ORIGINAL RESEARCH

Associations Between the Cyclic Guanosine Monophosphate Pathway and Cardiovascular Risk Factors: MESA

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***Background*—**cGMP mediates numerous cardioprotective functions and is a potential therapeutic target for cardiovascular disease. Preclinical studies suggest that plasma cGMP is reflective of natriuretic peptide stimulation. Epidemiologic associations between cGMP and natriuretic peptide, as well as cardiovascular disease risk factors, are unknown.

***Methods and Results*—**We measured plasma cGMP in 542 men and 496 women free of cardiovascular disease and heart failure in MESA (Multi-Ethnic Study of Atherosclerosis). Cross-sectional associations of N-terminal pro-B type natriuretic peptide, sex hormones, and cardiovascular disease/heart failure risk factors with log(cGMP) were analyzed using multivariable linear regression models. Mean (SD) cGMP was 4.7 (2.6) pmol/mL, with no difference between the sexes. After adjusting for cardiovascular risk factors, N-terminal pro-B type natriuretic peptide was significantly positively associated with cGMP ($P<0.05$). Higher blood pressure and lower estimated glomerular filtration rate were associated with higher cGMP ($P<0.05$). Triglyceride levels, total/high-density lipoprotein cholesterol ratio, presence of diabetes mellitus, and the homeostatic model assessment of insulin resistance were inversely associated with cGMP ($P<0.05$). Among women, free testosterone and dehydroepiandrosterone were inversely associated with cGMP, while sex hormone binding globulin was positively associated ($P<0.05$).

***Conclusions*—**In a community-cohort, plasma cGMP was associated with natriuretic peptide signaling. Higher blood pressure and greater renal dysfunction were positively associated with cGMP, while adverse metabolic risk factors were inversely associated. Increased androgenicity in postmenopausal women was inversely associated with cGMP. These novel associations further our understanding of the role of cGMP in a general population. **(*J Am Heart Assoc*. 2019;8:e013149. DOI: 10.1161/JAHA.119.013149.)**

Key Words: cardiovascular disease risk factors • cGMP • epidemiology • N-terminal pro-B type NP • sex hormones

Cyclic guanosine monophosphate (cGMP) is an intracellular second messenger that mediates a vast array of beneficial processes in the cardiovascular system. It stimulates left ventricular relaxation, counters maladaptive hypertrophy and remodeling, and induces relaxation of endothelial and smooth muscle cells.[1–3] Dysfunctional cGMP signaling has been implicated in the pathogenesis of cardiovascular disease (CVD) and heart failure (HF). There is considerable interest in enhancement of the cGMP signaling pathway to generate novel therapeutic strategies for acute decompensated HF, HF with preserved ejection fraction, and pulmonary arterial hypertension.[4–7]

Plasma cGMP has been used as a biomarker in clinical studies of cGMP pathway augmentation as therapies for CVD and HF; however, there is a lack of epidemiologic data on circulating cGMP and its pathway regulators.[6,8] To date, much of our understanding of cGMP signaling stems from in vitro or animal studies[6,9] or from small clinical studies of patients with CVD or

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Accompanying Tables S1 through S4 are available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.119.013149

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Clinical Perspective

What Is New?

- Plasma cGMP was positively associated with N-terminal pro-B type natriuretic peptide in a large, multiethnic community-based cohort.
- Adverse metabolic risk factors and increased androgenicity were inversely associated with cGMP, while higher blood pressure and greater renal dysfunction were positively associated.

What Are the Clinical Implications?

- Plasma cGMP is associated with natriuretic peptide signaling and can be a biomarker of this pathway in clinical trials of cGMP pathway stimulators for therapies in heart failure and cardiovascular disease.

HF.[9–11] cGMP synthesis is regulated through 2 pathways: one mediated by nitric oxide activation of soluble guanylyl cyclase and the other by natriuretic peptide (NP) activation of particulate guanylyl cyclase (pGC).[9] Animal studies suggest that plasma cGMP levels may reflect only the NP-pGC pathway, as cGMP stimulated by the NP-pGC is accessible at the plasma membrane and transported into the extracellular space, whereas cGMP produced by the nitric oxide/soluble guanylyl cyclase pathway is found in the cytosol.[12,13] However, whether this extends to a general human population is unknown.

Additionally, components of the NP-cGMP pathway have been shown to be involved in vasodilation and blood pressure regulation, as well as hemodynamic autoregulatory mechanisms in the kidney.[14,15] NP-cGMP pathway dysregulation has been shown to be associated with states of hyperlipidemia, insulin resistance, and androgenicity.[16–18] Whether plasma cGMP is associated with these CVD and HF risk factors in a general population is unknown.

We therefore measured plasma cGMP concentrations in a subset of participants from MESA (Multi-Ethnic Study of Atherosclerosis), a community-based cohort of individuals free of clinical CVD and HF at baseline. We aimed to investigate whether circulating cGMP levels would be correlated with NT-proBNP (N-terminal pro-B type NP). We also assessed whether cGMP would be associated with key CVD/HF risk factors in an effort to provide context for prior and future clinical studies of cGMP pathway stimulation as therapeutic strategies for CVD and HF.

Materials and Methods

Study Population

MESA is a prospective cohort study investigating subclinical CVD and its progression to clinical CVD. It enrolled 6814 men and women of 4 self-reported races/ethnicities (white, black, Hispanic, and Chinese-American) from 6 centers across the United States who were 45 to 84 years old at baseline and free of clinical CVD/HF (2000–2002).[19] The institutional review boards of all participating institutions approved the study, and all participants provided written informed consent. Data from the MESA study are available through the National Heart, Lung, and Blood Institute's Biologic Specimen and Data Repository.[20]

As part of an ancillary study, plasma cGMP levels were newly measured in 647 women who met the following inclusion/exclusion criteria: (1) available sex hormone data, (2) available cardiac magnetic resonance imaging data, (3) women with questionnaire-confirmed postmenopausal status, (4) exclusion of women on hormone therapy, and (5) exclusion of participants without sufficient samples in the blood repository (Figure 1). A random sample of 647 men were also selected for analysis. Of the total 1294 participants, individuals were excluded if they had unmeasurable cGMP levels (n=3), were missing exposure variables or key covariates (n=236), or were determined to be premenopausal women (n=17) by a previously established algorithm.[18] Ultimately, 1038 participants were included in our analysis. Baseline characteristics of these participants were similar to those of all participants in MESA (Table S1).

Measurement of cGMP

Early-morning fasting blood samples from the baseline exam were stored at −70°C. Plasma cGMP levels were measured from these samples using a competitive ELISA assay (Cayman Chemical, Ann Arbor, MI) at the Atherosclerosis Clinical Research Laboratory at Baylor College of Medicine (Houston, TX). The range of detection was 0.23 to 30 pmol/mL. The intra- and interassay coefficients of variation for a control pool with a mean cGMP level of 6.5 pmol/mL were 4.2% and 13.5%, respectively.

Measurement of NT-proBNP

NT-proBNP levels were primarily measured using the Elecsys proBNP immunoassay (Roche Diagnostics Corporation, Indianapolis, IN) at the University of California San Diego and the University of Vermont.[18] Additional measurements were performed using the Cobas e601 (Roche Diagnostics Corporation, Indianapolis, IN) at the University of Maryland.[21] There was harmonization of the NT-proBNP assays across the different Roche platforms. The range of measurements was 5 to 35 000 pg/mL. The intra-assay and interassay coefficients of variation, respectively, were 2.7% and 3.2% at 175 pg/mL, 2.4% and 2.9% at 35 pg/mL, 1.9% and 2.6% at 1068 pg/mL, and 1.8% and 2.3% at 4962 pg/mL.[18]

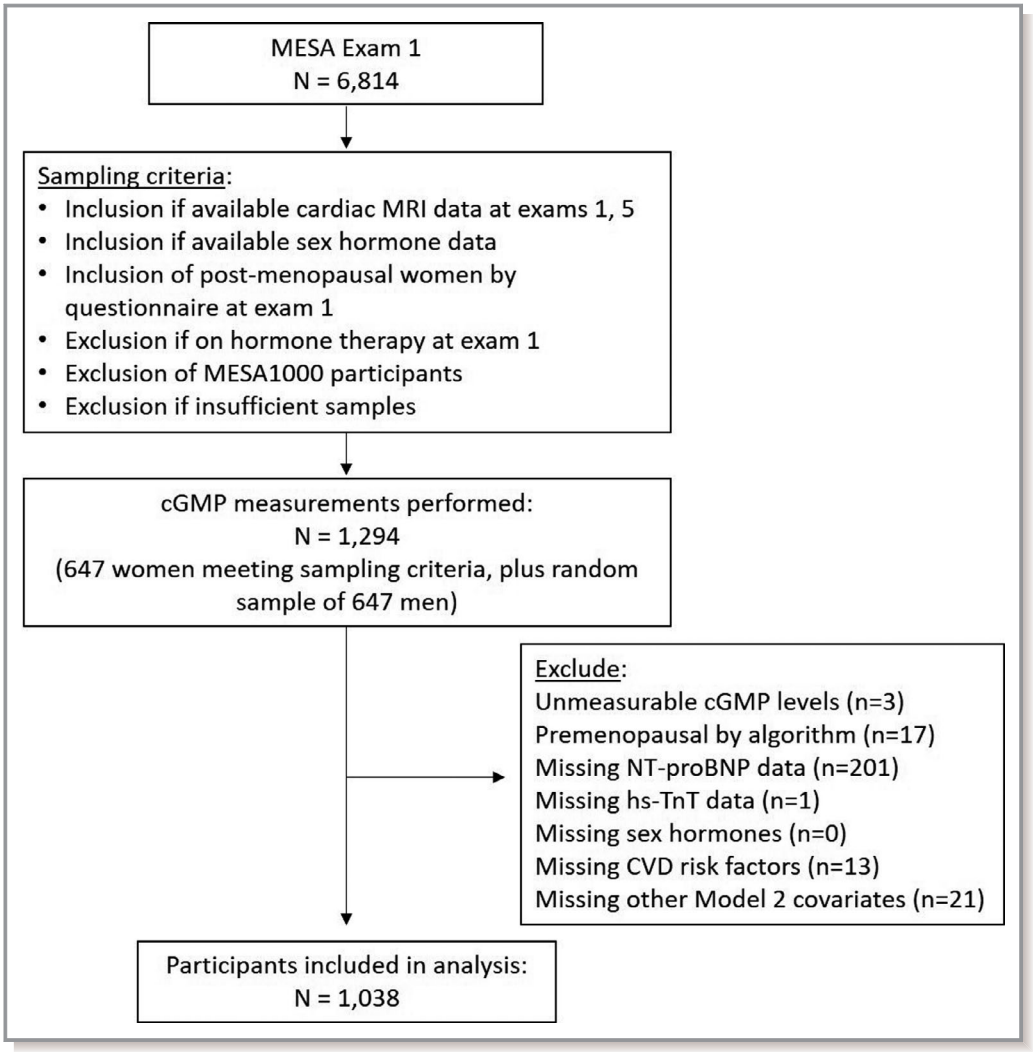

Figure 1. Selection of study sample. CVD indicates cardiovascular disease; hs-TnT, high-sensitivity cardiac troponin T; MESA, Multi-Ethnic Study of Atherosclerosis; MRI, magnetic resonance imaging; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

Measurement of Other Exposure Variables

Assessment of CVD risk factors was performed at the baseline exam using standardized questionnaires, physical exam, and laboratory measures, as described previously.[19] Hypertension was defined as systolic blood pressure (BP) ≥140 mm Hg, diastolic BP ≥90 mm Hg, or treatment with antihypertensive medication. Diabetes mellitus was defined as fasting glucose ≥126 mg/dL, self-reported diagnosis of diabetes mellitus, or treatment with hypoglycemic medication. Fasting total and high-density lipoprotein (HDL) cholesterol and triglycerides were measured at the Collaborative Studies Clinical Laboratory at Fairview–University Medical Center (Minneapolis, MN). Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation. The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using the equation: glucose (mmol/L)×insulin (mIU/L)/22.5. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation.[22]

Sex hormone measurements were performed at the Steroid Hormone Laboratory at the University of Massachusetts Medical Center (Worcester, MA) using serum samples stored at baseline.[18,23] Estradiol was measured using an ultrasensitive radioimmunoassay kit (Diagnostic System Laboratories, Webster, TX). Total testosterone and dehydroepiandrosterone (DHEA) were measured using radioimmunoassay kits, and sex hormone binding globulin (SHBG) was measured using the Immulite chemiluminescence enzyme immunometric assay (Diagnostic Products Corporation, Los Angeles, CA). Bioavailable testosterone was calculated using total testosterone and SHBG [total testosterone×(percent free testosterone×0.01)].[24] The intra-assay coefficients of variation were 10.5%, 12.3%, 11.2%, and 9.0% for estradiol, total testosterone, DHEA, and SHBG, respectively.[18]

Statistical Analysis

Baseline characteristics were summarized using means (standard deviation), medians (interquartile range), or percentages across tertiles of cGMP. Differences between groups were tested using ANOVA, Kruskal–Wallis, and chi-square tests as appropriate.

cGMP levels were log-transformed due to skewness and were modeled as the outcome variable for all assessments. Sex hormones, NT-proBNP, and homeostatic model assessment of insulin resistance values were also log-transformed due to skewness, as LOWESS plots demonstrated a more linear association with log(cGMP) when these exposure variables were modeled as log-transformed values. Multivariable-adjusted linear regression models were used to determine cross-sectional associations between sex hormones, NT-proBNP, and CVD risk factors with cGMP. Because β-coefficients of log-transformed values may be difficult to interpret, they were exponentiated and presented as ratios (95% CI) of the cGMP geometric means. Analyses involving the association of sex hormones with cGMP were stratified by sex, as sex hormone levels differ significantly between women and men, with nonoverlapping distributions. There were no significant interactions detected between sex and either NT-proBNP or the CVD risk factors (Table S2) in association analyses with cGMP. Squared partial correlations were calculated to determine the proportion of variance in cGMP that was explained by NT-proBNP, after accounting for the contribution from the other covariates. To assess for potential nonlinearity and to model a flexible dose-response relationship, we modeled free testosterone and NT-proBNP using restricted cubic splines (with knots at the 5th, 35th, 65th, and 95th percentiles of the free testosterone and NT-proBNP sample distributions).

We examined the association between NT-proBNP and cGMP using progressively adjusted models; selected covariates were determined a priori on the basis of potential confounding factors and intermediary variables determined by literature review. Model 1 adjusted for the demographic variables of age, sex, and race/ethnicity. Model 2 additionally adjusted for socioeconomic and behavioral/lifestyle factors of body mass index, education, smoking, and physical activity. Model 3 adjusted for Model 2 plus additional CVD risk factors of systolic BP, use of antihypertensive medication, total cholesterol, use of lipid-lowering therapy, diabetes mellitus, and eGFR.

We next examined the associations between traditional CVD/HF risk factors and cGMP. Models 1 and 2 were the same as that described for NT-proBNP. However, in Model 3, CVD risk factors that were evaluated as the exposure variables were excluded from adjustment. Model 4 adjusted for Model 3 variables plus NT-proBNP.

Finally, we examined the associations between sex hormones and cGMP using progressively adjusted models. Model 1 adjusted for age and race/ethnicity. Model 2 additionally adjusted for body mass index, education, smoking, physical activity, years since menopause (in women), and use of erectile dysfunction drugs (in men). Model 3 adjusted for Model 2 covariates, plus the CVD risk factors described above. Model 4 further adjusted for NT-proBNP.

Two-sided $P<0.05$ was considered statistically significant. All analyses were performed on STATA version 15 (StataCorp LP, College Station, TX).

Results

Baseline Characteristics

Baseline characteristics of the sample, stratified by cGMP tertiles, are shown in Table 1. There was no sex difference among the cGMP tertiles. Participants in higher tertiles of cGMP were significantly older. There was no difference in body mass index. Both systolic and diastolic BP were higher among participants with higher cGMP. Total and LDL cholesterol and triglycerides were lower among higher tertiles of cGMP, while HDL cholesterol was higher. NT-proBNP was higher among higher cGMP tertiles. For both men and women, free testosterone and DHEA were lower among those in higher tertiles of cGMP, while SHBG was higher.

Associations Between NP and cGMP

To evaluate the NP-cGMP pathway, we analyzed cross-sectional associations between NT-proBNP and cGMP (Table 2). NT-proBNP levels were positively associated with cGMP after adjusting for demographics, lifestyle, and CVD risk factors (ratio of cGMP geometric means of 1.20 [95% CI, 1.16–1.24] in Model 3). The relationship between NT-proBNP and cGMP was primarily linear and remained positive over the range of the NT-proBNP distribution in both women and men (Figure 2). Removing the effect of all other covariates in the fully adjusted model, NT-proBNP itself explained 9% of the variance in cGMP (Table 2).

Associations Between CVD Risk Factors and cGMP

Table 3 shows the cross-sectional associations between CVD biomarkers/risk factors and cGMP. Systolic BP, diastolic BP, and the presence of hypertension were all associated with higher cGMP levels after adjusting for demographic and lifestyle factors (Model 2). The positive association between systolic BP and cGMP remained significant after further adjustment for use of antihypertensive therapy and NT-proBNP (Model 4). Certain classes of antihypertensive medication, including β-blockers and angiotensin-converting

Table 1. Participant Characteristics at MESA Baseline Exam (2000–2002) by cGMP Tertiles

cGMP Tertiles	Overall (N=1038)	First Tertile (N=346)	Second Tertile (N=346)	Third Tertile (N=346)	*P* Value
Mean (SD)	4.7 (2.6)	2.6 (0.6)	4.2 (0.5)	7.5 (2.7)	
Range	0.2–23.6	0.2–3.4	3.4–5.0	5.0–23.6	
Age, y	63.3 (8.6)	61.0 (8.4)	63.8 (8.0)	65.0 (8.9)	<0.001
Men, n (%)	542 (52.2)	174 (50.3)	188 (54.3)	180 (52.0)	0.56
Race/ethnicity, n (%)					
Caucasian	350 (33.7)	107 (30.9)	114 (32.9)	129 (37.3)	<0.001
African American	266 (25.6)	49 (14.2)	85 (24.6)	132 (38.2)	
Chinese American	182 (17.5)	71 (20.5)	76 (22.0)	35 (10.1)	
Hispanic	240 (23.1)	119 (34.4)	71 (20.5)	40 (14.5)	
Body mass index, kg/m^2	27.7 (4.6)	27.9 (4.2)	27.7 (4.7)	27.4 (4.9)	0.44
Education, n (%)					
<High school	177 (17.1)	78 (22.5)	58 (16.8)	41 (11.8)	0.01
High school, technical school, or associate degree	458 (44.1)	148 (42.8)	149 (43.1)	161 (46.5)	
College, graduate or professional school	403 (38.8)	120 (34.7)	139 (40.2)	144 (41.6)	
Smoking, n (%)					
Never	586 (56.5)	224 (64.7)	179 (51.7)	183 (52.9)	0.01
Former	348 (33.5)	94 (27.2)	130 (37.6)	124 (35.8)	
Current	104 (10.0)	28 (8.1)	37 (10.7)	39 (11.3)	
Total moderate/vigorous physical activity, MET-min/wk*	4140 (5385)	4346.3 (5940)	3892.5 (5497.5)	4305 (4605)	0.22
Systolic BP, mm Hg	126.1 (20.4)	120.2 (18.3)	127.3 (18.0)	130.8 (23.2)	<0.001
Diastolic BP, mm Hg	72.6 (10.1)	71.3 (9.4)	73.1 (10.2)	73.3 (10.6)	0.02
Total cholesterol, mg/dL	195.1 (35.2)	198.4 (34.9)	196.6 (36.9)	190.3 (33.3)	0.01
HDL cholesterol, mg/dL	50.0 (14.1)	47.8 (12.4)	49.4 (14.0)	52.7 (15.4)	<0.001
LDL cholesterol, mg/dL	119.6 (31.5)	121.9 (31.0)	121.2 (32.8)	115.8 (30.4)	0.02
Triglycerides, mg/dL	127.3 (65.9)	143.3 (70.1)	129.6 (63.7)	109.1 (59.1)	<0.001
Diabetes mellitus, n (%)	107 (10.3)	47 (13.6)	29 (8.4)	31 (9.0)	0.048
HOMA-IR, mmol×mIU/L^2*	33.8 (27.4)	38.3 (33.9)	34.5 (24.7)	28.9 (21.9)	<0.001
eGFR, mL/min per 1.73 m^2	77.0 (14.8)	81.2 (14.2)	76.2 (14.0)	73.5 (15.1)	<0.001
NT-proBNP, pg/mL*	48.7 (77.6)	30.3 (51.0)	45.6 (69.2)	76.0 (98.6)	<0.001
Sex hormones*					
Total T, nmol/L					
Men	14.3 (5.8)	13.7 (5.3)	14.3 (5.6)	14.9 (6.4)	0.01
Women	0.9 (0.8)	0.9 (0.7)	1.0 (0.8)	0.9 (0.8)	0.73
Bioavailable T, nmol/L					
Men	5.2 (2.1)	5.1 (2.0)	5.2 (2.0)	5.3 (2.0)	0.53
Women	0.2 (0.2)	0.3 (0.2)	0.2 (0.3)	0.2 (0.2)	0.54
Free T, %					
Men	2.0 (0.6)	2.1 (0.6)	2.0 (0.6)	1.9 (0.6)	0.001
Women	1.5 (0.7)	1.6 (0.7)	1.5 (0.6)	1.4 (0.7)	0.004

Continued

Table 1. Continued

cGMP Tertiles	Overall (N=1038)	First Tertile (N=346)	Second Tertile (N=346)	Third Tertile (N=346)	*P* Value
Estradiol, nmol/L					
Men	0.1 (0.05)	0.1 (0.04)	0.1 (0.04)	0.1 (0.05)	0.15
Women	0.1 (0.04)	0.1 (0.04)	0.1 (0.05)	0.1 (0.03)	0.71
DHEA, nmol/L					
Men	12.4 (7.4)	13.3 (8.7)	12.6 (6.4)	10.7 (7.8)	0.002
Women	11.4 (8.0)	12.0 (8.1)	12.0 (7.9)	10.1 (7.2)	0.03
SHBG, nmol/L					
Men	40.5 (19.3)	38.0 (18.7)	40.0 (17.6)	44.5 (20.7)	<0.001
Women	49.4 (33.2)	44.2 (30.4)	49.2 (30.4)	53.1 (35.7)	0.004

BP indicates blood pressure; DHEA, dehydroepiandrosterone; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; LDL, low-density lipoprotein; MESA, Multi-Ethnic Study of Atherosclerosis; NT-proBNP, N-terminal pro-B type natriuretic peptide; SHBG, sex hormone binding globulin; T, testosterone.

*Data presented as mean (SD) or number (percentage), or median (interquartile range).

enzyme inhibitors, are known to increase cGMP levels.[25] These classes of medications were also associated with greater cGMP levels in our cohort; however, adjusting for these medications yielded similar results as adjusting for use of antihypertensive therapy as a whole (Tables S3 and S4). eGFR was inversely associated with cGMP, both when expressed as a continuous and categorical variable. HDL cholesterol was positively associated with cGMP levels, while total/HDL cholesterol ratio and triglycerides were inversely associated with cGMP, after adjusting for demographic and lifestyle risk factors and use of lipid-lowering therapy (Model 3). Total and LDL cholesterol were both inversely associated with cGMP, but only in the unadjusted model. The presence of diabetes mellitus and higher HOMA-IR scores were inversely associated with cGMP levels after full adjustment (Model 4).

Table 2. Cross-Sectional Associations of NT-proBNP and cGMP in Men and Women (N=1038)

	Ratio of cGMP Geometric Means (95% CI)*	R^2 or Partial R^2 of NT-proBNP
Unadjusted	1.19 (1.15–1.22)	0.11†
Model 1‡	1.23 (1.19–1.27)	0.13†
Model 2§	1.23 (1.19–1.27)	0.13†
Model 3‖	1.20 (1.16–1.24)	0.09†

NT-proBNP indicates N-terminal pro-B type natriuretic peptide.

*Per 1 SD greater log(NT-proBNP). Results are presented as exponentiated β coefficients to reflect ratio of cGMP geometric means (95% CI). Ratios >1 indicated a positive relationship; ratios <1 indicate an inverse relationship.

†Statistically significant results (P<0.05).

‡Model 1: adjusts for age, sex, and race/ethnicity.

§Model 2: adjusts for Model 1+body mass index, education, smoking, and physical activity.

‖Model 3: adjusts for Model 2+systolic blood pressure, antihypertensive medication, total cholesterol, lipid-lowering therapy, diabetes mellitus, and estimated glomerular filtration rate.

Associations between androgenic sex hormones and cGMP are displayed in Tables 4 and 5. Among women, higher free testosterone and DHEA were independently associated with lower cGMP levels after adjusting for demographics, lifestyle, and CVD risk factors (Table 4, Model 3). The inverse relationship between cGMP and free testosterone was generally linear over the range of the free testosterone distribution (Figure 3A). The statistical significance of the free testosterone and DHEA associations with cGMP was attenuated after adjusting for NT-proBNP (Table 4, Model 4). In contrast, higher SHBG was independently associated with higher cGMP after adjusting for CVD risk factors (Model 3). This relationship was also attenuated after further adjusting for NT-proBNP (Model 4).

Among men, in the unadjusted model, higher total testosterone and SHBG were associated with higher levels of cGMP, while free testosterone and DHEA were inversely associated with cGMP (Table 5). These associations were no longer statistically significant after adjusting for demographics, lifestyle, and CVD risk factors (Table 5; Figure 3B).

Discussion

In this study, we measured plasma cGMP concentrations in participants from a diverse, community-based cohort free of CVD/HF and investigated the associations between components of the NP-cGMP pathway. In addition, we assessed associations between traditional CVD/HF risk factors and cGMP. To our knowledge, this is the first study to comprehensively evaluate plasma cGMP in a general population, as prior work with cGMP has primarily been in in vitro and animal models or in smaller clinical studies.

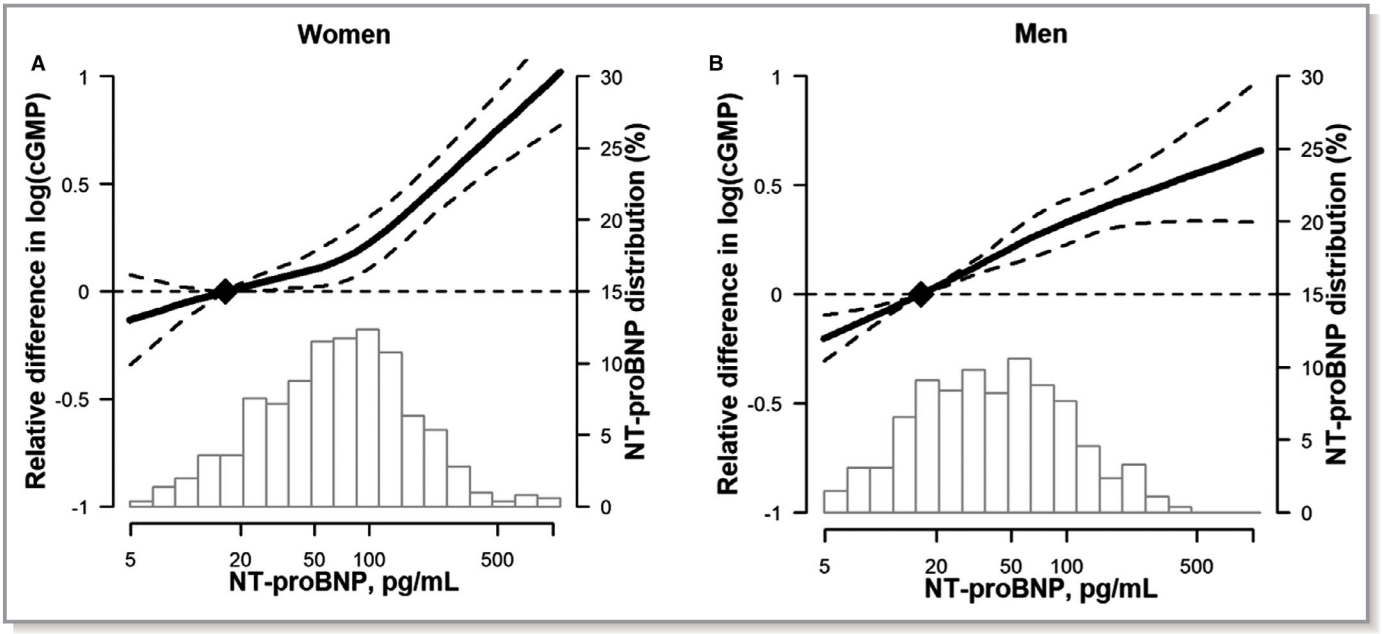

Figure 2. Associations between NT-proBNP and cGMP in women (**A**) and men (**B**) using restricted cubic splines. Analyses are adjusted for Model 2 covariates: age, race/ethnicity, body mass index, education, smoking, physical activity, and years since menopause (in women). Graphs represent the difference in log(cGMP) at various NT-proBNP levels relative to the reference (♦), with knots at the 5th, 35th, 65th, and 95th percentiles of the NT-proBNP distribution. 95% CI are represented by dotted lines. NT-proBNP indicates N-terminal pro-B type natriuretic peptide.

Associations Between NP and cGMP

Our study demonstrated a positive linear association between NT-proBNP and cGMP, even among individuals free of clinical HF. Previous animal studies have shown that cGMP synthesis is compartmentalized, such that the cGMP pool generated through NP-pGC signaling is accessible at the plasma membrane, whereas the pool generated through nitric oxide/soluble guanylyl cyclase stimulation is not.[12,26] One study of isolated rabbit aorta showed that both atrial NP and nitroprusside induced elevations in intracellular cGMP; however, extracellular levels of cGMP rose only with atrial NP stimulation, suggesting that release of cGMP into the extracellular space is specific for activation of the NP-pGC pathway.[27] It stands to reason that plasma cGMP levels may be primarily reflective of the cGMP synthesized from the NP/pGC pool; however, to our knowledge, this is the first study to examine and confirm this relationship in a human cohort.

NT-proBNP is synthesized in response to myocardial wall stress and serves to regulate ventricular volume by promoting vasodilation and natriuresis and by antagonizing effects of the renin-angiotensin-aldosterone system through cGMP signaling.[28] Augmentation of the NP system using drugs such as neprilysin inhibitors and nesiritide have demonstrated effectiveness in the treatment of HF.[29] Despite the cardioprotective effects of NT-proBNP, its levels are elevated in pathologic states such as HF because of its involvement in a negative feedback loop that drives compensatory increases in BNP in response to volume or pressure overload.[30] Like NT-proBNP, cGMP mediates a number of cardioprotective processes, but may also serve as a biomarker for disease states such as HF, particularly when measured from the plasma. Moreover, it has been shown that in HF patients, myocardial cGMP is reduced, while plasma cGMP is increased, mirroring the compensatory and pathologic NT-proBNP elevation in HF.[31,32]

CVD/HF Risk Factors and cGMP

The NP-cGMP pathway has been shown to regulate BP by stimulating vasodilation and natriuresis and by inhibiting the renin-angiotensin system.[14] However, little is known about the relationship between plasma levels of cGMP and hypertension. One study of endothelial dysfunction in 95 individuals with sleep apnea showed that cGMP levels were reduced in hypertensive compared with nonhypertensive patients.[33] In contrast, our study found that higher systolic and diastolic BP and the presence of hypertension were independently associated with higher cGMP levels. Adjustment for NT-proBNP attenuated these relationships, suggesting that the positive association between cGMP and BP may be related to the known positive association between NT-proBNP and hypertension. Prior literature has shown that NP secretion increases as a compensatory mechanism in the setting of pressure and volume overload and increased myocardial wall

Table 3. Cross-Sectional Associations Between CVD Biomarkers/Risk Factors and cGMP in Men and Women

	cGMP (pmol/mL)* N=1038				
	Unadjusted	Model 1†	Model 2‡	Model 3§	Model 4‖
Hypertension (Y/N)	1.21 (1.13, 1.28)¶	1.13 (1.07, 1.20)¶	1.14 (1.07, 1.21)¶	1.10 (1.01, 1.20)¶	1.06 (0.97, 1.14)
Systolic BP, mm Hg	1.12 (1.09–1.16)¶	1.09 (1.06–1.12)¶	1.10 (1.07–1.14)¶	1.09 (1.05–1.13)¶	1.05 (1.02–1.09)¶
Diastolic BP, mm Hg	1.04 (1.01–1.07)¶	1.03 (1.00–1.07)¶	1.04 (1.01–1.07)¶	1.03 (0.996–1.06)	1.02 (0.99–1.05)
eGFR, mL/min per 1.73 m^2	0.89 (0.86–0.92)¶	0.90 (0.87–0.93)¶	0.90 (0.87–0.93)¶	0.90 (0.87–0.93)¶	0.91 (0.88–0.94)¶
eGFR categories#					
≥90	Ref	Ref	Ref	Ref	Ref
60–89.9	1.17 (1.08–1.26)¶	1.11 (1.03–1.20)¶	1.11 (1.03–1.19)¶	1.11 (1.03–1.20)¶	1.11 (1.03–1.19)¶
<60	1.38 (1.23–1.53)¶	1.24 (1.10–1.39)¶	1.24 (1.10–1.39)¶	1.24 (1.10–1.39)¶	1.21 (1.08–1.35)¶
Total cholesterol, mg/dL	0.97 (0.94–1.00)¶	0.98 (0.96–1.01)	0.99 (0.96–1.01)	0.99 (0.96–1.02)	1.01 (0.98–1.04)
HDL cholesterol, mg/dL	1.08 (1.05–1.12)¶	1.07 (1.03–1.10)¶	1.06 (1.03–1.10)¶	1.07 (1.03–1.11)¶	1.05 (1.02–1.09)¶
Total/HDL ratio	0.92 (0.89–0.95)¶	0.95 (0.91–0.98)¶	0.95 (0.92–0.99)¶	0.95 (0.92–0.99)¶	0.98 (0.95–1.01)
LDL cholesterol, mg/dL	0.97 (0.94–1.00)¶	0.98 (0.95–1.01)	0.98 (0.95–1.01)	0.98 (0.96–1.01)	1.01 (0.98–1.04)
Triglycerides, mg/dL	0.88 (0.85–0.92)¶	0.94 (0.90–0.98)¶	0.94 (0.90–0.98)¶	0.95 (0.91–0.98)¶	0.97 (0.93–1.01)
Diabetes mellitus (Y/N)	0.93 (0.84–1.03)	0.89 (0.81–0.98)¶	0.90 (0.82–0.99)¶	0.87 (0.79–0.96)¶	0.91 (0.83–0.99)¶
HOMA-IR, mmol×mIU/L^2	0.92 (0.89–0.95)¶	0.93 (0.90–0.95)¶	0.92 (0.89–0.95)¶	0.92 (0.88–0.95)¶	0.95 (0.91–0.98)¶

BP indicates blood pressure; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; LDL, low-density lipoprotein.

*Per 1 SD greater continuous variables. Results are presented as exponentiated β coefficients to reflect ratio of cGMP geometric means (95% CI). Ratios >1 indicated a positive relationship; ratios <1 indicate an inverse relationship.

†Model 1: adjusts for age, sex, and race/ethnicity.

‡Model 2: adjusts for Model 1+education, smoking, body mass index, and physical activity.

§Model 3: adjusts for Model 2+antihypertensive medication and lipid-lowering therapy.

‖Model 4: adjusts for Model 3+log(NT-proBNP).

¶Statistically significant results ($P<0.05$).

#eGFR categories: ≥90, 60–89.9, <60 mL/min per 1.73 m^2.

stress, and elevated levels of NT-proBNP have been found to be associated with increased risk of hypertension in population studies.[34] Because cGMP is downstream of NP signaling, we speculate that compensatory increases in NP levels in the setting of hypertension and increased myocardial wall stress might translate into increases in plasma cGMP levels (Figure 4). However, additional research is needed to further evaluate this potential mechanism.

In terms of hemodynamic regulation of the kidney, as mentioned above, cGMP is known to inhibit renin release and counteract the renin-angiotensin-aldosterone system.[35] In a dog model of acute HF, NP pathway blockade resulted in a reduction in GFR.[36] In contrast, multiple studies in patients with and without clinical HF have demonstrated lower eGFR to be associated with higher NT-proBNP levels attributable to increased cardiac dysfunction and volume overload, as well as decreased clearance of NT-proBNP, in the setting of chronic kidney disease.[15,37] Our study similarly demonstrated that lower eGFR was associated with higher cGMP levels. This association mirrors the relationship between eGFR and NT-proBNP; thus, we speculate that cGMP levels may be elevated in states of renal dysfunction through NT-proBNP elevation (Figure 4). However, adjusting for NT-proBNP levels did not attenuate the association between eGFR and cGMP, suggesting that there may be other factors involved.

Hypercholesterolemia has been shown to impair endothelial function and promote atherogenesis, which is thought to occur through disruption of the nitric oxide–cGMP pathway.[38,39] The NP-cGMP pathway may also be dysregulated in the setting of hyperlipidemia.[16] Risks of elevated total cholesterol, LDL cholesterol, and triglycerides have been found to be reduced among elderly individuals with higher BNP.[40] A prior study showed that among a healthy cohort, urinary cGMP was inversely associated with total and non-HDL cholesterol.[38] In our study, a more favorable lipid profile (higher HDL cholesterol and lower total/HDL cholesterol ratio and triglycerides) was independently and positively associated with cGMP. Whether this reflects the cardioprotective effect of cGMP or whether adverse metabolic risk factors mediate a state of cGMP deficiency cannot be determined without additional studies on the directionality of these associations. Adjustment for NT-proBNP attenuated

Table 4. Cross-Sectional Associations Between Sex Hormones and cGMP in Women

	cGMP (pmol/mL)* N=496				
	Unadjusted	Model 1†	Model 2‡	Model 3§	Model 4‖
Total testosterone, nmol/L	1.02 (0.97–1.07)	1.01 (0.96–1.05)	1.00 (0.96–1.05)	0.99 (0.95–1.03)	1.01 (0.97–1.05)
Bioavailable testosterone, nmol/L	0.99 (0.94–1.04)	0.99 (0.94–1.04)	0.98 (0.93–1.03)	0.96 (0.92–1.01)	0.99 (0.95–1.04)
Free testosterone (%)	0.90 (0.85–0.96)¶	0.94 (0.88–1.00)	0.92 (0.86–0.99)¶	0.92 (0.86–0.99)¶	0.96 (0.90–1.02)
Estradiol, nmol/L	0.97 (0.91–1.03)	0.96 (0.91–1.02)	0.96 (0.90–1.02)	0.95 (0.89–1.01)	0.97 (0.92–1.03)
DHEA, nmol/L	0.93 (0.89–0.98)¶	0.95 (0.91–1.00)	0.96 (0.91–1.00)	0.95 (0.90–1.00)¶	0.97 (0.92–1.02)
SHBG, nmol/L	1.10 (1.04–1.17)¶	1.06 (1.00–1.12)	1.08 (1.01–1.15)¶	1.08 (1.01–1.14)¶	1.04 (0.98–1.11)

DHEA indicates dehydroepiandrosterone; SHBG, sex hormone binding globulin.
*Per 1 SD greater log(sex hormone levels). Results are presented as exponentiated β coefficients to reflect ratio of cGMP geometric means (95% CI). Ratios >1 indicate a positive relationship; ratios <1 indicate an inverse relationship.
†Model 1: adjusts for age and race/ethnicity.
‡Model 2: adjusts for Model 1+education, body mass index, smoking, physical activity, and years since menopause.
§Model 3: adjusts for Model 2+systolic BP, antihypertensive medication, total cholesterol, lipid-lowering therapy, diabetes mellitus, and estimated glomerular filtration rate.
‖Model 4: adjusts for Model 3+log(NT-proBNP).
¶Statistically significant results ($P<0.05$). Each hormone was modeled separately. No women were using hormone therapy in this sample.

the significance in the associations between total/HDL cholesterol ratio and triglycerides with cGMP, suggesting that the NP-cGMP pathway may play a role in these relationships (Figure 4).

Risk of diabetes mellitus and fasting plasma glucose have been shown to be inversely correlated with NT-proBNP levels.[17] Platelet-derived cGMP is reduced in patients with type 2 diabetes mellitus, but little is known about the relationship between plasma cGMP and insulin resistance.[41] Our study provides evidence on a population level that diabetes mellitus and insulin resistance are inversely associated with cGMP levels. Whether insulin resistance inhibits cGMP production, or cGMP improves glucose tolerance, or both, remains to be clarified with future research.

Androgens and cGMP

Our study demonstrated that among postmenopausal women, a more androgenic pattern of sex hormones (higher free testosterone and DHEA, lower SHBG) was associated with lower cGMP levels. The significance of these associations was attenuated after adjusting for NT-proBNP, suggesting that NT-proBNP may play a role in the relationship between androgenicity and plasma cGMP. Our group has previously shown

Table 5. Cross-Sectional Associations Between Sex Hormones and cGMP in Men

	cGMP (pmol/mL)* N=542			
	Unadjusted	Model 1†	Model 2‡	Model 3§
Total testosterone, nmol/L	1.07 (1.01–1.13)‖	1.07 (1.01–1.12)‖	1.05 (1.00–1.11)‖	1.05 (1.00–1.11)
Bioavailable testosterone, nmol/L	0.98 (0.93–1.04)	1.05 (1.00–1.10)	1.04 (0.99–1.09)	1.03 (0.98–1.09)
Free testosterone (%)	0.92 (0.88–0.96)‖	0.98 (0.94–1.03)	0.99 (0.95–1.04)	0.99 (0.94–1.04)
Estradiol, nmol/L	0.99 (0.95–1.04)	1.00 (0.95–1.04)	1.01 (0.96–1.05)	1.01 (0.97–1.05)
DHEA, nmol/L	0.92 (0.88–0.96)‖	0.96 (0.92–1.01)	0.96 (0.92–1.01)	0.98 (0.03–1.02)
SHBG, nmol/L	1.09 (1.04–1.14)‖	1.03 (0.98–1.07)	1.01 (0.97–1.06)	1.02 (0.97–1.06)

DHEA indicates dehydroepiandrosterone; SHBG, sex hormone binding globulin.
*Per 1 SD greater log(sex hormone). Results are presented as exponentiated β coefficients to reflect ratio of cGMP geometric means (95% CI). Ratios >1 indicated a positive relationship; ratios <1 indicate an inverse relationship.
†Model 1: adjusts for age and race/ethnicity.
‡Model 2: adjusts for Model 1+education, body mass index, smoking, physical activity, and erectile dysfunction drugs.
§Model 3: adjusts for Model 2+systolic BP, antihypertensive medication, total cholesterol, lipid-lowering therapy, diabetes mellitus, and estimated glomerular filtration rate.
‖Statistically significant results ($P<0.05$). Each hormone was modeled separately.

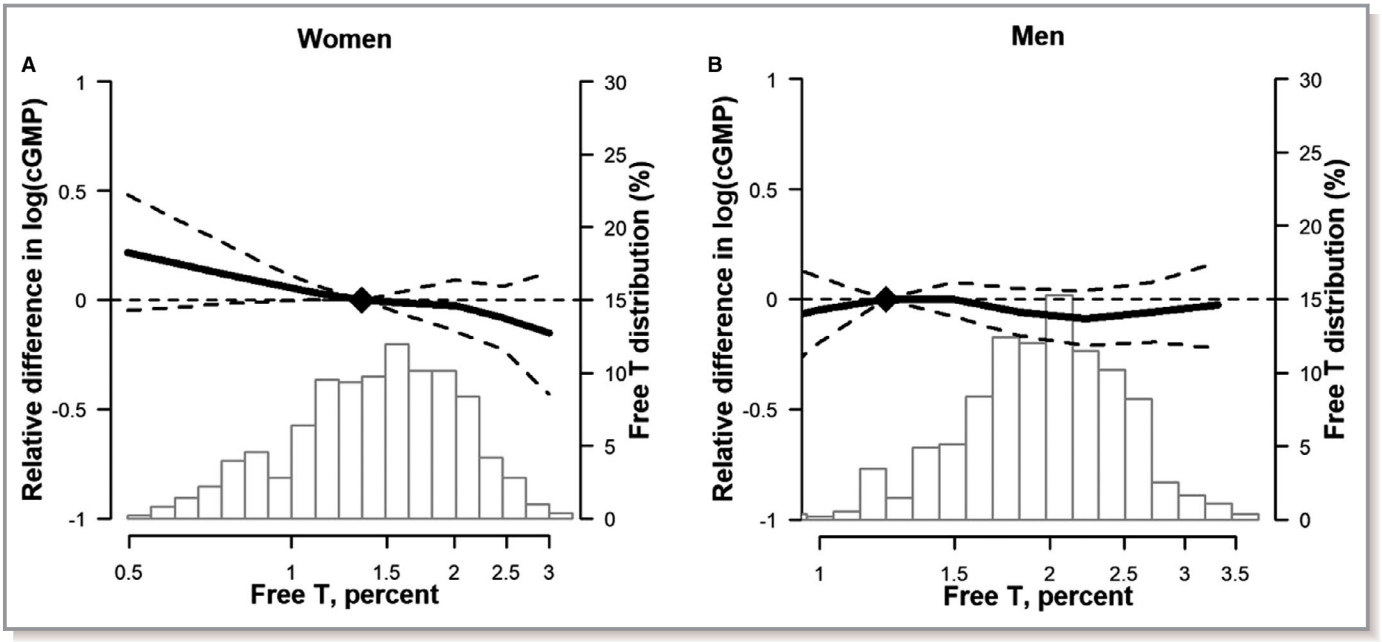

Figure 3. Associations between free T and cGMP in women (**A**) and men (**B**) using adjusted restricted cubic splines. Analyses are adjusted for Model 2 covariates: age, race/ethnicity, education, smoking, body mass index, physical activity, and years since menopause (in women). Graphs represent difference in log(cGMP) at various free T levels relative to the reference (♦), with knots at the 5th, 35th, 65th, and 95th percentiles of the free T distribution. 95% CI are represented by dotted lines. T indicates testosterone.

that androgenic sex hormones and NT-proBNP are also inversely related, and that increased androgenicity may mediate a state of NP deficiency.[18] It is thus possible that androgenicity may also confer a similar cGMP deficiency through the NP-cGMP pathway (Figure 4).

Strengths and Limitations

Our findings should be considered in the context of several limitations. First, cGMP was measured only once; thus, we were unable to evaluate associations between changes in

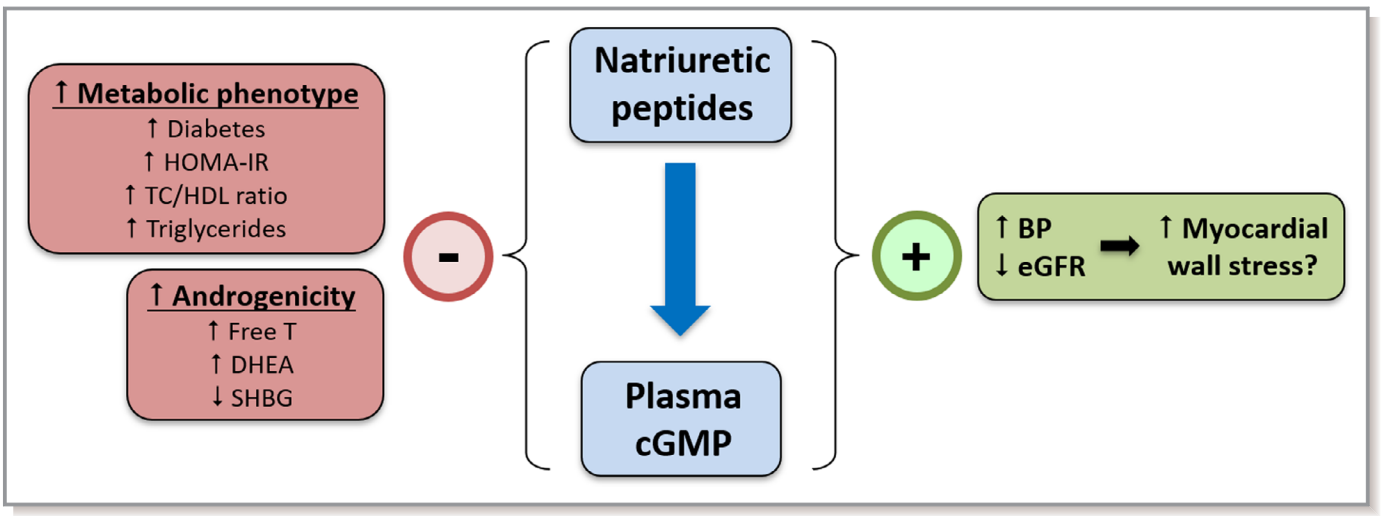

Figure 4. Summary of associations between sex hormones, CVD risk factors, and the cGMP pathway. Plasma cGMP is primarily reflective of stimulation from natriuretic peptide (NP). Increased androgenicity and metabolic risk factors are inversely associated with both NP and cGMP. On the other hand, higher blood pressure and greater renal dysfunction positively associated with NP and cGMP. Prior studies have suggested that these conditions are associated with higher BNP levels attributable to increased myocardial wall stress, which may also be a mechanism for the association of these conditions with higher cGMP levels. BP indicates blood pressure; DHEA, dehydroepiandrosterone; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; SHBG, sex hormone binding globulin; T, testosterone; TC, total cholesterol.

cGMP and CVD risk factors over time. Second, because cGMP levels were only measured among postmenopausal women in this study, additional studies would need to be performed to determine whether our findings can be generalized to premenopausal women. It is important to note that estradiol levels among postmenopausal women who were not taking hormone therapy were quite low, which limits full evaluation of the association between estradiol and cGMP; however, the primary aim of our ancillary study was to better understand cGMP levels in the postmenopausal state as a potential modulator of CVD risk in older women. Third, we also performed multiple comparisons because of the exploratory nature of this study. Fourth, the interassay coefficient of variation for cGMP was high, at 13.5%. However, increased assay variability would be expected to lead to null findings, and we found significant associations with cGMP despite increased variability in cGMP measurements. Finally, the cross-sectional and observational nature of our study precludes the ability to determine both temporality and causality. We can only speculate about biological mechanisms underlying these observed associations.

On the other hand, our study has a number of major strengths, including a large sample size and a multiethnic cohort that is well characterized, allowing for adjustment for numerous potential confounders.

Conclusions

cGMP is a cardioprotective second messenger whose signaling pathway is a target for novel CVD therapies. We found that NT-proBNP was positively associated with cGMP, suggesting that plasma cGMP levels are primarily regulated through the NP-pGC pathway. Higher blood pressure and greater renal dysfunction were positively associated with cGMP levels, while adverse metabolic risk factors and increased androgenicity were inversely associated with cGMP. These novel associations, not previously studied in a community cohort, further our understanding of cGMP on a population level and can serve as a basis for future studies on the cGMP pathway.

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Disclosures

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SUPPLEMENTAL MATERIAL

Table S1. Baseline characteristics of participants included in this study vs all participants at the MESA baseline exam (2000-2002).

		Included participants (n = 1,038)	All participants (n = 6,814)
Age, years		63.3 (8.6)	62.2 (10.2)
Men, n (%)		542 (52.2)	3213 (47.2)
Race/ethnicity, n (%)			
Caucasian		350 (33.7)	2622 (38.5)
African American		266 (25.6)	1892 (27.8)
Chinese American		182 (17.5)	804 (11.8)
Hispanic		240 (23.1)	1496 (22.0)
BMI, kg/m^2		27.7 (4.6)	28.3 (5.5)
Education, n (%)			
<High school		177 (17.1)	1225 (18.0)
High school, technical school, or associate degree		458 (44.1)	3173 (46.7)
College, graduate or professional school		403 (38.8)	2393 (35.2)
Smoking, n (%)			
Never		586 (56.5)	3418 (50.3)
Former		348 (33.5)	2487 (36.6)
Current		104 (10.0)	887 (13.1)
Total moderate/vigorous physical activity, MET-min/wk*		4140 (5385)	4020 (5520)
Systolic BP, mm Hg		126.1 (20.4)	126.6 (21.5)
Diastolic BP, mm Hg		72.6 (10.1)	71.9 (10.3)
Total cholesterol, mg/dl		195.1 (35.2)	194.2 (35.7)
HDL cholesterol, mg/dl		50.0 (14.1)	51.0 (14.8)
LDL cholesterol, mg/dl		119.6 (31.5)	117.2 (31.5)
Triglycerides, mg/dl		127.3 (65.9)	131.6 (88.8)
Diabetes, n (%)		107 (10.3)	859 (12.7)
eGFR, mL/min/1.73 m^2		77.0 (14.8)	77.7 (16.3)
HOMA-IR, mmol*mIU/L^2 *		33.8 (27.4)	34.3 (30.5)
NT-proBNP, pg/mL*		48.7 (77.6)	54.5 (88.5)
Sex hormones*			
Total T, nmol/L	*Men*	14.3 (5.8)	14.2 (6.4)
	Women	0.9 (0.8)	0.9 (0.7)
Bioavailable T, nmol/L	*Men*	5.2 (2.1)	5.2 (2.3)
	Women	0.2 (0.2)	0.2 (0.2)
Free T, %	*Men*	2.0 (0.6)	2.0 (0.7)
	Women	1.5 (0.7)	1.3 (0.8)
Estradiol, nmol/L	*Men*	0.1 (0.05)	0.1 (0.05)
	Women	0.1 (0.04)	0.1 (0.1)
DHEA, nmol/L	*Men*	12.4 (7.4)	12.6 (7.9)
	Women	11.4 (8.0)	10.3 (7.6)
SHBG, nmol/L	*Men*	40.5 (19.3)	40.8 (21.3)
	Women	49.4 (33.2)	59.0 (53.8)

Data presented as mean (SD) or number (percentage), or *median (IQR)
MESA, Multi-Ethnic Study of Atherosclerosis; BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; eGFR, estimated glomerular filtration rate; NT-proBNP, N-terminal pro-B type natriuretic peptide; T, testosterone; DHEA, dehydroepiandrosterone; SHBG, sex hormone binding globulin

Table S2. Interaction analyses of NT-proBNP and CVD risk factors with sex in regression models with cGMP (pmol/mL).

Variable	P of interaction with sex
NT-proBNP, pg/mL	0.83
Systolic BP, mmHg	0.55
Diastolic BP, mmHg	0.16
BMI, kg/m^2	0.16
Total cholesterol, mg/dl	0.21
HDL cholesterol, mg/dl	0.87
Total/HDL ratio	0.96
LDL cholesterol, mg/dl	0.24
Triglycerides, mg/dl	0.36
HOMA-IR, $mmol*mIU/L^2$	0.67
eGFR, $mL/min/1.73\ m^2$	0.89

NT-proBNP, N-terminal pro-B type natriuretic peptide; CVD, cardiovascular disease; cGMP, cyclic guanosine monophosphate; BP, blood pressure; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; eGFR, estimated glomerular filtration rate

Table S3. Summary of (unadjusted) cGMP and NT-proBNP levels by classes of antihypertensive, diabetes, and cholesterol medications.

	N	cGMP (pmol/mL)	P-value	NT-proBNP (pg/mL)	P-value
Beta blockers					
Yes	94	6.0 (3.3)	**0.0001**	152.8 (179.6)	**0.0001**
No	944	4.6 (2.5)		69.2 (80.7)	
ACE inhibitor					
Yes	117	5.4 (3.1)	**0.02**	98.3 (125.3)	**0.01**
No	921	4.7 (2.6)		74.0 (92.5)	
Calcium channel blocker					
Yes	136	5.3 (3.0)	**0.02**	88.3 (104.7)	**0.03**
No	902	4.7 (2.5)		75.0 (95.7)	
Nitrates					
Yes	2	3.9 (1.8)	0.70	68.6 (15.1)	0.54
No	1036	4.7 (2.6)		76.7 (97.0)	
Diabetes medication					
Insulin	8	4.4 (1.5)	0.57	62.7 (60.0)	0.70
Pills	65	4.8 (2.8)		79.4 (86.2)	
Statins					
Yes	172	4.7 (2.7)	0.43	73.5 (81.3)	0.72
No	866	4.8 (2.6)		77.4 (99.8)	

Results reported as mean (SD)
cGMP, cyclic guanosine monophosphate; NT-proBNP, N-terminal pro-B type natriuretic peptide; ACE, angiotensin converting enzyme

Table S4. Associations between hypertension and blood pressure with cGMP, with modified multivariable models to adjust for specific classes of antihypertensive medication.

	cGMP (pmol/mL)* N = 1038				
	Unadjusted	**Model 1**†	**Model 2**‡	**Model 3**§	**Model 4**‖
Hypertension (Y/N)	**1.21 (1.13, 1.28)**	**1.13 (1.07, 1.20)**	**1.14 (1.07, 1.21)**	**1.12 (1.04, 1.21)**	1.06 (0.99, 1.14)
Systolic BP, mmHg	**1.12 (1.09, 1.16)**	**1.09 (1.06, 1.12)**	**1.10 (1.07, 1.14)**	**1.09 (1.05, 1.12)**	**1.05 (1.02, 1.09)**
Diastolic BP, mmHg	**1.04 (1.01, 1.07)**	**1.03 (1.00, 1.07)**	**1.04 (1.01, 1.07)**	1.03 (0.997, 1.06)	1.02 (0.99, 1.05)

*Per 1 SD greater continuous variables. Results are presented as exponentiated beta coefficients to reflect ratio of cGMP geometric means (95% CI). Ratios >1 indicated a positive relationship; ratios <1 indicate an inverse relationship. Statistically significant results ($p < 0.05$) are in bold.

†Model 1: Adjusts for age, sex, race/ethnicity

‡Model 2: Adjusts for Model 1 + education, smoking, body mass index, physical activity

§Model 3: Adjusts for Model 2 + beta blocker use, ACE inhibitor use, calcium channel blocker use, lipid lowering therapy

‖Model 4: Adjusts for Model 3 + log(NT-proBNP)

cGMP, cyclic guanosine monophosphate; BP, blood pressure