Published in final edited form as:

J Am Coll Cardiol. 2019 March 26; 73(11): 1288–1296. doi:10.1016/j.jacc.2018.12.062.

Effect of Testosterone on Natriuretic Peptide Levels

Katherine N. Bachmann, MD, MSCI[1,2,3], **Shi Huang, PhD**[3,4], **Hang Lee, PhD**[5], **Laura E. Dichtel, MD, MHS**[6], **Deepak K. Gupta, MD, MSCI**[3,8], **John C. Burnett Jr., MD**[7], **Karen K. Miller, MD**[6], **Thomas J. Wang, MD**[*,3,8], and **Joel S. Finkelstein, MD**[9,*]

[1]Veterans Administration Tennessee Valley Healthcare System, Nashville, Tennessee;

[2]Division of Diabetes, Endocrinology, and Metabolism, Department of Medicine, Vanderbilt University Medical Center, Nashville, Tennessee;

[3]Vanderbilt Translational and Clinical Cardiovascular Research Center, Vanderbilt University School of Medicine, Nashville, Tennessee;

[4]Department of Biostatistics, Vanderbilt University Medical Center, Nashville, Tennessee;

[5]Biostatistics Center, Massachusetts General Hospital, Boston, Massachusetts;

[6]Neuroendocrine Unit, Massachusetts General Hospital/Harvard Medical School, Boston, Massachusetts;

[7]Cardiorenal Research Laboratory, Department of Cardiovascular Medicine, Mayo Clinic, Rochester, Minnesota;

[8]Division of Cardiovascular Medicine, Department of Medicine, Vanderbilt University Medical Center, Nashville, Tennessee;

[9]Endocrine Unit, Department of Medicine, Massachusetts General Hospital/Harvard Medical School, Boston, Massachusetts

Abstract

Background: Circulating natriuretic peptide (NP) levels are markedly lower in healthy men than women. A relative NP deficiency in men could contribute to their higher risk of hypertension and cardiovascular disease. Epidemiologic studies suggest testosterone may contribute to sex-specific NP differences.

Corresponding Author: Katherine N. Bachmann, M.D., M.S.C.I., Division of Diabetes, Endocrinology, and Metabolism, Vanderbilt University Medical Center, 2213 Garland Avenue, MRB IV Suite 7465, Nashville, Tennessee 37232, Telephone: 615-875-9520, Fax: 615-936-1667, Katherine.n.bachmann@vanderbilt.edu, Twitter: @VUMC_heart.

[*]T.J.W. and J.S.F. contributed equally to this work.

Disclosures: J.S.F. received an investigator-initiated grant from AbbVie Inc. Also, AndroGel was provided by AbbVie Inc. Zoladex and Arimidex were provided by AstraZeneca Pharmaceuticals LP. The remaining authors have nothing to disclose.

Tweet: Gender differences in heart health. New study finds testosterone lowers levels of natriuretic peptides, which are hormones that protect the heart.

Clinical Trial Registration: https://ClinicalTrials.gov, NCT00114114 (Joel S. Finkelstein).

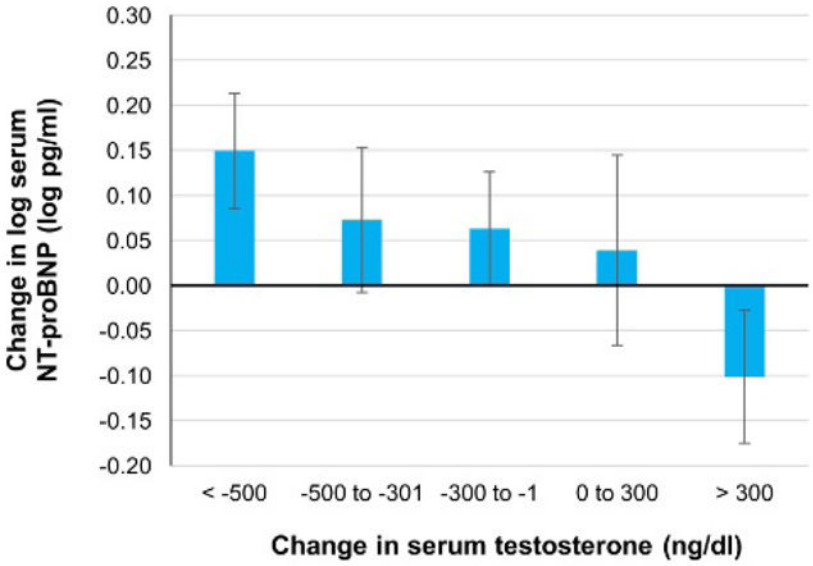
Objectives: We aimed to determine the effect of testosterone administration on NP levels using a randomized, placebo-controlled design.

Methods: 151 healthy men (aged 20–50 years) received goserelin acetate to suppress endogenous production of gonadal steroids, and anastrazole to suppress conversion of testosterone to estradiol. Subjects were randomized to placebo gel or 4 different doses of testosterone (1%) gel for 12 weeks. Serum N-terminal-pro-B-type natriuretic peptide (NT-proBNP) and total testosterone levels were measured at baseline and follow-up.

Results: Men who did not receive testosterone replacement (placebo gel group) after suppression of endogenous gonadal steroid production experienced a profound decrease in serum testosterone (median 540 to 36 ng/dl, p<0.0001). This was accompanied by an increase in median NT-proBNP (+8 pg/ml, p=0.02). Each 1 g increase in testosterone dose was associated with a 4.3% lower NT-proBNP at follow-up (95% confidence interval, −7.9% to −0.45%; p=0.029). An individual whose serum testosterone decreased by 500 ng/dl had a 26% higher predicted follow-up NT-proBNP than someone whose serum testosterone remained constant.

Conclusions: Suppression of testosterone production in men led to increases in circulating NT-proBNP, which were attenuated by testosterone replacement. Inhibition of NP production by testosterone may partly explain the lower NP levels in men.

Graphical Abstract



CENTRAL ILLUSTRATION: Changes in N-Terminal Pro–B-Type Natriuretic Peptide Levels With Respect to Changes in Serum Testosterone Levels

Central Illustration. Changes in NT-proBNP levels with respect to changes in serum testosterone levels. Changes in log serum NT-proBNP levels, with respect to changes in serum testosterone levels, from week 0 to week 12 are displayed. NT-proBNP, N-terminal pro b-type natriuretic peptide.

Condensed Abstract:

Natriuretic peptide (NP) levels are lower in men than women. These sex-related differences may be clinically important, since NPs appear to exert cardioprotective effects. Observational studies suggest testosterone may contribute to sex-specific NP differences, but randomized studies are limited. In this randomized, placebo-controlled trial, men underwent suppression of endogenous gonadal steroids and randomization to placebo gel or 4 different testosterone doses. Men receiving placebo gel experienced profound decreases in testosterone levels, accompanied by increases in NP levels. Higher testosterone doses were associated with lower follow-up NP levels. Inhibition of NP production by testosterone may contribute to sex-specific NP differences.

Keywords

natriuretic peptide; BNP; NT-proBNP; testosterone; hypertension; cardiovascular risk

Introduction

The prevalence of hypertension and cardiovascular disease is higher in men compared with women throughout most of the lifespan. However, the reasons for this are not well established. The cardiac natriuretic peptide (NP) system plays a major role in salt balance, blood pressure homeostasis, and cardiac remodeling. Atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) promote natriuresis, vasodilation, and inhibition of the renin-angiotensin-aldosterone system (1–3), leading to reduction of blood pressure. The protective role of the NP system in cardiovascular physiology is supported by a large body of evidence from experimental studies and human genetic investigations (4–6).

Interestingly, circulating NP levels are approximately 40% lower in healthy men compared with healthy women (7). This makes sex the single largest determinant of inter-individual variability in NP levels in healthy individuals (7). This phenomenon raises the possibility that a relative “NP deficiency” in men might contribute to their higher propensity to develop hypertension and cardiovascular disease.

Nonetheless, the reasons for sex-specific differences in NP levels are not firmly established. Cross-sectional data from epidemiologic studies suggest that testosterone might account for some of the differences in NP levels between the sexes (8,9). For instance, in a cross-sectional analysis of participants in the Framingham Heart Study, free testosterone concentrations were negatively associated with N-terminal pro B-type natriuretic peptide (NT-proBNP) levels in both men and women (8). In contrast, results of prior investigations on the association of estrogen concentrations with NPs have been inconclusive (8,10,11).

Moreover, data from human physiologic studies involving testosterone administration are limited, and confined to individuals with significant co-morbid conditions that might separately influence NP levels (12–14). Also, one of the challenges of evaluating the effects of testosterone supplementation is that testosterone undergoes conversion to serum estradiol via aromatization. This has made it difficult in prior studies to analyze the effects of serum testosterone, independent of estradiol, on the NP system. Therefore, we investigated the effect of testosterone replacement on NP levels in a randomized, placebo-controlled study, consisting of healthy men pre-treated with a gonadotropin releasing hormone (GnRH)

agonist to suppress endogenous production of gonadal steroids (testosterone and estradiol). Participants also received an aromatase inhibitor to suppress the conversion of testosterone to estradiol.

Methods

Subjects

Healthy men, aged 20 to 50 years, without a history of significant cardiac, renal, hepatic, or pulmonary disease, malignancy, or hyperthyroidism were enrolled in the study. Subjects taking testosterone, estrogens, or systemic glucocorticoids at the time of the screening visit were excluded. Full inclusion/exclusion criteria, subject clinical characteristics, testosterone levels, and estradiol levels have been published previously (15,16). The study protocol was approved by the Partners Institutional Review Board, and written informed consent was obtained from all participants.

Study design

This was a post-hoc analysis of a randomized, placebo-controlled trial; the design of the parent trial has been previously described (15,16). All subjects received goserelin acetate (Zoladex, provided by AstraZeneca) 3.6 mg subcutaneously at baseline and every 4 weeks throughout the study in order to suppress endogenous production of testosterone and estradiol, as well as anastrazole (Arimidex, provided by AstraZeneca) 1 mg daily in order to inhibit the aromatization of testosterone to estradiol. Subjects were randomly assigned to one of five testosterone replacement doses: 0 g (placebo gel), 1.25 g, 2.5 g, 5 g, or 10 g of a topical 1% testosterone gel (AndroGel, provided by AbbVie Inc.) daily. Originally, 202 men were enrolled and underwent randomization. 161 men completed the week 12 visit (15). NT-proBNP levels were available at week 0 (baseline, prior to receiving any study medications) and week 12 in 151 subjects. Because our primary endpoint was NT-proBNP levels, only those 151 subjects with NT-proBNP levels available at both baseline and week 12 are included in the analysis. The group of 151 subjects had similar baseline clinical characteristics and hormone levels as the entire cohort that underwent randomization.

Hormone measurements

During the trial, blood was collected, immediately processed for plasma and serum, and aliquoted. NT-proBNP was measured from serum samples that had been stored at −80 degrees Celsius for 5–11 years and had undergone no more than one prior thaw cycle. Serum NT-proBNP levels were measured using an automated double-incubation assay on the Roche Cobas e411 (Roche Diagnostics, Indianapolis, IN) with an interassay coefficient of variation <8%. The lower limit of detection of this assay was 5 pg/ml; levels below the lower limit of detection were assigned a value of 4 pg/ml. We elected to measure NT-proBNP rather the mature peptide (BNP, or C-terminal BNP), because NT-proBNP has a longer half-life in the circulation, and, thus, displays less intra-individual variability. Serum estradiol levels had been stored at −80 degrees Celsius, and were measured by liquid chromatography–tandem mass spectroscopy, with a threshold for detection of 1.25 pg/ml. Serum total testosterone levels were measured in real-time by a solid-phase chemiluminescent immunoassay with the use of an automated analyzer (ADVIA Centaur XP, Siemens) with a sensitivity of 20 ng/dl.

Free testosterone concentration was available in 90 of 151 subjects, and was estimated using total testosterone concentration, SHBG, and albumin using a previously validated method (17). We analyzed NT-proBNP and testosterone levels at 12 weeks in order to allow the testosterone levels to achieve an equilibrium after treatment with goserelin and testosterone gel. Goserelin can lead to transient increases in testosterone levels in the first 2–4 weeks after initiation. Further if increases in testosterone did occur early in response to goserelin, this should be balanced between groups due to the randomized design.

Medical comorbidities

Status of cardiovascular risk factors at baseline are reported. Hypertension was defined by the use of antihypertensive medications, or a systolic blood pressure ≥ 140 mmHg, or a diastolic blood pressure ≥ 90 mmHg at baseline. Dyslipidemia was defined by the use of dyslipidemia prescription, or LDL ≥ 190 mg/dl, at baseline. Diabetes was defined by use of antidiabetes medication at baseline, or baseline fasting glucose ≥ 126 mg/dl. Pre-diabetes was defined by reported history of pre-diabetes, or baseline fasting glucose between 100–125 mg/dl.

Statistical Analysis

Clinical characteristic and hormone level data are reported as median, (lower quartile, upper quartile) for continuous variables and as percentages for categorical variables. Clinical characteristics and hormone concentrations at baseline were compared between the testosterone dose groups using the Kruskal-Wallis test for continuous variables and the Pearson Chi-square test for categorical variables. Changes in hormone concentrations (Figure 1; **Central Illustration**) are presented in box plots displaying the median, interquartile range, and upper and lower adjacent values.

The relationship of NT-proBNP with testosterone dose was analyzed using multivariable linear regression. Because the distribution of NT-proBNP was highly skewed, it was log-transformed before statistical analysis. First, multivariable linear regression was conducted, with Week 12 log NT-proBNP as the dependent variable, and with testosterone dose and baseline log NT-proBNP as the predictor variables. Testosterone dose was treated as a continuous predictor for analyses determining the effect of dose on outcomes. Next, we performed a second regression analysis that also included age, BMI, and race as predictors. We had pre-specified that we would adjust for age, BMI, and race, as these variables are known to be important determinants of NP levels in large population cohort studies (7,8,18–20). Next, to analyze the relationship of NT-proBNP levels with serum testosterone levels, we performed multivariable linear regression, using change in log serum NT-proBNP level (week 12 minus week 0 level) as the dependent variable, and using change in serum testosterone level, baseline serum testosterone level, and baseline log serum NT-proBNP as the covariates. We then performed a second regression analysis that also included age, BMI, and race as covariates. This model enabled us to calculate the relative difference of predicted NT-proBNP level at week 12 in an individual given a specified change in serum testosterone level, relative to an individual whose serum testosterone level remained constant.

In addition, we constructed a hypothesized path model using a structural equation model, in order to examine the effects of testosterone dose on NT-proBNP through serum testosterone levels. In this type of two-wave mediation analysis (21), the path coefficients are estimated simultaneously. The product of coefficients method (22) was employed to test whether serum testosterone levels mediated the effects of testosterone dose on NT-proBNP levels. For example, the product of the two pathways A*B (Figure 2) is the effect of testosterone dose on NT-proBNP levels as mediated by serum testosterone levels. Because the product of regression coefficients was usually not normally distributed, we employed 1000 bootstraps to obtain 95% confidence intervals of the product of regression coefficients (23). Mediation is assumed if the confidence interval for this product does not include zero.

Two-sided *p*-values <0.05 were considered statistically significant. Data for continuous variables in figures are reported as means and standard errors of the mean. R version 3.3.1 (R foundation of Statistical Computing, Vienna, Austria) was used for statistical analyses. K.N.B. had full access to all data presented in the current manuscript and takes responsibility for the data integrity and data analysis.

Results

Clinical Characteristics

At baseline, there were no significant differences in age, BMI, or race across testosterone dose groups (Table 1). In this cohort of subjects without significant cardiovascular disease, the occurrence of cardiovascular risk factors (smoking, hypertension, dyslipidemia, diabetes, and pre-diabetes) was low (Online Table 1) and balanced between testosterone dose groups. Similarly, use of medications that could influence natriuretic peptide levels through salt and water balance was low and evenly distributed across randomization groups (Online Table 2).

Changes in gonadal steroid levels

At baseline, there were no significant differences in gonadal steroid levels or NT-proBNP levels across testosterone dose groups (Table 1). As expected, men who did not receive testosterone replacement (placebo gel group) after suppression of endogenous gonadal steroid production experienced a profound decrease in serum testosterone levels (median 540.5 to 36 ng/dl, p<0.0001), with week 12 serum testosterone levels comparable to those found in women. In contrast, men who received 5 g or 10 g daily doses of testosterone replacement achieved week 12 serum testosterone levels comparable to those found in healthy men. Changes in serum testosterone from week 0 to week 12 are displayed in Figure 1A. Estradiol levels decreased significantly in all dose groups (p<0.0001) due to the aromatase inhibitor, as expected. Although week 12 estradiol levels were higher in men who received higher testosterone doses, final estradiol levels were very low in all men (<10 pg/ml) from a clinical perspective (Table 1).

Relationships between testosterone and natriuretic peptides

Men who received placebo gel experienced a significant increase in median NT-proBNP (+8 pg/ml, p= 0.02). Median changes in NT-proBNP were 0 (p= 0.8), +7 (p= 0.02), 0 (p= 0.5), and −2 (p= 0.41) pg/ml in men receiving 1.25 g, 2.5 g, 5 g, and 10 g of daily testosterone

replacement, respectively. Changes in NT-proBNP from week 0 to week 12 with respect to testosterone dose are shown in Figure 1B. Week 12 log NT-proBNP, adjusted for baseline level, had a marginally significant, negative association with testosterone dose (p= 0.05). After adjustment for baseline NT-proBNP, age, BMI, and race, week 12 log NT-proBNP had a significant, inverse association with testosterone dose. Each 1 g increment in testosterone dose was associated with a 4.3% lower NT-proBNP level at week 12 (95% confidence interval, −7.97% to −0.49%; p=0.028). These findings were attenuated when testosterone dose was analyzed as a categorical variable (5 dose groups; p=0.15) rather than as a continuous variable. Of note, black individuals had a higher proportion of week 12 NT-proBNP levels below the assay's lower limit of detection (<5 pg/ml) compared with non-blacks (35% vs. 15.6%, respectively, p = 0.035), which is consistent with findings in large cohort studies of lower NP levels in blacks (18,19). Because the proportion of detectable NT-proBNP levels was greater among non-blacks, we performed a subgroup analysis among these individuals. Among non-blacks (N = 128), Week 12 log NT-proBNP, adjusted for baseline level, had a significant negative association with testosterone dose (p = 0.011), which persisted after adjustment for age and BMI.

Because we postulated that the effect of testosterone supplementation on NT-proBNP would occur through changes in circulating testosterone levels, we examined the association between week 12 serum testosterone and NT-proBNP. At week 12, log serum testosterone had a significant inverse correlation with log NT-proBNP level (r = −0.20, p = 0.014). This association persisted after adjustment for baseline testosterone and NT-proBNP, age, BMI, and race (partial r = −0.23, p = 0.01). Also, the higher the serum testosterone level at week 12, the more likely that the week 12 BNP level was below the assay's lower limit of detection. An increment in week 12 serum testosterone by 500 ng/dl was associated with an odds ratio of 1.60 (95% confidence interval, 1.08–2.37; p=0.018) for having an undetectably low NT-proBNP level at week 12. These relationships remained significant after adjustment for baseline NT-proBNP and testosterone levels, age, BMI, and race.

Changes in NT-proBNP, with respect to changes in serum total testosterone levels, from week 0 to week 12 are displayed in the **Central Illustration**. Changes in serum total testosterone and NT-proBNP, controlling for baseline levels, were inversely associated with each other (p= 0.005); this association persisted after adjustment for age, BMI, and race (p= 0.004). In the subset of men with free testosterone levels available, similar results were obtained. In multivariable models derived from these data, an individual whose serum testosterone decreased by 500 ng/dl had a 23% higher predicted week 12 NT-proBNP level, relative to an individual whose serum testosterone remained constant.

Next, we constructed a conceptual path model to examine the effect of testosterone dose on NT-proBNP levels through dose's impact on circulating testosterone levels (Figure 2). The negative relationship of testosterone dose and NT-proBNP was due to dose's impact on serum testosterone levels, which had a negative association with NT-proBNP (p= 0.01). These relationships remained significant after adjusting for age, BMI, and race.

Discussion

We conducted a randomized controlled trial of men treated with GnRH agonists and allocated to various doses of testosterone replacement or placebo. We found that testosterone replacement was associated with lower NT-proBNP levels in a dose-dependent manner, and changes in circulating testosterone concentrations were inversely associated with changes in NT-proBNP levels. Our results support the hypothesis that testosterone reduces circulating NT-proBNP, consistent with the epidemiologic observation that men have lower circulating NP levels than women. Given the important role of the NPs in cardiovascular homeostasis, our data have potentially important implications for sex-related differences in the risk of hypertension and cardiovascular disease.

Comparison with prior studies

Several smaller studies have investigated the effect of testosterone supplementation on NP levels. In men with type 2 diabetes mellitus, high cardiovascular risk, and mildly low testosterone levels (≤346 ng/dl), those randomized to testosterone supplementation had decreases in NT-proBNP levels compared with placebo-treated men (13). In another study of 53 women with androgen deficiency due to hypopituitarism, those randomized to testosterone replacement experienced decreases in NT-proBNP levels compared with those randomized to placebo (12). In contrast, in a small nonrandomized study of men with metabolic syndrome and testosterone deficiency, testosterone supplementation over a shorter duration of 9 weeks was not associated with a change in NT-proBNP levels (14).

Aside from its substantially larger sample size, the present study differs from the prior investigations in several important respects. First, we focused on healthy men, because comorbidities such as diabetes mellitus or cardiovascular disease can influence the NP system independently. Prevalence of cardiovascular risk factors was low, and only a very small minority of subjects were taking medications that could potentially influence sodium homeostasis or renal function (Online Table 2). Second, the use of a GnRH agonist in all subjects, followed by randomization to multiple doses of testosterone, resulted in final circulating testosterone levels that spanned an extremely wide range (19 – 3,546 ng/dl). Across this extensive range, final circulating testosterone levels exhibited a negative relationship with final NT-proBNP levels, and furthermore, changes in circulating testosterone levels had a negative relationship with changes in NT-proBNP levels. Third, the concomitant use of an aromatase inhibitor reduced the confounding effect of estrogen, which increases with testosterone supplementation. The suppression of estrogen levels in our study differs from normal physiology, but allowed us the opportunity to determine the independent effects of testosterone on NP levels, which had been very challenging in prior studies.

Implications of findings

Importantly, our results lend insight into mechanisms underlying the well-documented sex-specific differences in NP levels (7,8). Serum total testosterone levels range from 325–800 ng/dl in healthy men (8,24), and 15–50 ng/dl in healthy women (8). With our protocol, we achieved a broad range of final circulating testosterone levels that encompassed the low levels observed in women as well as the higher levels observed in men. Our results suggest

that decreases in testosterone levels comparable to the differences between healthy men and women are associated with about 25% higher NT-proBNP levels. In observational studies, NP levels are about 30–60% higher in healthy women than men (7). Therefore, our findings suggest that the sex-specific differences in NP levels are explained partly, but not completely, by differences in testosterone levels between men and women.

Given that natriuretic peptides have been found to protect against hypertension and adverse cardiac remodeling (5), the sex disparities in circulating NP levels have important implications for cardiovascular health. However, relating absolute changes in NT-proBNP levels to biological actions is challenging, because the natriuretic peptide hormones are regulated by a negative feedback loop, so circulating levels represent the net effect of efferent and afferent influences on the heart. Further, testosterone may be only one factor affecting production and secretion of natriuretic peptides from the heart. That said, Mendelian Randomization studies involving genetic variants affecting NP levels suggest that relative changes in circulating NP levels as small as 10–20% can lead to clinically significant differences in the risk of hypertension (5). The changes in NP concentrations observed in the present study are substantially larger than those attributable to described common genetic variants.

Based on our findings, we hypothesize that the lower NP levels in men reflect a decrease in NP production, rather than an increase in NP clearance. This is because NT-proBNP, in contrast to mature BNP, is not cleared by the NP clearance receptor or by neprilysin (25). Prior experimental data support that androgens may negatively regulate NP production. Testosterone suppresses ANP secretion induced by atrial distention in isolated perfused rat atria (26). Moreover, androgen suppression appears to stimulate NP production; orchiectomy in male rats leads to increases in atrial ANP contents and plasma ANP concentrations, and the latter is reversed by testosterone replacement (27). On the other hand, some studies in cardiomyocytes and rodents have not supported a negative effect of androgens on NP production (28,29).

Study limitations

Our study has a few limitations. First, approximately 25 percent of the NT-proBNP values were below the assay's lower limit of detection, which reduced our statistical power. This may partly explain why the association of testosterone dose and NT-proBNP was attenuated in models with testosterone dose as a categorical variable. Analyzing NT-proBNP as a dichotomous variable (detectable versus undetectable) yielded consistent, significant results. Also, in a sensitivity analysis excluding subjects with undetectably low NT-proBNP values, similar results were obtained. A second limitation is that circulating NP levels offer an indirect index of NP production, secretion, and clearance. Unfortunately, there is no direct way to fully interrogate the NP system in humans at the present time. Third, serum NT-proBNP levels were not available at both baseline and week 12 in all men who were originally randomized in the study. Because our primary endpoint was the change in NT-proBNP levels, we included only the 151 individuals who had NP levels at both baseline and week 12. These 151 individuals had similar baseline characteristics as the entire original cohort. Next, circulating levels of the mature natriuretic peptides, ANP and BNP, were not

measured. Notably, sex-related differences in the natriuretic peptides follow a consistent pattern for ANP, BNP, and their pro-peptides (7–9,30,31). Further, levels of the pro-peptides and mature peptides for ANP and BNP are highly correlated (32). While the Roche assay has cross-reactivity with circulating proBNP, both proBNP and NT-proBNP reflect natriuretic peptide production, rather than clearance, and NT-proBNP and mature BNP are produced from proBNP in a one-to-one ratio. Finally, the reduction in NP levels due to testosterone supplementation may have been partially offset by testosterone's tendency to cause salt and water retention, which would tend to bias our results toward the null.

Conclusions

Our findings suggest that testosterone reduces circulating NP levels and indicate that gonadal steroids may play a role in the development of a relative NP deficiency in men compared with women. Further investigation is needed to elucidate the mechanisms by which testosterone suppresses NP levels, such as whether testosterone influences the transcription of genes involved in NP production or regulation. Moreover, as the NP system exerts cardioprotective effects, future studies are warranted to determine whether addressing the relative NP deficiency in men can reduce cardiovascular risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Funding:

This research was supported by the National Institutes of Health [grant numbers R01 AG030545, K24 DK02759, M01-RR-01066, 1 UL1 RR025758, T32DK007061, T32DK007028, K24 HL092902, R01 HL102780, K23 DK113220]; an investigator-initiated grant from AbbVie Inc.; Vanderbilt University Medical Center Faculty Research Scholars award, and Career Development Award #IK2 CX001678 from the United States (U.S.) Department of Veterans Affairs Clinical Sciences Research and Development (CSR&D) Program. AndroGel was provided by AbbVie, Zoladex and Arimidex were provided by AstraZeneca Pharmaceuticals LP.

Abbreviations:

ANP	atrial natriuretic peptide
BNP	B-type natriuretic peptide
GnRH	gonadotropin releasing hormone
NP	nariuretic peptide
NT-proBNP	N-terminal pro B-type natriuretic peptide
T	testosterone

References

1. Clerico A, Iervasi G, Mariani G. Pathophysiologic relevance of measuring the plasma levels of cardiac natriuretic peptide hormones in humans. [Review] [125 refs]. Hormone & Metabolic Research 1999;31:487–498. [PubMed: 10569249]

2. de Bold AJ, Bruneau BG, Kuroski de Bold ML. Mechanical and neuroendocrine regulation of the endocrine heart. CardiovascRes 1996;31:7–18.

3. Levin ER, Gardner DG, Samson WK. Natriuretic peptides. [Review] [67 refs]. New England Journal of Medicine 1998;339:321–328. [PubMed: 9682046]

4. Vasan RS, Glazer NL, Felix JF et al. Genetic variants associated with cardiac structure and function: a meta-analysis and replication of genome-wide association data. JAMA 2009;302:168–78. [PubMed: 19584346]

5. Newton-Cheh C, Larson MG, Vasan RS et al. Association of common variants in NPPA and NPPB with circulating natriuretic peptides and blood pressure. Nat Genet 2009;41:348–53. [PubMed: 19219041]

6. Tamura N, Ogawa Y, Chusho H et al. Cardiac fibrosis in mice lacking brain natriuretic peptide. Proc Natl Acad Sci U S A 2000;97:4239–44. [PubMed: 10737768]

7. Wang TJ, Larson MG, Levy D et al. Impact of age and sex on plasma natriuretic peptide levels in healthy adults. AmJCardiol 2002;90:254–258.

8. Lam CS, Cheng S, Choong K et al. Influence of sex and hormone status on circulating natriuretic peptides. Journal of the American College of Cardiology 2011;58:618–26. [PubMed: 21798425]

9. Ying W, Zhao D, Ouyang P et al. Sex Hormones and Change in N-Terminal Pro-B-Type Natriuretic Peptide Levels: The Multi-Ethnic Study of Atherosclerosis. J Clin Endocrinol Metab 2018;103:4304–4314. [PubMed: 30137406]

10. Celik O, Sahin I, Celik N et al. Diagnostic potential of serum N-terminal pro-B-type brain natriuretic peptide level in detection of cardiac wall stress in women with polycystic ovary syndrome: a cross-sectional comparison study. Hum Reprod 2007;22:2992–8. [PubMed: 17855407]

11. Yasui T, Matsui S, Saijo A et al. Association of N-terminal pro B-type natriuretic peptide and sex hormone-binding globulin in non-obese peri- and postmenopausal women. Clin Chim Acta 2010;411:280–4. [PubMed: 19945451]

12. Lin E, McCabe E, Newton-Cheh C et al. Effects of transdermal testosterone on natriuretic peptide levels in women: a randomized placebo-controlled pilot study. Fertility and sterility 2012;97:489–93. [PubMed: 22137497]

13. Gianatti EJ, Hoermann R, Lam Q, Dupuis P, Zajac JD, Grossmann M. Effect of testosterone treatment on cardiac biomarkers in a randomized controlled trial of men with type 2 diabetes. Clin Endocrinol (Oxf) 2016;84:55–62. [PubMed: 26120052]

14. Poliwczak AR, Tylinska M, Broncel M. Testosterone therapy improves the heart rate turbulence without effect on NT-proBNP level in men with metabolic syndrome. Horm Metab Res 2014;46:116–9. [PubMed: 24062090]

15. Finkelstein JS, Lee H, Burnett-Bowie SA et al. Gonadal steroids and body composition, strength, and sexual function in men. The New England journal of medicine 2013;369:1011–22. [PubMed: 24024838]

16. Finkelstein JS, Lee H, Leder BZ et al. Gonadal steroid-dependent effects on bone turnover and bone mineral density in men. The Journal of clinical investigation 2016;126:1114–25. [PubMed: 26901812]

17. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 1999;84:3666–72. [PubMed: 10523012]

18. Gupta DK, Claggett B, Wells Q et al. Racial differences in circulating natriuretic peptide levels: the atherosclerosis risk in communities study. J Am Heart Assoc 2015;4.

19. Gupta DK, de Lemos JA, Ayers CR, Berry JD, Wang TJ. Racial Differences in Natriuretic Peptide Levels: The Dallas Heart Study. JACC Heart Fail 2015;3:513–519. [PubMed: 26071618]

20. Wang TJ, Larson MG, Levy D et al. Impact of obesity on plasma natriuretic peptide levels. Circulation 2004;109:594–600. [PubMed: 14769680]

21. MacKinnon DP, Goldberg L, Clarke GN et al. Mediating mechanisms in a program to reduce intentions to use anabolic steroids and improve exercise self-efficacy and dietary behavior. Prev Sci 2001;2:15–28. [PubMed: 11519372]

22. MacKinnon DP. Introduction to statistical mediation analysis. New York: Lawrence Erlbaum Associates, 2008.

23. Shrout PE, Bolger N. Mediation in experimental and nonexperimental studies: new procedures and recommendations. Psychol Methods 2002;7:422–45. [PubMed: 12530702]
24. Harman SM, Metter EJ, Tobin JD, Pearson J, Blackman MR, Baltimore Longitudinal Study of A. Longitudinal effects of aging on serum total and free testosterone levels in healthy men. Baltimore Longitudinal Study of Aging. J Clin Endocrinol Metab 2001;86:724–31. [PubMed: 11158037]
25. Hall C NT-ProBNP: the mechanism behind the marker. J Card Fail 2005;11:S81–3. [PubMed: 15948107]
26. Deng Y, Kaufman S. The influence of reproductive hormones on ANF release by rat atria. Life Sci 1993;53:689–96. [PubMed: 8355570]
27. Hwu CM, Tsai SC, Lau CP et al. Increased concentrations of atrial and plasma atrial natriuretic peptide in castrated male rats. Life Sci 1993;52:205–12. [PubMed: 8355561]
28. Hong M, Yan Q, Tao B et al. Estradiol, progesterone and testosterone exposures affect the atrial natriuretic peptide gene expression in vivo in rats. Biol Chem Hoppe Seyler 1992;373:213–8. [PubMed: 1534483]
29. Marsh JD, Lehmann MH, Ritchie RH, Gwathmey JK, Green GE, Schiebinger RJ. Androgen receptors mediate hypertrophy in cardiac myocytes. Circulation 1998;98:256–261. [PubMed: 9697826]
30. Lew J, Sanghavi M, Ayers CR et al. Sex-Based Differences in Cardiometabolic Biomarkers. Circulation 2017;135:544–555. [PubMed: 28153991]
31. Redfield MM, Rodeheffer RJ, Jacobsen SJ, Mahoney DW, Bailey KR, Burnett JC Jr. Plasma brain natriuretic peptide concentration: impact of age and gender. Journal of the American College of Cardiology 2002;40:976–82. [PubMed: 12225726]
32. Gegenhuber A, Struck J, Poelz W et al. Midregional pro-A-type natriuretic peptide measurements for diagnosis of acute destabilized heart failure in short-of-breath patients: comparison with B-type natriuretic peptide (BNP) and amino-terminal proBNP. Clin Chem 2006;52:827–31. [PubMed: 16543387]

CLINICAL PERSPECTIVES

Competency in Medical Knowledge:

Natriuretic peptides (NP) play a protective role in cardiovascular physiology. By reducing circulating NP levels, testosterone may contribute to relative NP deficiency in men, helping to explain gender-based differences in cardiovascular risk.

Translational Outlook:

Further research is needed to determine whether raising natriuretic peptide levels in men can decrease their risk of hypertension and cardiovascular disease.

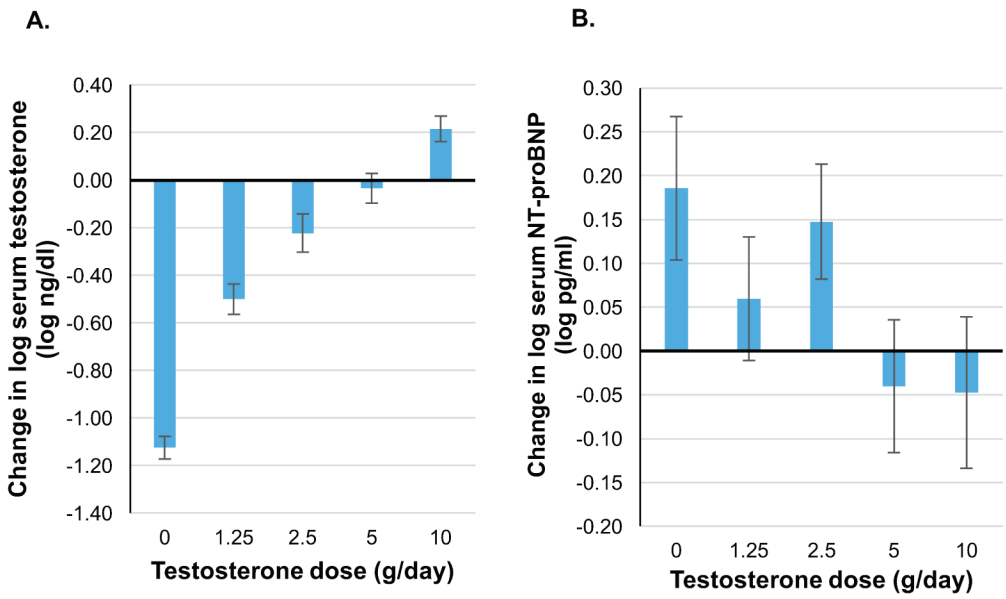
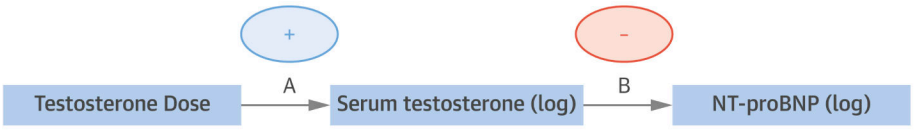


Figure 1. Changes in serum testosterone and NT-proBNP levels with respect to testosterone dose.
Changes in log serum testosterone levels (panel A) and changes in log serum NT-proBNP levels (panel B) from week 0 (baseline) to week 12 with respect to testosterone dose are displayed. NT-proBNP, N-terminal pro b-type natriuretic peptide.



"NT-proBNP (log)" = Week 12 log NT-proBNP level, adjusted for baseline NT-proBNP and log serum T
"Serum testosterone (log)" = Week 12 log serum testosterone level, adjusted for baseline level

Path	Description	Effect Size: Beta [95% CI]	SE	p-value
A	Effect of testosterone dose on log serum T	0.103 [0.086, 0.119]	0.009	< 0.001
B	Effect of log serum T on log NT-proBNP	-0.134 [-0.236, -0.038]	0.05	0.01
A*B	Effect of testosterone dose, as mediated by log serum T, on log NT-proBNP	-0.014 [-0.024, -0.003]	0.005	0.01

Figure 2. Conceptual Path Model: Testosterone dose impacts NT-proBNP levels through its effects on serum testosterone levels.

Higher testosterone doses led to higher serum testosterone levels at week 12 (Path A), which were associated with lower NT-proBNP levels at week 12 (Path B). The negative relationship of testosterone dose and NT-proBNP levels (Path A*B) was driven by the negative association of serum testosterone levels with NT-proBNP levels (Path B). These relationships remained significant after adjusting for age, BMI, and race. NT-proBNP, N-terminal pro b-type natriuretic peptide; SE, standard error; T, testosterone

Table 1.

Clinical characteristics and hormone levels

	Testosterone Dose (g/day)				
	0	**1.25**	**2.5**	**5**	**10**
	(N=22)	**(N=29)**	**(N=31)**	**(N=38)**	**(N=31)**
Age (years)	34.5 (28.5, 40.8)	32.0 (28.0, 39.0)	33.0 (26.0, 38.0)	33.5 (30.0, 38.0)	32.0 (30.0, 37.0)
Race, % White	68%	66%	65%	84%	84%
BMI (kg/m^2), Week 0	28.1 (25.7, 30.3)	27.2 (22.9, 30.9)	25.9 (23.4, 29.7)	27.3 (25.3, 30.4)	27.0 (24.4, 28.6)
BMI (kg/m^2), Week 12	28.0 (25.8, 30.2)	27.4 (24.5, 31.6)	26.5 (24.0, 30.1)	27.6 (26.2, 30.5)	27.4 (23.5, 29.9)
Systolic BP, mmHg, Week 0	122 (116, 127)	119 (114, 124)	118 (110, 124)	120 (116, 128)	114 (111, 121)
Diastolic BP, mmHg, Week 0	76 (71, 80)	74 (68, 78)	73 (66, 80)	77 (67, 81)	72 (68, 78)
Serum testosterone (ng/dl), Week 0	540 (437, 628)	562 (405, 636)	523 (383, 606)	467 (371, 619)	482 (386, 562)
Serum testosterone (ng/dl), Week 12	36 (32, 43)	160 (125, 251)	298 (184, 480)	536 (271, 724)	818 (502, 1027)
Serum estradiol (pg/ml), Week 0	33.5 (27.0, 40.0)	29.0 (24.0, 36.0)	28.0 (25.0, 34.5)	30.0 (23.3, 33.8)	25.0 (20.0, 33.0)
Serum estradiol (pg/ml), Week 12	0.9 (0.5, 1.1)	1.0 (0.8, 1.2)	1.1 (0.8, 1.8)	1.6 (1.3, 2.4)	2.0 (1.4, 2.7)
Serum NT-proBNP (pg/ml), Week 0	10.5 (4.0, 25.5)	15.0 (4.0, 31.0)	12.0 (4.0, 18.0)	11.5 (4.4, 42.5)	17.0 (4.0, 32.5)
Serum NT-proBNP (pg/ml), Week 12	19.5 (8.4, 30.5)	14.0 (7.9, 33.0)	19.0 (7.7, 32.5)	13.5 (5.2, 32.0)	12.0 (5.2, 24.5)

NS, not significant. NT-proBNP, N-terminal pro b-type natriuretic peptide. BP, blood pressure.