higher NT-proBNP levels among individuals free of clinical CVD are associated with an increased risk of incident CVD(16), HF(17), and cardiovascular mortality(18,19).

BNP and NT-proBNP levels are higher in women than men in the general population.(20-22) Several studies have proposed the use of sex- and age-specific reference ranges for BNP and NT-proBNP levels, where reference limits are higher for women and older individuals.(20,21,23,24) The etiology behind this sex difference has not been fully elucidated, but prior studies have demonstrated an association between sex hormones and NT-proBNP levels. Analyzing the relationship between NT-proBNP and sex hormones may help clarify the role of sex hormones in CVD/HF pathogenesis. Current data on the exact associations between BNP and sex hormones are controversial. Initial studies, particularly in women using estrogen therapy, have proposed that estrogen may have a stimulating effect on the natriuretic peptide (NP) system.(20,25-27) More recent studies measuring endogenous sex hormones have suggested that androgens may play a larger role in BNP regulation by inhibiting its production.(28,29) These studies are predominantly cross-sectional and limited in the number of sex hormones measured. Additionally, the association between sex hormone levels and change in NT-proBNP levels over time is unknown.

Therefore, to address these knowledge gaps, we used data from a large, multi-ethnic community-based cohort of individuals free of CVD and HF at baseline to analyze both the cross-sectional and longitudinal associations between sex hormones (total testosterone [T], bioavailable T, free T, dehydroepiandrosterone [DHEA], sex hormone binding globulin [SHBG], and estradiol) and NT-proBNP, separately for women and men. We hypothesized that a more androgenic profile of sex hormones would be inversely associated with NT-proBNP levels in

both cross-sectional and longitudinal analyses, and that the associations would be stronger in women than in men.

Materials and Methods

Data availability statement

The Multi-Ethnic Study of Atherosclerosis (MESA) cohort participates in the National Heart, Lung, and Blood Institute's Biologic Specimen and Data Repository (BioLINCC). The MESA data are available upon request, including data from exams 1 to 5, used in this analysis. Requests for data can be made through the following website:

https://biolincc.nhlbi.nih.gov/studies/mesa/

Study population

The MESA is a prospective community-based cohort study investigating the characteristics of subclinical CVD and its progression into clinical CVD. It enrolled 6,814 men and women of four self-reported race/ethnicities (White, Black, Hispanic, and Chinese-American), who were 45-84 years old at the time of the baseline exam (2000-2002).(30) MESA participants were recruited at six centers across the United States and were free of CVD/HF at baseline. The study was approved by the institutional review boards of all participating institutions, and all participants provided written informed consent.

Our study included cross-sectional and longitudinal analyses (**Figure 1**). Premenopausal women (n=514) were excluded, as sex hormone levels differ significantly between pre- and post-menopausal women. Participants with missing data for sex hormones (n=209), baseline NT-proBNP (n=975), and covariates from our second model (n=57), described below, were also excluded. Ultimately, 5,059 participants were included in the cross-sectional analysis. Of this sample, those that had at least one repeat measure of NT-proBNP at exam 3 (2004-05) and/or exam 5 (2010-2012) were included in our longitudinal analysis (n=4,389).

Measurement of sex hormones

Early morning fasting blood samples were collected and stored at -70^{o}C at the time of the baseline exam (exam 1). Sex hormones were measured from stored serum samples at the Steroid Hormone Laboratory at the University of Massachusetts Medical Center (Worcester, MA). Ultrasensitive radioimmunoassay kits from Diagnostic System Laboratories (Webster, TX) were used to measure estradiol. Total T and DHEA were measured using radioimmunoassay kits, and SHBG was measured via a chemiluminescence enzyme immunometric assay using Immulite kits from Diagnostic Products Corporation (Los Angeles, CA). Bioavailable T and free T were calculated using total T and SHBG concentrations, by equations described by Sodergard et al.(31) Intra-assay coefficients of variation for estradiol, total T, DHEA, and SHBG were 10.5%, 12.3%, 11.2%, and 9.0%, respectively.

Measurement of NT-proBNP

NT-proBNP levels were measured from blood samples that were collected at the time of exams 1, 3, and 5, and stored at -70^{o}C. NT-proBNP measurements from a subset of exam 1 and 3 participants, plus participants at exam 5, were performed using the Elecsys proBNP immunoassay (Roche Diagnostics Corporation, Indianapolis, IN) at the University of California San Diego and the University of Vermont.(32) Additional measures of NT-proBNP were performed in the remaining samples from exams 1 and 3 using the Cobas e601 (Roche Diagnostics Corporation, Indianapolis, IN) at the University of Maryland.(33) NT-proBNP assays were harmonized across different Roche platforms. The measurement range was 5 to

35,000pg/mL, and the intra- and inter-assay coefficients of variation were as follows: 2.7% and 3.2% at 175pg/mL, 2.4% and 2.9% at 355pg/mL, 1.9% and 2.6% at 1068pg/mL, and 1.8% and 2.3% at 4962pg/mL.(32)

Measurement of other covariates

Socio-demographic and lifestyle characteristics, as well as CVD risk factors, were obtained at exam 1 using standardized questionnaires, physical exam, and laboratory measures as described previously.(30) Physical activity was defined as the total amount of moderate plus vigorous intentional exercise performed in a usual week and summed as MET-minutes/week. Menopausal status was determined from a combination of self-reported status, age, age of menopause, and history of hysterectomy or oophorectomy.(34) Due to missing self-reported menopausal status data, as well as inconsistencies within the data, an algorithm was created to more accurately delineate the sample of postmenopausal women [shown in **Supplemental Figure**(35)]. Participants were deemed postmenopausal if they met one or more of the following criteria: 1) age ≥55 years (86% of cross-sectional sample); 2) age <55 years without history of hysterectomy with available menopause age data (10%); 3) age <55 years with self-reported postmenopausal status, but no menopause age data, without history of hysterectomy (0.04%); 4) age <55 with history of hysterectomy and bilateral oophorectomy if not already deemed postmenopausal by any previous criteria (4%); 5) age <55 with history of hysterectomy without bilateral oophorectomy with reported hysterectomy at age > menopause age (0.08%). As stated above, only postmenopausal women were included in this analysis.

Body mass index (BMI) was calculated by weight (kg) divided by height (m) squared. Seated blood pressure was measured using a Dinamap automated oscillometric sphygmomanometer. Hypertension was defined as systolic blood pressure (SBP) ≥140mmHg, diastolic blood pressure (DBP) ≥90mmHg, or treatment with antihypertensive medication. Diabetes was defined as a fasting glucose ≥126mg/dL, self-reported diagnosis of diabetes, or treatment with hypoglycemic medications. Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI equation.(36)

Statistical analysis

All analyses were performed stratified by sex, since sex hormone levels differ significantly between women and men, with non-overlapping distributions. Continuous variables were summarized as means (standard deviation [SD]), or medians (interquartile range [IQR]) in the case of skewed distributions. Categorical variables were expressed as percentages.

Cross-sectional and longitudinal associations between sex hormones and NT-proBNP were determined using multivariable-adjusted linear mixed effects models. Because sex hormone and NT-proBNP distributions were skewed, we used log-transformed values for both. Sex hormones were modeled individually as continuous measures, and the 90th vs 10th percentiles of their log-transformed distributions were compared for contrast. For NT-proBNP, participants could contribute 2 or 3 data points for longitudinal analyses. Among our sample, 3,085 participants had all 3 data points (exams 1, 3, and 5), and 1,304 participants had data at exam 1 plus one follow-up measurement at either exam 3 or 5. Cross-sectional associations were represented by coefficients of the sex hormones, which estimate the difference in NT-proBNP at baseline by varying sex hormone levels. Longitudinal associations were represented by coefficients of interactions between sex hormones and time since baseline, which estimate the rate of change in NT-proBNP associated with sex hormones. The longitudinal analyses adjusted for baseline NT-proBNP values in the mixed effects models. Because log-transformed values of sex hormones and NT-proBNP may be difficult to interpret, the beta-coefficients from the linear mixed effects

models were then exponentiated and presented as ratios (95% CI) of the NT-proBNP geometric means.

We utilized three models that were progressively adjusted for potential confounders. Model 1 adjusted for demographic factors, including age, race/ethnicity, and MESA site. Model 2 included all covariates in model 1, plus lifestyle, socioeconomic, body composition, and hormonal factors, including education, smoking, alcohol consumption, BMI, and physical activity, as well as use of hormone therapy (HT) and years since menopause in women. Model 3 included all covariates of model 2, plus CVD risk factors, including diabetes, SBP, use of antihypertensive medications, total and high-density lipoprotein (HDL) cholesterol, use of lipid-lowering therapy, and eGFR.

We additionally modeled sex hormones continuously using restricted cubic splines (with knots at the 5th, 35th, 65th, and 95th percentiles of the sex hormone sample distributions) to assess for potential non-linearity and to model a flexible dose-response relationship between sex hormones and NT-proBNP. Sensitivity analyses were performed to exclude women on HT at baseline, participants with reduced left ventricular ejection fraction (LVEF) at baseline (defined as <50% as assessed by cardiac MRI), and participants with incident clinical CVD events between exams 1 and 5. Given inherent interest in differences by race/ethnicity in this multi-ethnic cohort, results were also stratified by race/ethnic groups in exploratory analyses.

Two-sided P values <0.05 were considered statistically significant. All analyses were performed on STATA version 14 (StataCorp LP, College Station, Texas).

Results

Participant characteristics

Baseline characteristics of the cross-sectional sample (n=5,059), stratified by sex, are shown in **Table 1**. Women were slightly older than men (mean age 65.1 and 62.3 years, respectively). NT-proBNP levels were higher in women than men at baseline (median 79.9 and 38.5pg/ml, respectively). Women had higher BMI, participated in less physical activity, and were less likely to be smokers than men. Women also had higher SBP and total cholesterol and were less likely to have diabetes. Approximately one-third (32.9%) of women were receiving HT. Baseline characteristics of the longitudinal sub-sample (n=4,389) are presented in **Supplemental Table 1**(35) and are similar to those of the cross-sectional sample.

Cross-sectional analysis

Table 2 shows the adjusted cross-sectional associations between baseline sex hormones and baseline levels of NT-proBNP. Among women, higher total T, bioavailable T, free T, and DHEA levels were independently associated with lower NT-proBNP (Model 1). These inverse associations remained significant after further adjusting for lifestyle factors and hormonal status (Model 2), and for other CVD risk factors (Model 3). The corresponding ratios (95% CI) of the NT-proBNP geometric means per 1 SD increment in sex hormone levels in the fully adjusted model (Model 3) were: total T 0.96 (0.92, 0.99), bioavailable T 0.88 (0.84, 0.91), free T 0.81 (0.78, 0.85), and DHEA 0.90 (0.87, 0.94). SHBG, on the other hand, was associated with higher NT-proBNP levels, with significance persisting after adjusting for the same covariates (ratio 1.24 [1.18, 1.29]). Estradiol levels were not significantly associated with NT-proBNP in women in cross-sectional analyses. These results were generally consistent after stratifying by race/ethnicity [**Supplemental Table 2** (35)].

Among men, in the fully adjusted model (Model 3), bioavailable T (0.94 [0.91, 0.98]), free T (0.85 [0.82, 0.89]), and DHEA (0.92 [0.89, 0.96]) were associated with lower NT-

proBNP. Estradiol was also significantly associated with lower NT-proBNP levels (0.94 [0.91, 0.98]). Similar to the relationship in women, SHBG levels in men were significantly associated with higher NT-proBNP (1.17 [1.12, 1.21]). These results were also generally consistent after stratifying by race/ethnicity [**Supplemental Table 2** (35)].

Longitudinal analysis

Table 3 shows the association between baseline sex hormones and adjusted change in NT-proBNP over a 10-year follow-up (2000-2002 to 2010-2012). Among women, greater baseline total T (ratio 1.06 [1.02, 1.11]), bioavailable T (1.13 [1.09, 1.18]), free T (1.16 [1.11, 1.20]), and DHEA levels (1.04 [1.00, 1.08]) were associated with a greater increase in NT-proBNP even after adjusting for demographic, lifestyle, and CVD risk factors (Model 3). In contrast, SHBG and estradiol were inversely associated with change in NT-proBNP after adjustment for the above covariates (0.86 [0.83, 0.90] and 0.95 [0.91, 0.99], respectively).

All above associations in women were preserved after excluding 22 women with reduced LVEF at baseline [**Supplemental Table 3** (35)]. After excluding 691 women who were receiving HT at baseline, bioavailable T, free T, and estradiol levels were associated with greater longitudinal increase in NT-proBNP, and SHBG levels were associated with less change in NT-proBNP [**Supplemental Table 4** (35)]. The pattern of findings also remained consistent after excluding 116 women with incident CVD and HF events during follow-up [**Supplemental Table 5** (35)], as well as after stratifying by race/ethnicity [**Supplemental Table 6** (35)].

In men, greater free T and estradiol were associated with a greater increase in NT-proBNP over 10-years (**Table 3**), even after adjusting for demographic, lifestyle, and CVD risk factors (Model 3). The adjusted ratios were 1.04 [1.00, 1.09] for free T and 1.08 [1.03, 1.13] for estradiol. Findings were consistent after excluding 57 men with reduced LVEF at baseline [**Supplemental Table 3** (35)], after excluding 197 men with incident CVD and HF during follow-up [**Supplemental Table 5** (35)], and after stratifying by race/ethnicity [**Supplemental Table 6** (35)].

To test for a non-linear relationship between sex hormones and NT-proBNP, we modeled the associations using adjusted restricted cubic splines (**Figures 2, 3**). Among women, those with free T in the 10th percentile had higher baseline NT-proBNP levels than those with free T in the 90th percentile (**Figure 2A,** time=0 years). However, notably, the longitudinal increase and slope of the rise in NT-proBNP was greater among women with the highest free T. Despite having lower NT-proBNP levels at baseline, women with free T in the 90th percentile had a steeper increase in their NT-proBNP levels such that their NT-proBNP levels were not significantly different from those in women with free T in the 10th percentile by the end of follow-up (**Figure 2A**, time=11 years). In terms of estradiol, the differences in baseline and longitudinal NT-proBNP levels in women with 10th percentile compared to 90th percentile estradiol levels were not statistically significant (**Figure 3A**).

Among men, those with baseline free T in the 10th percentile had higher NT-proBNP levels over the course of follow-up than those with free T in the 90th percentile (**Figure 2B**). Men with estradiol in the 90th percentile had lower NT-proBNP levels at baseline, but had greater increase in their NT-proBNP levels such that over the course of follow-up, their NT-proBNP levels were similar to that of men with estradiol in the 10th percentile (**Figure 3B**).

We additionally examined the associations of sex (women vs. men) with baseline and longitudinal change in NT-proBNP levels (**Table 4**). After adjusting for demographic and lifestyle covariates and CVD risk factors, women had greater adjusted geometric means of NT-proBNP levels at baseline (women: 68.1pg/mL [65.5, 70.9], men: 39.7 [38.3, 41.2]), but men

demonstrated a greater increase in NT-proBNP levels over the 10-year follow-up period (women: 1.71 [1.64, 1.79], men: 2.43 [2.34, 2.53]). The results were similar after stratifying by race/ethnicity [**Supplemental Table 7** (35)].

Discussion

In a large, multi-ethnic cohort of men and women free of baseline clinical CVD/HF, we found that women had higher levels of NT-proBNP at baseline, but men had a greater increase in NT-proBNP over 10-years of follow-up, after accounting for age and CVD risk factors. Additionally, we found that a more androgenic pattern of sex hormones (higher bioavailable T, free T, and DHEA, and lower SHBG) was associated with lower baseline NT-proBNP levels in men and postmenopausal women. These cross-sectional associations suggest a potential mechanism for the higher levels of NT-proBNP observed in women compared to men, seen in our study and in other studies in the general population.(20-22) Unexpectedly, we found that the longitudinal associations between sex hormones and change in NT-proBNP were the opposite of their cross-sectional associations. In women, a more androgenic and less estrogenic pattern of sex hormones (higher total T, bioavailable T, free T, and DHEA, and lower SHBG and estradiol) were positively associated with change in NT-proBNP levels over 10-years. In men, higher estradiol and higher free T levels were associated with greater increase in NT-proBNP. Fewer sex hormones demonstrated significant longitudinal associations with NT-proBNP in men than in women, suggesting that the associations between sex hormones and change in NT-proBNP may be more robust in women compared to men. Overall, our results show that while women with a more androgenic pattern of sex hormones have lower NT-proBNP levels at baseline, they also experience a greater increase in NT-proBNP over time.

Cross-sectional vs. longitudinal associations between NT-proBNP and sex hormones

NT-proBNP mediates various cardioprotective effects by stimulating vasodilation and natriuresis and inhibiting hypertrophy and fibrosis. States of NP deficiency, including obesity and African-American race, have been associated with adverse cardiovascular outcomes, including hypertension and HF.(37-39) Our cross-sectional analyses demonstrated that a more androgenic pattern of sex hormones was associated with lower levels of NT-proBNP in women and men, suggesting that having a more androgenic pattern of sex hormones may represent another state of NP deficiency. This may contribute the rise in CVD/HF risk in women after menopause, when women experience a decrease in estrogens and develop relative androgen excess.

To our knowledge, the longitudinal relationship between sex hormones and NT-proBNP has not been previously studied. Our analysis showed that, in women, a more androgenic pattern of sex hormones was associated with a *greater* increase in NT-proBNP over time. This was consistent with our finding that men experienced a greater longitudinal change in NT-proBNP levels than women. While NT-proBNP plays a favorable role in cardioprotective and homeostatic functions, elevated levels of NT-proBNP are seen in acute HF and acute coronary syndrome and are used clinically as markers of pathology. Studies have suggested that HF patients demonstrate BNP resistance and suffer from relative BNP deficiency, resulting in activation of compensatory systems that increase NT-proBNP levels to restore homeostasis.(11) In acute HF, BNP undergoes abnormal processing into less biologically active forms that are indistinguishable from active forms on standard assays. As a consequence, HF patients experience blunted physiological effects of BNP, despite having significantly elevated NT-proBNP values.(40,41) A prior MESA study found that the inverse relationship between NT-

proBNP and various CVD risk factors, including BMI, lipids, and insulin resistance, was present at NT-proBNP levels <100 pg/mL, but not present at levels ≥100.(42)

Taken together, current evidence suggests that while NT-proBNP exerts cardioprotective effects to maintain volume homeostasis, and while states of NP deficiency are associated with higher risk of CVD, elevated levels of NT-proBNP predict poorer outcomes. The apparently paradoxical roles of NT-proBNP as both a cardioprotective hormone and a biomarker of CVD and HF are likely due to the involvement of NT-proBNP in a negative feedback loop, where there is a compensatory increase in NT-proBNP levels in the setting of pathology.(39) In our study, at baseline, a more androgenic pattern of sex hormones was associated with lower NT-proBNP, which may represent a state of NP deficiency. Over the course of follow-up, a more androgenic and less estrogenic pattern of sex hormones was associated with greater change in NT-proBNP. Women with the highest percentile of free T had the lowest levels of NT-proBNP at baseline but had the greatest increase in NT-proBNP over time. The rise in NT-proBNP over time in these women may reflect the development of incident subclinical CVD and subsequent compensatory increase in NT-proBNP levels, particularly in those with relative NP deficiency at baseline. The association between androgenic sex hormones and greater change in NT-proBNP may also help explain the higher incidence of CVD in men and postmenopausal women compared to premenopausal women, who have relatively lower androgen levels. Additionally, our results suggest that higher levels of androgenic hormones may identify individuals who may have greater CVD risk.

Our findings also suggest that the associations of androgens with NT-proBNP levels are more robust in women than in men, which was previously unrecognized. This suggests that sex hormone levels may impact change in NT-proBNP in women more so than in men, which mirrors the increase in the risk of heart failure with preserved ejection fraction (HFpEF) in women compared to men.

Role of androgens versus estrogen in NP regulation

Whether the suppressive effect of androgens or the stimulatory effect of estrogens is the primary driver in sex hormone regulation of BNP is unclear, as there is evidence for both from prior studies. A study of men with systolic HF demonstrated that total and free T were inversely associated with NT-proBNP.(43) In women, higher androgens have been found to be associated with lower NT-proBNP, while estrogen status did not affect NT-proBNP levels.(28,29,44) A cross-sectional analysis of premenopausal women in the Dallas Heart Study found that higher levels of SHBG and lower levels of T were correlated with higher BNP and NT-proBNP levels.(28) Additionally, the Framingham Heart Study showed that higher SHBG and lower free T were associated with higher NT-proBNP in both women and men cross-sectionally.(29) These prior studies have proposed that men may have an NP deficiency compared to women. Our cross-sectional results extend these findings by showing that higher androgen levels were associated with lower NT-proBNP in women and men across a range of androgenic sex hormones. Our results also suggest that a more androgenic sex hormone pattern may mediate the state of NP deficiency in both men and women.

In terms of estrogen modulation of the NP system, NP levels have been found across multiple studies to be higher in women using HT compared to those not on HT.(20,26,27,45) However, other studies have argued that estrogen is not primarily responsible for sex differences in BNP.(28,29,44) Of note, many of these studies were limited by the inability to measure endogenous estrogen levels in both men and women, and estrogen status in women was inferred from menopausal status, HT use, and measurement of other hormones in the gonadal axis.

Our ability to measure estradiol directly in both men and women allows us to discern the differences in the relationship between estradiol and NT-proBNP in men and women, after adjustment for important clinical confounders. Our study demonstrates that, among women, only androgens, not estrogen, were associated with NT-proBNP in cross-sectional analyses. This supports the hypothesis that primarily androgens, rather than estrogens, influence cross-sectional NT-proBNP levels in postmenopausal women. Of note, postmenopausal women not on HT have lower estradiol levels than men. In men, estradiol was actually associated with lower cross-sectional levels of NT-proBNP. One possible explanation is that estradiol levels are higher in obese men due to increased aromatization in adipose tissue, and obesity is associated with lower levels of NT-proBNP.(46,47) However, the inverse association between estradiol and NT-proBNP remained after adjusting for BMI. Additional studies are needed to elucidate the etiology of the relationship between estradiol and NT-proBNP in men.

Strengths and limitations

The inclusion of a longitudinal design was a strength of our study, as it allowed us to assess the change in NT-proBNP over time in relation to baseline sex hormone levels. Additional strengths include a large sample size, a multi-ethnic cohort, and the ability to evaluate six different sex hormone levels and various important confounding clinical variables.

Limitations of this study include having only a single measure of sex hormone levels at baseline. We were thus unable to analyze whether there were any associations between changes in sex hormone levels and changes in NT-proBNP. MESA enrolled predominantly postmenopausal women, and we excluded the few premenopausal women for the analyses presented here; therefore, we were unable to examine whether relationships between sex hormone and NT-proBNP differ prior to the menopausal transition. The observational nature of our study precludes the ability to determine causality. Longitudinal analyses are limited by temporal bias from attrition. Finally, the aim of this present study was to examine the relationships of sex hormones with NT-proBNP, an important biomarker related to ventricular wall stress, volume homeostasis, and LV hypertrophy, to gain insight into sex differences in NT-proBNP levels, HFpEF risk, and the female predominance of both. This complements our previous work in MESA where we examined the relationship of sex hormones with 10-year change in cardiac structure/function(7) and aortic stiffness(9) and with incident clinical events including CVD, HF, and HFpEF.(10)

Conclusions

In summary, we found that a more androgenic pattern of sex hormones was independently associated with lower NT-proBNP levels in cross-sectional analyses in men and postmenopausal women. This association may help explain sex differences in the distribution of NT-proBNP and may contribute to the NP deficiency in men relative to women. In longitudinal analyses, a more androgenic pattern of sex hormones was more strongly associated with a greater increase in NT-proBNP levels in women, with only free T being associated with NT-proBNP in men. This relationship may be a mechanism for the increased risk of CVD and HF seen in women after menopause. Additional research is needed to further explore whether longitudinal changes in NT-proBNP levels seen in our study are correlated with longitudinal changes in sex hormones. The impact of menopause on changes in NT-proBNP levels over time should also be explored. Furthermore, future studies should aim to determine whether sex hormones directly play a role in biological pathways of BNP synthesis and clearance in a causal fashion. Lastly, the dual role of NT-proBNP as both a cardioprotective hormone and a biomarker of CVD and HF, as

well as the role of sex hormones in delineating these processes, should be further explored. This would provide a step toward improved clinical CVD risk stratification and prognostication based on sex hormone and NT-proBNP levels.

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Trial Registration

MESA is not a clinical trial. However the cohort design is registered at clinicaltrials.gov as follows: https://clinicaltrials.gov/ct2/show/NCT00005487

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Figure 1: Selection of study sample

Figure 2: Longitudinal associations between free T and NT-proBNP in men and women using restricted cubic splines[*, †]. *Adjusted for model 2 covariates: age, race/ethnicity, MESA site, education, smoking, alcohol consumption, body mass index, and physical activity; in women, also adjusts for use of hormone therapy and years since menopause. † Graphs represent baseline and longitudinal change in NT-proBNP, with knots at the 5th, 35th, 65th, and 95th percentiles of the free T distribution. 95% CI are represented by dotted lines. Abbreviations: T, testosterone; NT-proBNP, N-Terminal pro-B-type Natriuretic Peptide.

Figure 3: Longitudinal associations between estradiol and NT-proBNP in men and women using restricted cubic splines[*, †]. *Adjusted for model 2 covariates: age, race/ethnicity, MESA site, education, smoking, alcohol consumption, body mass index, and physical activity; in women, also adjusts for use of hormone therapy and years since menopause. † Graphs represent baseline and longitudinal change in NT-proBNP, with knots at the 5th, 35th, 65th, and 95th percentiles of the estradiol distribution. 95% CI are represented by dotted lines. Abbreviations: NT-proBNP, N-Terminal pro-B-type Natriuretic Peptide.

Table 1: Characteristics of study participants at MESA baseline exam (2000-2002)

	Women (n = 2,371)	Men (n = 2,688)	Overall (n = 5,059)
Age, years	65.1 (9.0)	62.3 (10.3)	63.6 (9.8)
Race/ethnicity, n (%)			
White	928 (39.1)	1,081 (40.2)	2,009 (39.7)
Chinese-American	310 (13.1)	363 (13.5)	673 (13.3)
Black	577 (24.3)	619 (23.0)	1,196 (23.6)
Hispanic	556 (23.5)	625 (23.3)	1,181 (23.3)
Education, n (%)			
<High school	544 (22.9)	427 (15.9)	971 (19.2)
High school, technical school, or associate degree	1,181 (49.8)	1,137 (42.3)	2,318 (45.8)
College, graduate or professional school	646 (27.2)	1,124 (41.8)	1,770 (35.0)
Cardiovascular risk factors			
BMI, kg/m^2	28.6 (6.0)	27.8 (4.5)	28.2 (5.3)
Systolic BP, mmHg	129.4 (23.6)	125.7 (19.1)	127.5 (21.4)
Diastolic BP, mmHg	68.9 (10.2)	74.8 (9.3)	72.0 (10.2)
Antihypertension medication, n (%)	992 (41.8)	947 (35.2)	1,939 (38.3)
Current smoking, n (%)	234 (9.9)	383 (14.2)	617 (12.2)
Total intentional exercise, MET-	3390.0 (1665.0 - 6360.0)	4361.3 (2130.0 - 8332.5)	3845.0 (1890.0 - 7290.0)

min/week*			
Total cholesterol, mg/dl	201.7 (36.0)	188.4 (34.9)	194.6 (36.0)
HDL cholesterol, mg/dl	56.7 (15.4)	45.0 (11.8)	50.5 (14.8)
LDL cholesterol, mg/dl	118.2 (32.0)	116.6 (31.0)	117.3 (31.5)
Triglycerides, mg/dl	133.2 (76.0)	137.3 (98.5)	135.4 (88.7)
Lipid lowering medication, n (%)	445 (18.8)	440 (16.4)	885 (17.5)
Diabetes, n (%)	295 (12.4)	363 (13.5)	658 (13.0)
eGFR, mL/min/1.73 m2	74.7 (15.8)	78.0 (16.0)	76.5 (16.0)
Sex hormones			
Total T, nmol/L*	0.9 (0.6 - 1.3)	14.2 (11.3 - 17.7)	8.0 (0.9 - 14.6)
Bioavailable T, nmol/L*	0.2 (0.1 - 0.3)	5.2 (4.2 - 6.5)	2.9 (0.2 - 5.3)
Estradiol, nmol/L*	0.1 (0.0 - 0.2)	0.1 (0.1 - 0.1)	0.1 (0.1 - 0.1)
DHEA, nmol/L*	10.1 (6.8 - 14.4)	12.6 (9.1 - 17.1)	11.3 (8.0 - 16.0)
Free T, %*	1.3 (0.9 - 1.7)	2.0 (1.6 - 2.3)	1.7 (1.2 - 2.1)
SHBG, nmol/L*	59.9 (40.6 - 94.9)	40.8 (31.4 - 52.6)	46.9 (34.1 - 67.7)
Hormone therapy, n (%)	763 (32.9)	NA	763 (32.9)
NT-proBNP, pg/mL *	79.9 (41.7 - 154.2)	38.5 (15.7 - 81.7)	56.0 (24.1 - 117.8)

*Data represented as median (IQR)
MESA, Multi-Ethnic Study of Atherosclerosis; BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; eGFR, estimated glomerular filtration rate; T, testosterone; DHEA, dehydroepiandrosterone; SHBG, sex hormone binding globulin; NT-proBNP, N-Terminal pro-B-type Natriuretic Peptide.

Table 2: Cross-sectional associations between sex hormone levels and NT-proBNP levels at MESA baseline exam (2000-2002)

Sex hormones*	**Ratio of NT-proBNP (pg/mL)***					
	Women (n = 2,371)			Men (n = 2,688)		
	Model 1†	Model 2‡	Model 3§	Model 1†	Model 2‡	Model 3§
Total T (nmol/L)	**0.94 (0.91, 0.98)**	**0.96 (0.93, 1.00)**	**0.96 (0.92, 0.99)**	1.02 (0.99, 1.06)	1.02 (0.98, 1.06)	1.01 (0.97, 1.05)
Bioavailable T (nmol/L)	**0.86 (0.83, 0.89)**	**0.87 (0.84, 0.90)**	**0.88 (0.84, 0.91)**	**0.95 (0.92, 0.99)**	**0.95 (0.91, 0.99)**	**0.94 (0.91, 0.98)**
Free T (%)	**0.82 (0.80, 0.85)**	**0.80 (0.76, 0.83)**	**0.81 (0.78, 0.85)**	**0.86 (0.83, 0.90)**	**0.87 (0.83, 0.90)**	**0.85 (0.82, 0.89)**
Estradiol (nmol/L)	1.03 (0.99, 1.07)	1.00 (0.96, 1.05)	0.98 (0.94, 1.03)	**0.95 (0.91, 0.99)**	**0.95 (0.91, 0.99)**	**0.94 (0.91, 0.98)**
DHEA (nmol/L)	**0.89 (0.86, 0.92)**	**0.90 (0.86, 0.93)**	**0.90 (0.87, 0.94)**	**0.92 (0.88, 0.95)**	**0.91 (0.87, 0.95)**	**0.92 (0.89, 0.96)**
SHBG (nmol/L)	**1.22 (1.18, 1.26)**	**1.26 (1.21, 1.32)**	**1.24 (1.18, 1.29)**	**1.15 (1.11, 1.20)**	**1.15 (1.11, 1.20)**	**1.17 (1.12, 1.21)**

* Per 1 standard deviation greater log-transformed sex hormone levels. Results are presented as exponentiated beta coefficient to reflect ratio of NT-proBNP geometric means (95% CI) at baseline. Statistically significant results ($p < 0.05$) are in bold. Each hormone was modeled separately.
† Model 1: Adjusted for age, race/ethnicity, and MESA site.
‡ Model 2: Adjusted for model 1, plus education, smoking, alcohol consumption, body mass index, and physical activity. In women, also adjusts for use of hormone therapy and years since menopause.
§ Model 3: Adjusted for model 2, plus systolic blood pressure, use of antihypertensive medications, total cholesterol, HDL cholesterol, use of lipid-lowering therapy, diabetes, and eGFR.
Abbreviations: NT-proBNP, N-Terminal pro-B-type Natriuretic Peptide; MESA, Multi-Ethnic Study of Atherosclerosis; T, testosterone; DHEA, dehydroepiandrosterone; SHBG, sex hormone binding globulin.

Table 3: Longitudinal associations between sex hormone levels and 10-year change in NT-proBNP from MESA exam 1 (2000-2002) to MESA exam 5 (2010-2012)

Sex hormones*	**Ratio of 10-year Change in NT-proBNP (pg/mL)***					
	Women (n = 2,041)			Men (n = 2,348)		
	Model 1†	Model 2‡	Model 3§	Model 1†	Model 2‡	Model 3§
Total T (nmol/L)	**1.07 (1.03, 1.11)**	**1.06 (1.02, 1.11)**	**1.06 (1.02, 1.11)**	0.98 (0.93, 1.02)	0.98 (0.93, 1.02)	0.98 (0.93, 1.02)

Bioavailable T (nmol/L)	**1.14 (1.10, 1.19)**	**1.13 (1.09, 1.18)**	**1.13 (1.09, 1.18)**	0.99 (0.94, 1.03)	0.98 (0.94, 1.03)	0.98 (0.94, 1.03)
Free T (%)	**1.16 (1.12, 1.21)**	**1.16 (1.11, 1.21)**	**1.16 (1.11, 1.20)**	**1.04 (1.00, 1.09)**	**1.04 (1.00, 1.09)**	**1.04 (1.00, 1.09)**
Estradiol (nmol/L)	**0.94 (0.91, 0.98)**	**0.95 (0.91, 0.99)**	**0.95 (0.91, 0.99)**	**1.08 (1.03, 1.13)**	**1.08 (1.03, 1.13)**	**1.08 (1.03, 1.13)**
DHEA (nmol/L)	**1.05 (1.01, 1.09)**	**1.04 (1.00, 1.08)**	**1.04 (1.00, 1.08)**	0.99 (0.95, 1.04)	0.99 (0.95, 1.04)	0.99 (0.95, 1.03)
SHBG (nmol/L)	**0.86 (0.83, 0.89)**	**0.86 (0.83, 0.90)**	**0.86 (0.83, 0.90)**	0.95 (0.91, 1.00)	0.96 (0.92, 1.00)	0.96 (0.92, 1.00)

* Per 1 standard deviation greater log-transformed sex hormone levels. Results are presented as exponentiated beta coefficient to reflect ratio of NT-proBNP geometric means (95% CI) in change in NT-proBNP from 2000-2002 to 2010-2012. Statistically significant results ($p < 0.05$) are in bold. Each hormone was modeled separately.
† Model 1: Adjusted for age, race/ethnicity, and MESA site.
‡ Model 2: Adjusted for model 1, plus education, smoking, alcohol consumption, body mass index, and physical activity. In women, also adjusts for use of hormone therapy and years since menopause.
§ Model 3: Adjusted for model 2, plus systolic blood pressure, use of antihypertensive medications, total cholesterol, HDL cholesterol, use of lipid-lowering therapy, diabetes, and eGFR.
Abbreviations: NT-proBNP, N-Terminal pro-B-type Natriuretic Peptide; MESA, Multi-Ethnic Study of Atherosclerosis; T, testosterone; DHEA, dehydroepiandrosterone; SHBG, sex hormone binding globulin.

Table 4: Baseline and 10-year change in NT-proBNP levels* in men and postmenopausal women

	Baseline NT-proBNP (pg/mL)	10-year change in NT-proBNP (pg/mL)
Women	68.1 (65.5, 70.9)	1.71 (1.64, 1.79)
Men	39.7 (38.3, 41.2)	2.43 (2.34, 2.53)
Difference (Women – Men)	28.4 (31.5, 25.3)	-1.42 (-1.34, -1.51)

*Geometric mean of NT-proBNP, adjusted for age, race/ethnicity, MESA site, education, smoking, alcohol consumption, body mass index, and physical activity.
Abbreviations: NT-proBNP, N-Terminal pro-B-type Natriuretic Peptide; MESA, Multi-Ethnic Study of Atherosclerosis;

Figure 1:

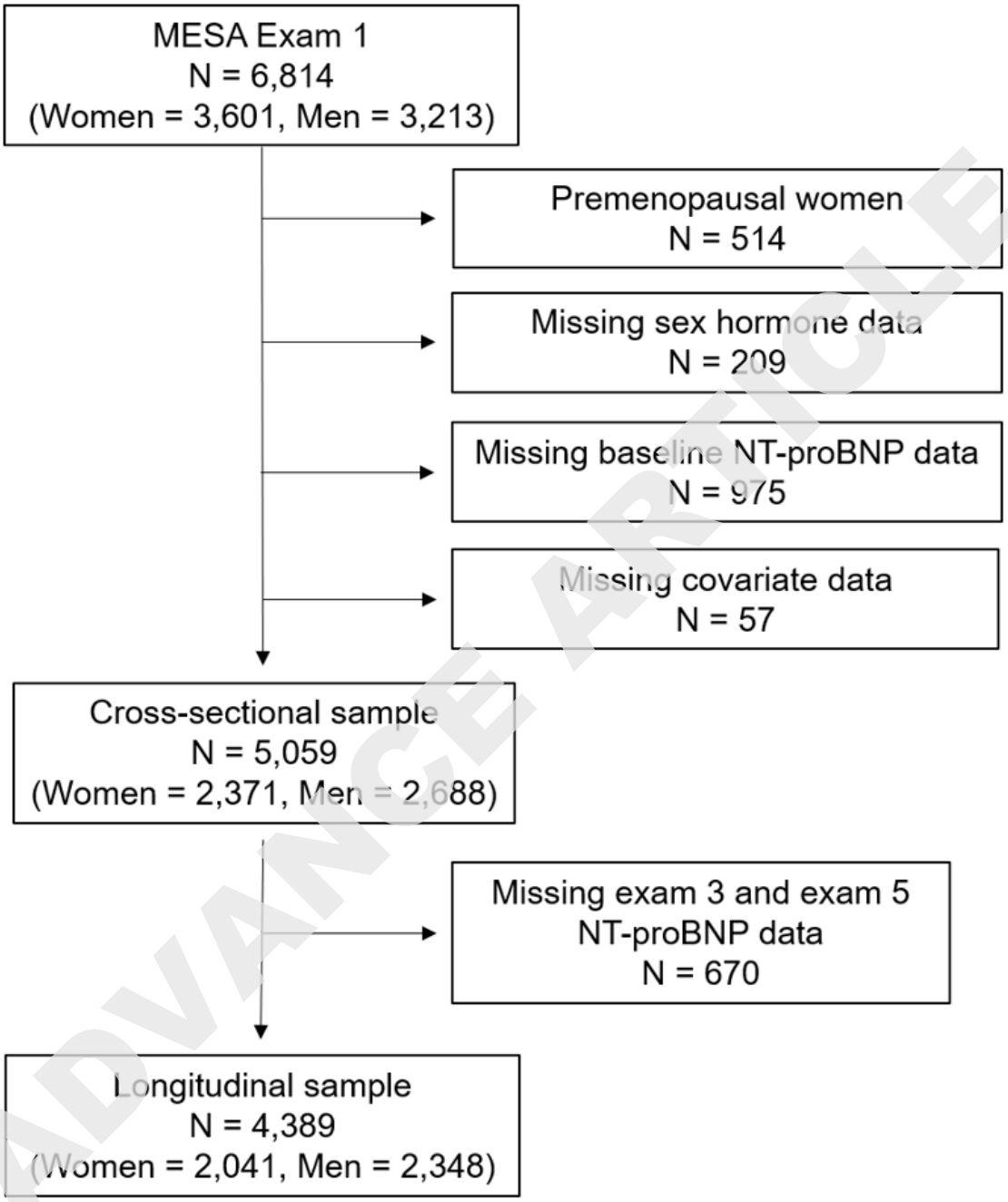

Figure 2:

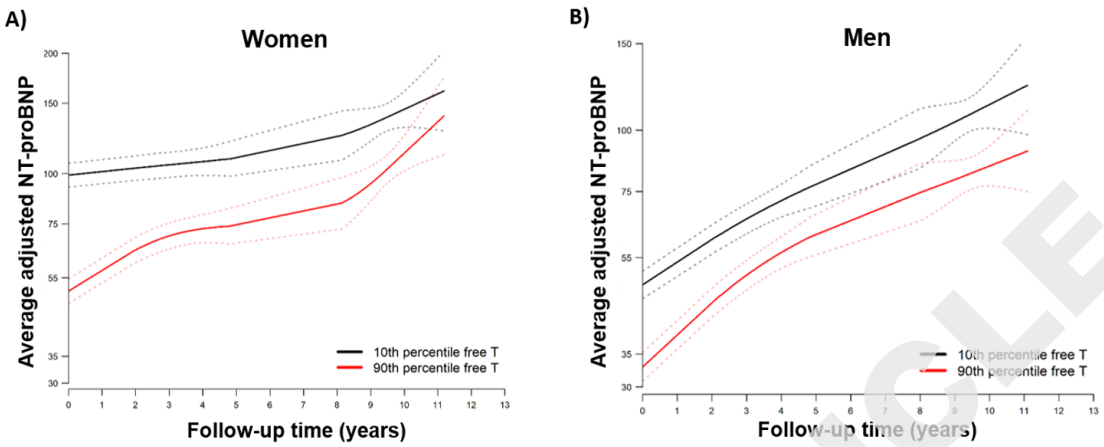

Figure 3: