

Influence of Sex and Hormone Status on Circulating Natriuretic Peptides

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- Objectives** The aim of this study was to assess the relationship between sex hormones and natriuretic peptide levels in community-based adults.
- Background** Women have higher circulating natriuretic peptide concentrations than men, but the mechanisms for these sex-related differences and the impact of hormone therapy are unclear. Experimental studies suggest that androgens may suppress natriuretic peptide secretion.
- Methods** We measured N-terminal pro-B-type natriuretic peptide (NT-proBNP), total testosterone, and sex hormone-binding globulin plasma levels in 4,056 men and women (mean age 40 ± 9 years) from the Framingham Heart Study Third-Generation cohort. Sex/hormone status was grouped as: 1) men; 2) post-menopausal women not receiving hormone replacement therapy; 3) pre-menopausal women not receiving hormonal contraceptives; 4) post-menopausal women receiving hormone replacement therapy; and 5) pre-menopausal women receiving hormonal contraceptives.
- Results** Circulating NT-proBNP levels were associated with sex/hormone status (overall $p < 0.0001$). Men had lower NT-proBNP levels than women of all menopause or hormone groups, and women receiving hormonal contraceptives had higher NT-proBNP levels than women who were not receiving hormone therapy (all $p < 0.0001$). These relationships remained significant after adjusting for age, body mass index, and cardiovascular risk factors. Across sex/hormone status groups, free testosterone (FT) levels decreased and sex hormone-binding globulin levels increased in tandem with increasing NT-proBNP levels. In sex-specific analyses, NT-proBNP levels decreased across increasing quartiles of FT in men (p for trend < 0.01) and women (p for trend < 0.0001). Adjustment for FT markedly attenuated the association between sex/hormone status and NT-proBNP concentrations.
- Conclusions** These findings suggest that lower levels of circulating androgens and the potentiating effect of exogenous female hormone therapy contribute to the higher circulating NT-proBNP concentrations in women. (J Am Coll Cardiol 2011;58:618–26) © 2011 by the American College of Cardiology Foundation

The importance of understanding the effects of sex and hormone therapy on the cardiovascular system is underscored by the pronounced sex differences in the prevalence of cardiovascular disease, the increase in cardiovascular events in women following menopause, and concerns regarding the safety of hormone replacement therapy (HRT) in post-menopausal women (1–3). Studies of circulating

cardiovascular biomarkers, such as the natriuretic peptides (NPs) (4–8), may provide a biological basis to better

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understand these sex-related differences in cardiovascular risk. The NPs exert hormonal (vasodilation, natriuresis, and

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aldosterone and endothelin suppression) and autocrine/paracrine (antihypertrophic, antifibrotic, and proangiogenic) protective cardiovascular effects. Sex is one of the strongest determinants of concentrations of circulating B-type natriuretic peptide (BNP) or N-terminal pro-B-type natriuretic peptide (NT-proBNP) in population-based studies, with women having consistently higher circulating levels than men (4–7). Nonetheless, the mechanisms underlying the sex-based difference in circulating NPs have not been established. Further, the effect of menstrual status, menopausal status, or exogenous hormone therapy on plasma NP concentrations in women remains unclear (6,8–10).

Previous studies have suggested that sex hormones play an important role in the regulation of NPs (11). On one hand, estrogens have been shown to exert a stimulating effect on the NP system (9,12) and are therefore postulated to mediate the “NP excess” in women compared with men. On the other hand, recent experimental (13,14) and cross-sectional human data (8,15) suggest that androgens may exert an inhibitory effect on the NP system, thus accounting for the lower NP levels in men and potentially explaining the lack of cardiovascular protection in men compared with women (8). However, prior clinical studies of the influence of androgens on NPs were limited to studying women (8,16) or children (15) and did not fully characterize both endogenous (menstrual phase) and exogenous (hormone therapy) variations. Further, previous investigations did not include measures of insulin resistance, which is a potential confounder because hyperinsulinemia is known to be associated with both sex hormones (hyperandrogenemia) and lowered NP concentrations (17–20).

We aimed to test the hypothesis that free testosterone (FT) is an important determinant of the relationship between sex/hormone status and circulating NPs in adults from the general population. Specifically, we hypothesized that higher FT levels in men compared with women and in women not receiving hormone therapy compared with those receiving hormone therapy may be related to lower circulating concentrations of NPs. To achieve our aim, we measured levels of testosterone and its primary binding protein, NP levels, and insulin measures in a large, community-based sample of predominantly middle-aged adults grouped by sex, menstrual status, and the presence or absence of hormone therapy.

Methods

Study sample. The Framingham Heart Study (21) is a community-based cohort investigation that began in 1948 with the recruitment of the Original Cohort, recruited a second generation in 1971 consisting of offspring of the Original Cohort and their spouses (Offspring Cohort), and most recently added a third generation in 2002 consisting of children of the Offspring Cohort (Third-Generation Cohort). Participants of the youngest cohort (Third-Generation Cohort), in whom plasma NT-proBNP concentrations were measured, were eligible for the current study. Those with prevalent heart failure (prior diagnosis of

heart failure based on Framingham criteria [22]), myocardial infarction, or serum creatinine >2 mg/dl were excluded from the present investigation (n = 26). The index examinations took place during April 2002 to July 2005. All women had menstrual histories recorded by trained physicians using standardized questionnaires, including details regarding reproductive history, first day of the last menstrual period, frequency and regularity of menstrual cycles, and current usage of any hormone therapy. Participants were instructed to bring all of their current medications (taken within 1 month) with them to the examination, allowing verification by the examining physician. Anthropometric, blood pressure, and cardiovascular risk factor data were obtained at the index visit using standard protocols. All participants provided written informed consent, and protocols were approved by the Boston University Medical Center Institutional Review Board.

Classification of sex/hormone status. Menopause was defined as the cessation of periods (in the absence of pregnancy) for at least 1 year because of naturally occurring (as opposed to surgical or medical) causes. Among postmenopausal women, those receiving HRT were defined by self-reported current usage of female hormone therapy, as well as validation by direct assessment of all of the participants' current medications by the examining physician. Among premenopausal women, those receiving hormonal contraceptives (HC) were defined by self-reported current usage of hormone-containing oral pills, injections, or implants for birth control or medical indications, as well as validation by direct assessment of all of the participants' current medications by the examining physician. Based on these definitions, sex/hormone status was classified into 5 categories (Fig. 1):

- Men
- Postmenopausal women not receiving HRT
- Premenopausal women not receiving HC
- Postmenopausal women receiving HRT currently
- Premenopausal women receiving HC currently

In secondary analyses, we estimated the menstrual phase (follicular vs. luteal vs. midcycle) at the index visit among premenopausal women with regular ongoing menstrual cycles (cycle lengths 28 to 30 days) who were not receiving HC. This determination was based on the fact that the interval from ovulation to menstruation (luteal phase) is fixed at 14 days, whereas the interval from the start of menstruation to ovulation (follicular phase) can vary de-

Abbreviations and Acronyms

BNP = B-type natriuretic peptide
FT = free testosterone
HC = hormonal contraceptives
HOMA = homeostatic model assessment
HRT = hormone replacement therapy
IR = insulin resistance
NP = natriuretic peptide
NT-proBNP = N-terminal pro-B-type natriuretic peptide
SHBG = sex hormone-binding globulin

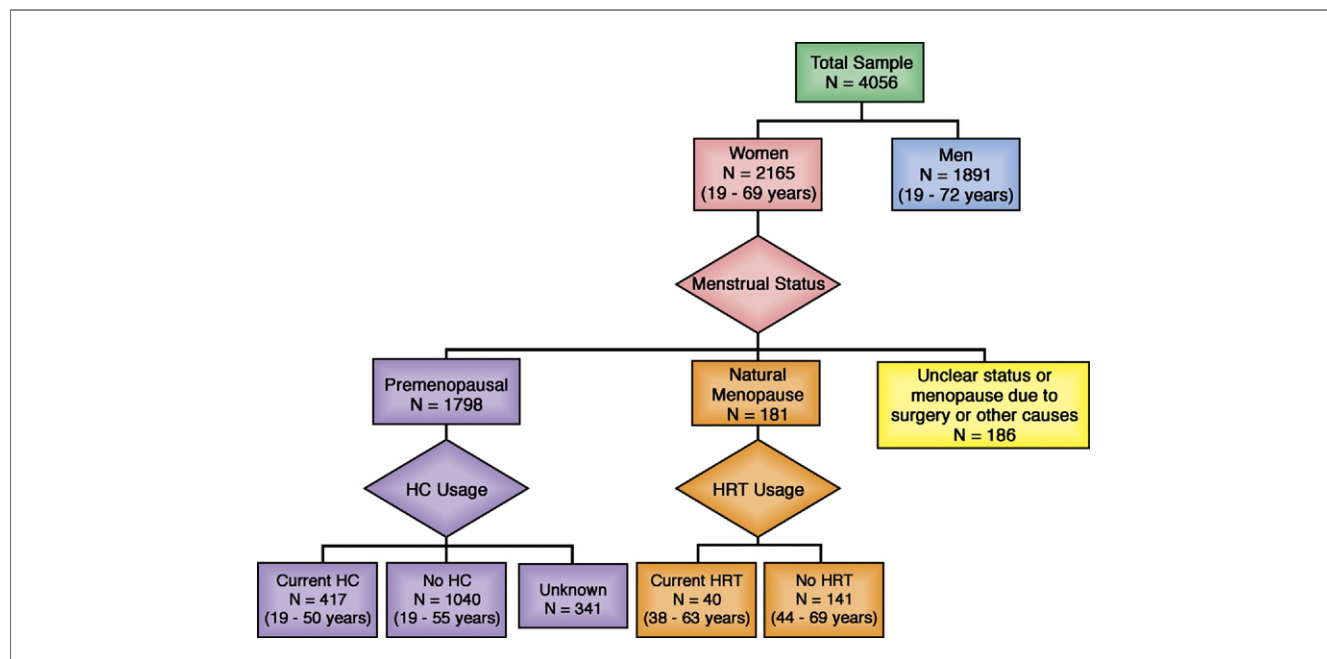


Figure 1 Classification of Sex/Hormone Status

The flow chart illustrates the classification of sex/hormone status into 1) men; 2) post-menopausal women not receiving hormone replacement therapy (HRT); 3) premenopausal women not receiving hormonal contraceptives (HC); 4) current HRT users; and 5) current HC users. The age range of participants in each group is indicated in brackets.

pending on cycle length, as previously established (23) and widely applied in similar studies (24–27). Thus, using the date of the examination, date of onset of the last menstrual period, and menstrual cycle length (specifically ascertained at the index examination), we derived the duration of the follicular phase in each regularly cycling woman, allowing 4 days between the follicular and luteal phases for the mid-cycle phase. The calculated durations were then used to assess whether NT-proBNP measurement on the date of the index visit occurred in the follicular, midcycle, or luteal phase of the menstrual cycle.

Measurement of circulating NT-proBNP, testosterone, and SHBG. Venous blood was drawn under fasting conditions between 8:00 and 9:00 AM. Samples were immediately stored at -70°C and analyzed in batches in 2009, allowing minimization of interassay variability and effects of temporal drift in the laboratory measurements. Plasma NT-proBNP levels were measured using a standard immunoassay (Roche Diagnostics, Indianapolis, Indiana), with a measurement range of 5 to more than 35,000 ng/l and intra-assay coefficient of variation of 2.7%. Serum total testosterone concentrations were quantified using a validated liquid chromatography–tandem mass spectrometry assay, with a lower detection limit of 2 ng/dl. Sex hormone-binding globulin (SHBG) concentrations were measured using an immunofluorometric assay (DELFLIA-Wallac, Inc., Turku, Finland). Total testosterone concentrations are influenced by multiple factors, such as obesity in men and oral contraceptive therapy in women, and may not reflect androgen activity. We thus calculated FT using the law of

mass action equation (28,29) and used FT in subsequent analyses.

Measurement of insulin resistance. Insulin resistance (IR) was calculated using the homeostatic model assessment (HOMA) equation: $\text{HOMA-IR} = (\text{FPG} \times \text{FPI})/22.5$, for which FPG = fasting plasma glucose (mmol/l) and FPI = fasting plasma insulin (mU/l) measured by enzyme-linked immunosorbent assay (Millipore Corporation, Billerica, Massachusetts) (30).

Statistical analyses. The association between log NT-proBNP and sex/hormone status was assessed using general linear models followed by pairwise comparisons between sex/hormone status groups with Bonferroni correction. The model was adjusted for covariates known to influence NT-proBNP concentrations (age, body mass index, systolic blood pressure, diastolic blood pressure, serum creatinine, and presence of antihypertensive medications, diabetes mellitus, and current smoking). We similarly assessed the association of log FT and log SHBG with sex/hormone status. Of a total of 2,165 women (Fig. 1), menopause status was unclear or not due to natural causes in 186, HC usage was unknown in 341, and FT/SHBG measurements or clinical covariates were missing in a further 25, leaving 1,613 women in the final multivariable models for sex/hormone status.

To assess the association between log NT-proBNP and circulating androgens, we used sex-pooled and sex-specific general linear models, in which the dependent variable was log NT-proBNP and predictors were log FT or log SHBG (separately), hormone status (among women; categories 2 to

Table 1 Baseline Characteristics

	All (N = 4,056)	Men (n = 1,891)	Women (n = 2,165)
Age, yrs	40 ± 9	40 ± 9	40 ± 9
Body mass index, kg/m ²	26.9 ± 5.6	28.0 ± 4.7	26.0 ± 6.1
Systolic blood pressure, mm Hg	117 ± 14	121 ± 13	113 ± 14
Diastolic blood pressure, mm Hg	75 ± 10	78 ± 9	73 ± 9
Serum creatinine, mg/dl	0.79 ± 0.15	0.90 ± 0.13	0.70 ± 0.11
Hypertension	16	21	12
Antihypertensive medications	8	10	7
Diabetes mellitus	3	4	2
Smoking	17	18	16
NT-proBNP, ng/l	28.1 (14.1, 52.6)	16.2 (8.1, 28.8)	42.9 (25.7, 72.2)
Log NT-proBNP	3.3 ± 1.0	2.7 ± 0.9	3.7 ± 0.8
FT, pg/ml	5.3 (2.1, 114.5)	119.0 (95.0, 152.0)	2.2 (1.4, 3.2)
Total testosterone, ng/dl	49.6 (23.7, 600.2)	617.7 (487.5, 786.1)	24.6 (17.7, 34.3)
SHBG, nmol/l	55.1 (35.2, 96.1)	37.0 (26.7, 50.2)	89.7 (58.7, 132.7)
HOMA-IR	0.98 (0.70, 1.49)	1.01 (0.78, 1.66)	0.89 (0.65, 1.31)

Values are mean ± SD, %, or median (25th, 75th percentiles). To convert FT concentration to SI units (pmol/l), multiply values in pg/ml by 3.47; to convert total testosterone concentration to SI units (nmol/l), multiply values in ng/dl by 0.0347.

FT = free testosterone; HOMA-IR = homeostatic model assessment of insulin resistance; NT-proBNP = N-terminal pro-B-type natriuretic peptide; SHBG = sex hormone-binding globulin.

5 of sex/hormone status as defined above), and covariates (listed above). We tested for interaction between log FT or log SHBG and hormone status among women and adjusted models for HOMA-IR among participants without diabetes. To further assess the association among sex/hormone status, log NT-proBNP, and circulating androgen levels, we analyzed multivariable models (sex pooled and in women alone), in which the dependent variable was log NT-proBNP and predictors sequentially included clinical covariates (listed above), sex/hormone status groups (defined above), and log FT. We assessed the beta coefficients for each sex/hormone status group, compared with the referent group of pre-menopausal women receiving HC, before and after adding log FT to the model. We also compared the type I sum of squares with the type III sum of squares for the class variable sex/hormone status to assess the percent variability in NT-proBNP levels due to sex/hormone status before (type I sum of squares) and after (type III sum of squares) the addition of log FT to the model, adjusting for all other covariates. Of a total of 2,165 women and 1,891 men, FT measurements were available for 2,144 women and 1,880 men, whereas SHBG measurements were available for 2,147 women and 1,880 men. Of these, there were no missing clinical covariates (age, body mass index, systolic blood pressure, diastolic blood pressure, serum creatinine, antihypertensive medications, diabetes mellitus, and smoking) in 2,123 women with FT measurements, 2,126 women with SHBG measurements, and 1,871 men. In addition to clinical covariates, HOMA-IR measurements were also available for 1,857 women with FT measurements, 1,859 women with SHBG measurements, and 1,650 men.

In the subgroup of pre-menopausal women with regular menstrual cycles in the absence of HC, we compared log NT-proBNP concentrations among menstrual phases (follicular, midcycle, and luteal) using general linear models with

Bonferroni correction in pairwise comparisons among phases. We similarly compared log FT and log SHBG concentrations among menstrual phase groups. Models were adjusted for covariates known to influence NT-proBNP concentrations (listed above).

All analyses were performed using SAS software. Statistical significance was determined at a p value of <0.05 (Bonferroni-adjusted significance level required p < 0.05 [number of possible comparisons] in cases of multiple comparisons).

Results

Baseline characteristics. Characteristics of the study sample (N = 4,056; mean age 40 years) are shown in Table 1. As expected in a predominantly middle-aged, community-based sample, the prevalence of cardiovascular risk factors was low, and the majority (83%) of women were pre-menopausal (Fig. 1). Among pre-menopausal women, 23% (n = 417) were currently receiving HC; among post-menopausal women, 21% (n = 40) were currently receiving HRT. HRT consisted predominantly of oral combination therapy with estrogen and progesterone (n = 34 [85%]). Of note, the age range of participants was relatively narrow, even among post-menopausal women.

Association between sex/hormone status and circulating NT-proBNP levels. Log NT-proBNP was strongly associated with sex/hormone status (age-adjusted p < 0.0001) (Fig. 2A), with the lowest concentrations in men and highest concentrations in pre-menopausal women receiving HC. These relationships remained unchanged after adjusting for age, body mass index, systolic blood pressure, diastolic blood pressure, serum creatinine, and presence of antihypertensive medications, diabetes mellitus, and smok-

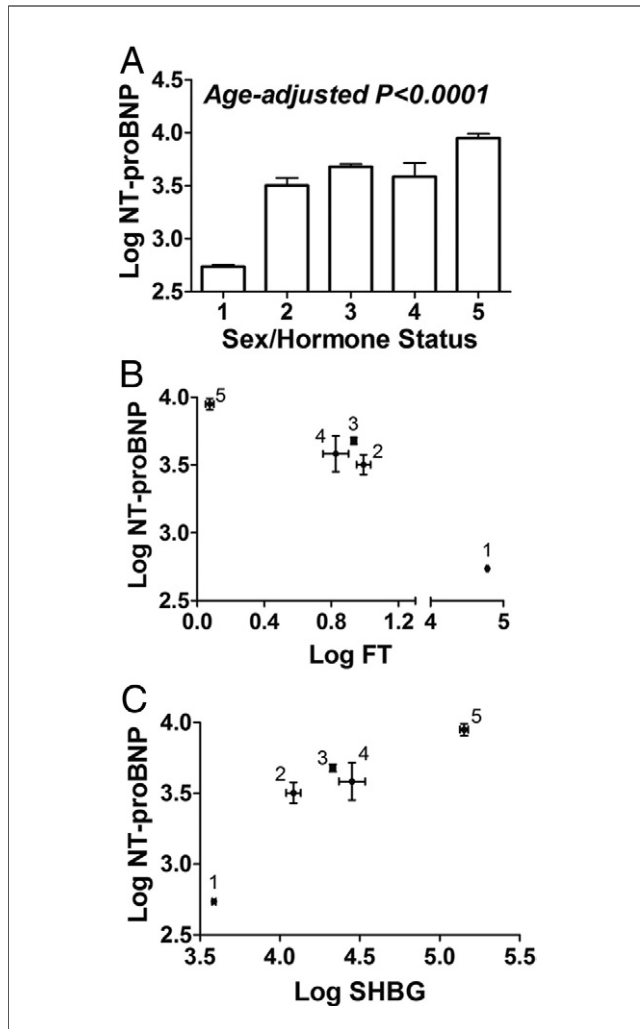


Figure 2 Association of Sex/Hormone Status With Circulating NT-proBNP, Testosterone, and SHBG

(A) Columns and bars represent the age-adjusted least squares means and SE of log N-terminal pro-B-type natriuretic peptide (NT-proBNP) by sex/hormone status (1 = men, 2 = post-menopausal women not receiving HRT, 3 = pre-menopausal women not receiving HC, 4 = post-menopausal HRT users, and 5 = pre-menopausal HC users). The p value shown is for the association between NT-proBNP and sex/hormone status overall; for pairwise comparisons within sex/hormone status groups, please refer to the text. (B) Bivariate plot showing the age-adjusted least squares means and SE of log free testosterone (FT) (horizontal axis) and log NT-proBNP (vertical axis) in each sex/hormone status group. (C) Bivariate plot showing the age-adjusted least squares means and SE of log sex hormone-binding globulin (SHBG; horizontal axis) and log NT-proBNP (vertical axis) in each sex/hormone status group. Abbreviations as in Figure 1.

ing. In pairwise comparisons, men had lower plasma NT-proBNP levels than women regardless of menopause status or hormone therapy (Bonferroni-corrected $p < 0.0001$). Among pre-menopausal women, levels of NT-proBNP were higher in those receiving HC (Bonferroni-corrected $p < 0.0001$). Among post-menopausal women, there was no difference in circulating NT-proBNP levels between current HRT users and nonusers (multivariable-adjusted $p = 0.39$). In additional analyses including an interaction term between year of blood collection and sex, there was no evidence for an interaction.

Association among circulating NT-proBNP, FT, and SHBG levels. As expected, men had the highest FT concentrations, whereas women receiving HC had the lowest FT and highest SHBG concentrations. Across sex/hormone status groups, FT levels decreased and SHBG levels increased in tandem with increasing NT-proBNP levels (Figs. 2B and 2C).

In sex-stratified analyses, NT-proBNP levels were lower in the highest quartiles of FT in both men (Fig. 3A) and women (Fig. 3B). Log NT-proBNP was inversely related to log FT and directly related to log SHBG in both men and women (Table 2). After adjustments for age, body mass index, systolic blood pressure, diastolic blood pressure, serum creatinine, antihypertensive medications, diabetes, and smoking, the association between log NT-proBNP and log FT was significant in women ($p < 0.0001$) but not men ($p = 0.78$). Each unit increase in log FT was associated with a 20% decrease in NT-proBNP levels among women, adjusting for clinical covariates. Results were similar after further adjustments for HOMA-IR among participants without diabetes. In both sexes, log NT-proBNP was related to log SHBG in multivariable analyses ($p < 0.0001$). Each unit increase in log SHBG was associated with a 19% increase in NT-proBNP levels among men and a 40% increase in NT-proBNP levels among women, adjusting for clinical covariates. There was no interaction between menopause or hormone status and log FT or log SHBG.

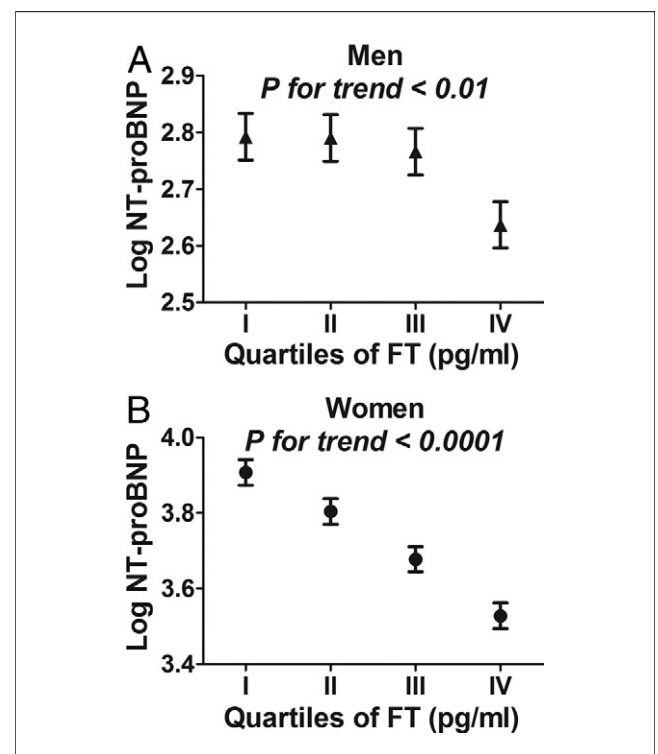


Figure 3 Association Between Circulating NT-proBNP and FT Levels

Least squares means and SE of log NT-proBNP are shown for each sex-specific quartile (I to IV) of FT in men (triangles; A) and women (circles; B), respectively. Abbreviations as in Figure 2.

Table 2 Association Among Circulating NT-proBNP, Testosterone, and SHBG

Association With Log NT-proBNP	Men		Women	
	Beta* (SE)	p Value	Beta* (SE)	p Value
Entire sample (single variable)				
Log FT	-0.30 (0.11)	0.007	-0.54 (0.05)	<0.0001
Log SHBG	0.23 (0.03)	<0.0001	0.23 (0.02)	<0.0001
Entire sample (multivariable)†				
Log FT	-0.03 (0.11)	0.78	-0.47 (0.06)	<0.0001
Log SHBG	0.12 (0.03)	<0.0001	0.25 (0.02)	<0.0001
Nondiabetic patients only (multivariable)‡				
Log FT	-0.05 (0.12)	0.65	-0.45 (0.06)	<0.0001
Log SHBG	0.13 (0.03)	0.0001	0.24 (0.02)	<0.0001

*Beta coefficients represent the mean change in log NT-proBNP per 1 SD difference in log FT or log SHBG; For single variable analyses, n = 1,880 in men (both models), 2,144 in women (model containing log FT), and 2,147 in women (model containing log SHBG). †Adjusted for age, body mass index, systolic blood pressure, diastolic blood pressure, serum creatinine, antihypertensive medications, diabetes mellitus, and smoking; n = 1,871 in men (both models), 2,123 in women (model containing log FT), and 2,126 in women (model containing log SHBG). ‡Adjusted for age, body mass index, systolic blood pressure, diastolic blood pressure, serum creatinine, antihypertensive medications, smoking, and HOMA-IR; n = 1,650 in men (both models), 1,857 in women (model containing log FT), and 1,859 in women (model containing log SHBG).

Abbreviations as in Table 1.

In the entire sample, sex/hormone status explained 38% of the variability in NT-proBNP levels in multivariable models adjusted for age, body mass index, systolic blood pressure, diastolic blood pressure, serum creatinine, and presence of antihypertensive medications, diabetes mellitus, and smoking. This effect was larger than the contribution of any other clinical covariate. The addition of log FT to multivariable models adjusted for age, body size, and cardiovascular risk factors led to attenuation of the differences in log NT-proBNP among sex/hormone status groups (Table 3). After log FT was added to the multivariable model, clinical sex/hormone status only explained 1% of the variability of NT-proBNP levels.

In women alone, the addition of log FT to multivariable models similarly led to attenuation of the differences in log NT-proBNP levels among menopause or hormone therapy

groups (Table 3). Menopause and hormone status explained 14% of the variability in NT-proBNP levels in women and in multivariable models adjusted for clinical covariates but only 2% after log FT was added to the multivariable model. Thus, the vast majority of the variability in NT-proBNP due to menopause and hormone status in women appeared to be attributable to differences in FT concentrations.

In analyses restricted to participants not receiving any antihypertensive therapy, the associations among sex/hormone status, NT-proBNP, and FT were unchanged (not shown).

Subgroup analysis by menstrual phase. Among 546 premenopausal women with regular menstrual cycles in the absence of HC (Table 4), NT-proBNP levels were lower in the midcycle phase than in the follicular or luteal phase (p = 0.014 for midcycle vs. follicular phase; p = 0.015 for midcycle vs. luteal phase; Bonferroni-corrected p < 0.05 for

Table 3 Multivariable Models Assessing the Association Among Sex/Hormone Status, Androgens, and NT-proBNP Concentrations

Association With Log NT-proBNP	Model Without FT		Model With FT	
	Beta* (SE)	p Value	Beta* (SE)	p Value
Entire sample (N = 3,484)				
Men	-1.12 (0.05)	<0.0001	-0.51 (0.14)	0.0004
Post-menopausal women without HRT	-0.46 (0.09)	<0.0001	-0.35 (0.09)	0.0001
Pre-menopausal women without HC	-0.25 (0.05)	<0.0001	-0.14 (0.05)	0.008
Post-menopausal women with HRT	-0.34 (0.14)	0.015	-0.24 (0.14)	0.083
Pre-menopausal women with HC	Referent	Referent	Referent	Referent
Log FT	—	—	-0.27 (0.06)	<0.0001
Women only (n = 1,613)				
Post-menopausal women without HRT	-0.35 (0.09)	0.0001	-0.17 (0.10)	0.073
Pre-menopausal women without HC	-0.22 (0.05)	<0.0001	-0.05 (0.06)	0.385
Post-menopausal women with HRT	-0.25 (0.14)	0.069	-0.09 (0.14)	0.499
Pre-menopausal women with HC	Referent	Referent	Referent	Referent
Log FT	—	—	-0.43 (0.07)	<0.0001

*Beta coefficients represent the mean difference in log NT-proBNP in the corresponding sex/hormone status group compared with that in premenopausal women receiving hormonal contraceptives (HC) (referent group) or mean change in log NT-proBNP per 1 SD difference in log FT, adjusted for age, body mass index, systolic blood pressure, diastolic blood pressure, serum creatinine, antihypertensive medications, diabetes mellitus, and smoking.

HC = hormonal contraceptives; HRT = hormone replacement therapy; other abbreviations as in Table 1.

Table 4 Subgroup Analysis by Menstrual Phase

Multivariable-Adjusted* Least-Squares Mean (SE)	Menstrual Phase			p Value		
	Follicular (n = 262)	Midcycle (n = 70)	Luteal (n = 214)	Follicular vs. Midcycle	Follicular vs. Luteal	Luteal vs. Midcycle
Log NT-proBNP	3.71 (0.05)	3.45 (0.09)	3.71 (0.05)	0.014	0.97	0.015
Log FT	0.91 (0.03)	1.05 (0.06)	0.93 (0.04)	0.045	0.642	0.100
Log SHBG	4.33 (0.03)	4.38 (0.05)	4.33 (0.03)	0.348	0.943	0.386

*Adjusted for age, body mass index, systolic blood pressure, diastolic blood pressure, serum creatinine, antihypertensive medications, diabetes mellitus, and smoking. Abbreviations as in Table 1.

both) in multivariable models. There was a corresponding trend toward higher FT levels in the midcycle phase compared with that in the follicular or luteal phase ($p = 0.045$ for midcycle vs. follicular phase; $p = 0.099$ for midcycle vs. luteal phase). When log FT was added to multivariable models, the association between menstrual phase and log NT-proBNP became nonsignificant. There was no association between SHBG levels and menstrual phase.

Discussion

Principal findings. In a large sample of men and women from the general community, sex and exogenous hormone therapy were the largest determinants of variation in circulating NT-proBNP levels. Men had lower plasma NT-proBNP concentrations than women regardless of menopause status or hormone therapy, whereas women receiving HC had higher NT-proBNP concentrations than women without hormone therapy. Among sex/hormone status groups, men also had the highest FT and lowest SHBG concentrations; conversely, women receiving HC had the lowest FT and highest SHBG concentrations. In both sexes, increasing NT-proBNP levels were related to decreasing FT and increasing SHBG concentrations. Accounting for FT greatly attenuated the differences in circulating NT-proBNP levels among sex/hormone status groups, even after adjustments for known clinical covariates and restricting analyses to women. These findings are consistent with the hypothesis that androgens suppress NT-proBNP and suggest that differences in FT concentrations may largely explain the sex- and hormone-related differences in circulating NPs.

Androgen regulation of NP concentrations. Several lines of evidence suggest that testosterone may exert a suppressive effect on the NP system and thus mediate a “BNP deficiency” in men compared with women. In male rats, orchiectomy produced marked increases in plasma NP levels, and testosterone replacement restored NP concentrations to baseline (14). In isolated perfused rat atria, testosterone suppressed volume-stimulated release of atrial NP (13). However, contradictory results have also been reported regarding the effect of testosterone on atrial NP gene expression (31) and synthesis in cultured rat myocytes (32). In clinical studies, inverse correlations between BNP and FT levels have been observed in women in the Dallas Heart Study (8) and in a small Japanese study (16). Androgen

receptor antagonism for prostate cancer has been associated with large increases in levels of NT-proBNP (33). In male children and adolescents, associations of decreasing FT, increasing SHBG, and increasing NT-proBNP levels have been noted (15).

Our findings extend prior observations to a large community-based cohort of middle-aged men and women. The demonstrated association between NT-proBNP and androgens offers a potential unifying explanation for sex and hormonal status-related differences in NP concentrations. In men, low BNP concentrations may be related to the suppressive effects of high concentrations of FT. A nonlinear relationship with NT-proBNP at such high concentrations of FT may explain the failure to detect a statistically significant relationship between FT and NT-proBNP in men following multivariable adjustment. In women, FT concentrations are more than 50-fold lower than that in men and are exquisitely sensitive to SHBG concentrations. **Estrogen regulation of NP concentrations.** Previous studies have also shown that estrogens may exert a stimulatory effect on the NP system (11). In female rats, pretreatment with estradiol and progesterone stimulated atrial NP gene expression (31). In post-menopausal women, administration of estrogens produced a rise in plasma levels of BNP (9). A complex interplay of factors has been postulated, in which estrogens modulate NP production via known effects on the renin-angiotensin system (12).

Measurements of estradiol and estrone were not available for the current study, limiting our conclusions regarding the role of estrogens in mediating the observed difference in NT-proBNP levels. However, according to the hypothesis of a stimulatory effect of estrogens on NPs, we would have expected post-menopausal women and men to have similar NT-proBNP levels given their similar estrogen levels, yet NT-proBNP concentrations were much higher in the former. Similarly, based on higher endogenous estrogens in pre-menopausal than post-menopausal women, we would have expected higher NT-proBNP levels in the former, but levels were similar in the 2 groups. Lastly, given that the midcycle (ovulatory) phase of the menstrual cycle is associated with higher estrogen levels compared with the follicular or luteal phases, higher NT-proBNP levels would be expected midcycle; however, levels were paradoxically lower in women at midcycle. Interestingly, each of the above observations can potentially be explained by variation in FT, which is highest in men, comparable in pre- and post-

menopausal women, and higher during the midcycle phase than the follicular or luteal phase. These findings are consistent with prior studies showing that circulating NT-proBNP concentrations are not correlated with measured estradiol concentrations in females (15,16).

Nonetheless, in the absence of direct measurements of estrogens, a role for estrogen-stimulated increases in NT-proBNP cannot be excluded. An explanation based purely on androgen suppressive effects would be inadequate to account for the known rapid fall in NP concentrations during the first year of life or the similar levels of NT-proBNP in male adolescents and male pre-pubertal children (34). In aggregate, it is likely that both the stimulatory effects of estrogens and inhibitory effects of testosterone contribute to the regulation of BNP concentrations during the life course. The relative concentrations of these sex hormones may also be an important factor. Additional population-based studies including pre- and post-pubertal individuals of both sexes are warranted.

Circulating NPs and use of hormone therapies. Previous studies examining the impact of HRT on BNP in postmenopausal women have produced conflicting results (6,8–10), and none have examined the impact of HC on circulating BNP in pre-menopausal women. Our study indicates that usage of HC in pre-menopausal women is associated with higher circulating NT-proBNP levels compared with no usage. This may be due to direct stimulatory effects of estrogens on the NP system, a reduction in FT-mediated suppression of NT-proBNP secondary to increased SHBG from oral estrogens, or indirect effects of oral estrogens and progestins acting via the renin-angiotensin system to modulate NP levels (12). Consistent with previous studies (8,16), we did not detect a significant association between naturally occurring menopause and NT-proBNP concentrations after accounting for age, although the relatively small number of post-menopausal women in our middle-aged sample may have limited our statistical power to detect a difference. We were similarly unable to demonstrate a significant association between usage of HRT and circulating NT-proBNP, in contrast to findings from Olmsted County (6). This could be due to small numbers of HRT users in our current sample because we included younger women who were recruited following publication of the Women's Health Study and the nationwide reduction in HRT prescription rates (2,35). Of note, the effects of exogenous female sex hormone therapies are known to vary with the route of administration (36), formulation (37), and composition; for example, progestogens in HC may exert both androgenic and antiandrogenic effects (38). These details were not available in our study but represent important areas for future study.

Study strengths and limitations. Strengths of this study include the large sample size, community-based design, careful recording of menstrual history, standardized examinations with routine blood collection, and uniform ascertainment of cardiovascular risk factors including IR. Measurements of estrogens and details regarding the individual

hormone components of HC were not available. The accurate detection of an ovulatory cycle or hormonal changes in reproductive aging in women requires specific measurements of female sex hormones and gonadotropins. A role for estrogen-stimulated increases in circulating BNP cannot be excluded based on these data. FT was not directly measured, but estimated FT concentrations from total testosterone (by mass spectrometry) and SHBG (by radioimmunoassay) correlate well with direct measurements by equilibrium dialysis (39–42). Biologically active atrial NP (43) and BNP (44) provide physiologically meaningful information but are less practical for measurement in large, ambulatory cohorts composed of predominantly healthy individuals, in part because of the high proportion of values censored by the detection limit of the mature NP assays (4,5). We acknowledge the potential for residual confounding by unmeasured comorbidities and their pharmacological therapies, as well as the limited ability to draw conclusions regarding causality from these observational data. Nonetheless, our findings are consistent with experimental data on the effects of testosterone on NPs.

Conclusions

Circulating NT-proBNP levels were related to sex and exogenous hormone therapy in men and women from the general community. Suppression of NPs by androgens may account for sex- and hormone-related differences in NT-proBNP concentrations. Given the known cardioprotective effects of BNP (45), further studies are warranted to elucidate how these mechanisms may contribute to the well-described sex-related differences in cardiovascular risk.

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