ORIGINAL RESEARCH

Cyclic Guanosine Monophosphate and Risk of Incident Heart Failure and Other Cardiovascular Events: the ARIC Study

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***Background*—**Cyclic guanosine monophosphate (cGMP) is a second messenger regulated through natriuretic peptide and nitric oxide pathways. Stimulation of cGMP signaling is a potential therapeutic strategy for heart failure with preserved ejection fraction (HFpEF) and atherosclerotic cardiovascular disease (ASCVD). We hypothesized that plasma cGMP levels would be associated with lower risk for incident HFpEF, any HF, ASCVD, and coronary heart disease (CHD).

***Methods and Results*—**We conducted a case–cohort analysis nested in the ARIC (Atherosclerosis Risk in Communities) study. Plasma cGMP was measured in 875 participants at visit 4 (1996–1998), with oversampling of incident HFpEF cases. We used Cox proportional hazard models to assess associations of cGMP with incident HFpEF, HF, ASCVD (CHD+stroke), and CHD. The mean (SD) age was 62.4 (5.6) years and median (interquartile interval) cGMP was 3.4 pmol/mL (2.4–4.6). During a median follow-up of 9.9 years, there were 283 incident cases of HFpEF, 329 any HF, 151 ASCVD, and 125 CHD. In models adjusted for CVD risk factors, the hazard ratios (95% CI) associated with the highest cGMP tertile compared with lowest for HFpEF, HF, ASCVD, and CHD were 1.88 (1.17–3.02), 2.18 (1.18–4.06), 2.84 (1.44–5.60), and 2.43 (1.19–5.00), respectively. In models further adjusted for N-terminal-proB-type natriuretic peptide, associations were attenuated for HFpEF and HF but remained statistically significant for ASCVD (2.56 [1.26–5.20]) and CHD (2.25 [1.07–4.71]).

***Conclusions*—**Contrary to our hypothesis, higher cGMP levels were associated with incident CVD in a community-based cohort. The associations of cGMP with HF or HFpEF may be explained by N-terminal-proB-type natriuretic peptide, but not for ASCVD and CHD. **(*J Am Heart Assoc*. 2020;9:e013966. DOI: 10.1161/JAHA.119.013966.)**

Key Words: cardiovascular disease • coronary heart disease • cyclic GMP • heart failure • heart failure with preserved ejection fraction

Although progress in clinical management of heart failure (HF) has resulted in decreased mortality, the prognosis of HF with preserved ejection fraction (HFpEF) remains largely the same.[1] HFpEF accounts for 50% of all HF cases, and it is projected to outgrow HF with reduced ejection fraction (HFrEF) over the next decade.[2,3] Therefore, factors that influence the pathogenesis of HFpEF may identify potential pharmacotherapy targets and have a direct impact in the prevention or treatment of HFpEF.

Cyclic guanosine monophosphate (cGMP) is an intracellular second messenger generated by guanylyl cyclases linked to either nitric oxide (NO)[4] or natriuretic peptide (NP)[5] signaling. It primarily signals by binding to and activating protein kinase G, and by interactions with other phosphodiesterases that can regulate the companion second messenger cyclic adenosine monophosphate. These effectors in turn contribute to a broad range of cardiovascular effects, including reducing vascular motor tone, antifibrotic and antihypertrophic signaling,[6] and

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Accompanying Tables S1 through S4 are available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.119.013966

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Clinical Perspective

What Is New?

- In a community-based cohort of men and women, plasma cyclic guanosine monophosphate levels are associated with increased risk of incident heart failure with preserved ejection fraction and other cardiovascular diseases.
- The associations of cyclic guanosine monophosphate with heart failure outcomes may be explained by N-terminal-proB-type natriuretic peptide, but associations with other cardiovascular diseases may follow different pathways.

What Are the Clinical Implications?

- Plasma cyclic guanosine monophosphate levels reflect upstream N-terminal-proB-type natriuretic peptide signaling.
- Abnormalities in the natriuretic peptide-cGMP signaling pathway precede the development of heart failure with preserved ejection fraction and other cardiovascular end points; these may potentially be targets for therapeutic interventions.

increases in protein quality control.[7,8] Alterations in the cGMP signaling cascade have been implicated in several cardiovascular disorders, and new pharmacological approaches to stimulate cGMP synthesis are being evaluated as potential therapeutic agents for HFrEF,[9,10] HFpEF,[11] and other cardiovascular diseases (CVD).[12]

Population-based studies on cGMP are largely limited to cross-sectional evaluations of the association between plasma cGMP and CVD risk factors in small samples.[13–17] To our knowledge, the association of plasma cGMP levels with the risk of developing incident CVD events has not been assessed in prospective studies. Using a case–cohort design, we measured plasma cGMP in a subset of women and men in the ARIC (Atherosclerosis Risk in Communities) study to evaluate the association between cGMP levels and CVD end points. Our primary hypothesis was that cGMP levels would be inversely associated with the risk of incident HFpEF. Our secondary hypothesis was that cGMP levels would also be inversely associated with the incidence of atherosclerotic cardiovascular disease (ASCVD), coronary heart disease (CHD), and any HF outcomes. We also assessed whether these associations differed by sex and race, and whether they were independent of NT-proBNP (N-terminal pro B-type natriuretic peptide), an upstream factor regulating NP-cGMP signaling.

Methods

Data Availability Statement

The ARIC cohort participates in the National Heart, Lung, and Blood Institute's Biologic Specimen and Data Repository (BioLINCC). The ARIC data are available upon request through BioLINCC (https://biolincc.nhlbi.nih.gov/studies/aric/).

Case–Cohort Design

The ARIC Study is an ongoing, prospective, predominantly biracial, community-based study investigating risk factors for CVD.[18] Women and men 45 to 64 years of age were sampled in 1987–1989 from 4 US communities: Forsyth County, NC; Jackson, MS; Minneapolis suburbs, MN; and Washington County, MD. Follow-up visits were conducted in 1990–1992 (Visit 2), 1993–1995 (Visit 3), 1996–1998 (Visit 4), 2011–2013 (Visit 5), and 2016–2017 (Visit 6). The ARIC study has been approved by institutional review boards at all centers, and written informed consent was provided by all participants.

Among 9706 participants attending Visit 4 and free of HF at that visit, we conducted a case–cohort analysis based on 332 cases who developed HFpEF between January 1, 2005 (the date of onset for HFpEF adjudication in ARIC) and December 31, 2013 (the administrative censoring date by the time of case selection), as well as a random sample of 700 participants attending Visit 4 and free of HF at that visit (selected using a simple random sampling approach; 25 participants in the random cohort sample were also included in the group of incident HFpEF cases). Visit 4 served as the baseline for this analysis. We then excluded participants with depleted plasma sample (n=19), unmeasurable plasma cGMP levels (n=13), participants with self-reported race other than black or white (n=2), blacks from the Minneapolis and Washington County centers (n=6), and participants with prevalent CVD at Visit 4 (n=92).

The final study sample comprised 875 participants (Figure 1), including 283 participants who developed incident HFpEF, 329 participants who developed any HF, 151 participants who developed incident ASCVD, 125 participants who developed CHD, and 617 participants in the random cohort sample. Of note, association of cGMP with HFrEF was not examined because of few incident HFrEF cases (n=32). The reason for the greater number of HFpEF cases compared with HFrEF is that our study design specifically selected for HFpEF as our case status in this case–cohort design, given our a priori interest in understanding mechanisms for HFpEF development.

Measurement of cGMP

Plasma cGMP was determined from samples collected at visit 4 (1996–1998) and stored at −80°C until cGMP measurement was performed in 2017. cGMP concentrations were assessed at the Atherosclerosis Clinical Research Laboratory at Baylor College of Medicine using a competitive ELISA assay (Cayman Chemical Company, MI), with the addition of an

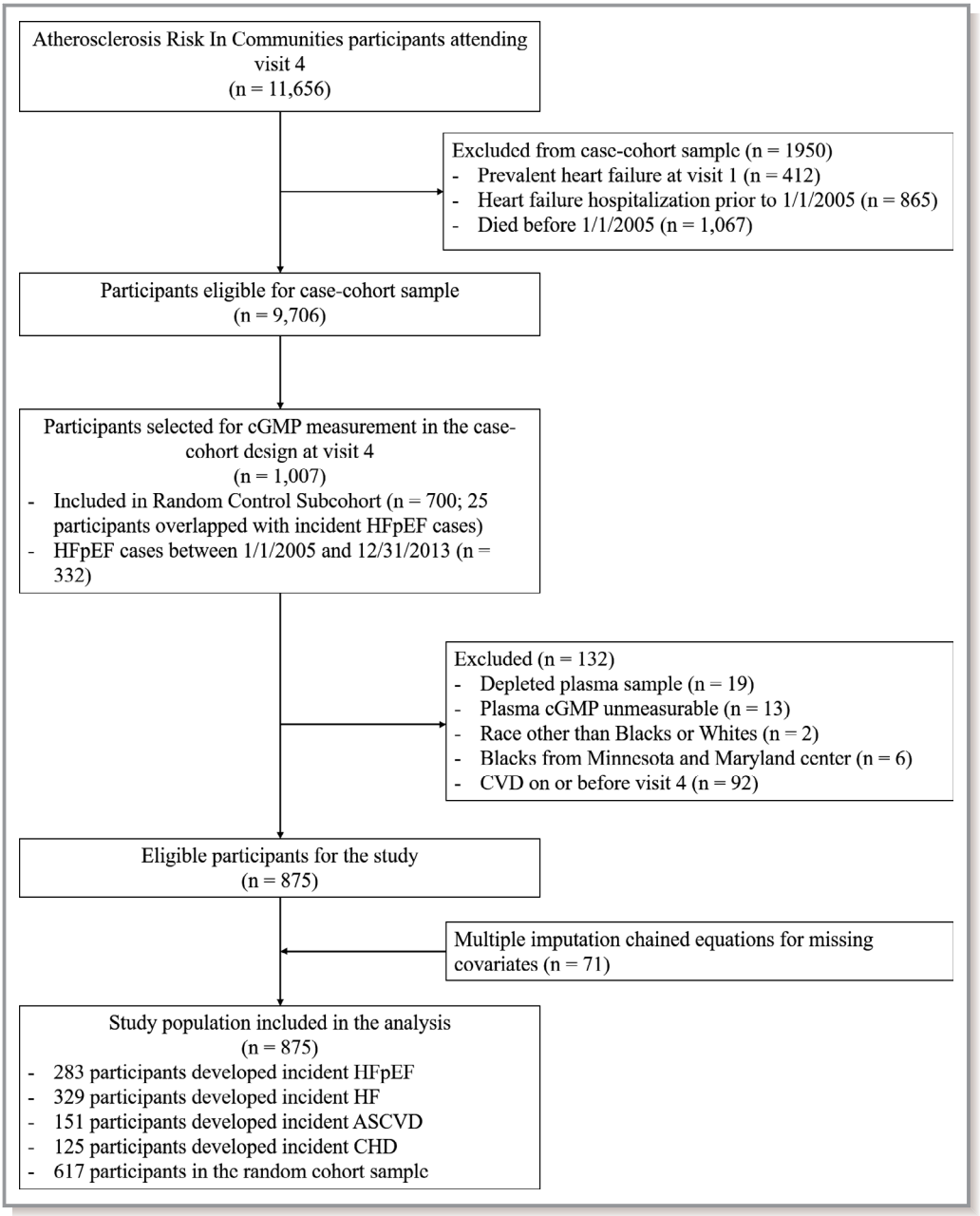

Figure 1. Flowchart of study participants. ASCVD indicates atherosclerotic cardiovascular disease; cGMP, cyclic guanosine monophosphate; CHD, coronary heart disease; CVD, cardiovascular disease; HFpEF, heart failure with preserved ejection fraction.

optional acetylation procedure per manufacturer protocol. Intra- and interassay coefficients of variation were 4.2% and 13.5%, respectively.

Outcome Assessment

The primary outcome of this study is HFpEF. The secondary outcomes are any HF, ASCVD, and CHD. Participants were followed for CVD-related events from visit 4 (1996–1998) through December 31, 2016 (the administrative censoring date by the time of data analysis). Incident ASCVD and HF events were identified through annual follow-up telephone interviews, local hospital discharge lists, and death records from the National Death Index.[19] Information on all hospitalizations was extracted by trained staff and validated by physician reviewers.

HF was defined as the first HF hospitalization (*International Classification of Diseases, Ninth Revision* [*ICD-9*] code 428) or death related to HF (*ICD-9* code 428 or *ICD-10* code I-50).[20] HF types were classified based on left ventricular ejection

fraction results from inpatient or pre-admission tests. HFpEF was defined as a normal or mildly decreased systolic function (left ventricular ejection fraction ≥50%) within 2 years of reviewer assessment.[21] The median (interquartile range) difference of the EF and HF admission date was 1 (0–2) days.

CHD was defined as definite or probable myocardial infarction, definite fatal CHD, or cardiac procedure (percutaneous coronary interventions, bypass surgery, or coronary revascularization). ASCVD was defined as CHD or ischemic stroke (definite or probable embolic or thrombotic brain infarction).

All ASCVD and HF events were adjudicated by a physician panel using standardized criteria through review of death certificates, hospital discharge summaries, physician notes, and clinical, laboratory, and/or imaging data. While ASCVD, CHD, and any HF events have been ascertained since the ARIC baseline visit, the adjudication for the HF subtypes of HFpEF and HFrEF only became available for HF cases occurring after January 1, 2005.

Risk Factor Assessment

CVD risk factors were collected at baseline and at each follow-up visit. Sports physical activities were assessed via a modified Baecke questionnaire at visit 3.[22] Weight and height were measured in light clothing. Sitting blood pressure measurements were taken 3 times after 5 minutes of rest during each visit using a random-zero sphygmomanometer. Blood pressure measurements at visit 4 were calculated as average of the first and second measurements (second and third measurements in previous visits).[23] Diabetes mellitus was defined by self-report of a physician diagnosis, a fasting blood glucose level ≥126 mg/dL, a nonfasting blood glucose level ≥200 mg/dL, or use of hypoglycemic medications. Total cholesterol, high-density lipoprotein cholesterol, and triglyceride levels were measured using standardized enzymatic methods. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation based on serum creatinine.[24] Plasma NT-proBNP was measured using an electrochemiluminescent immunoassay on an automated Cobas e411 analyzer (Roche Diagnostics).[25]

Statistical Analysis

The study end points were the development of incident HFpEF, HF, ASCVD, and CHD. Participants were followed from visit 4 until the development of a study end point, death, dropout, or until December 31, 2016 (the administrative censoring date by the time of data analysis). We used multiple imputation with chained equations to impute missing covariates (8%). Since adjudication for HFpEF cases was only available after January 1, 2005, we specified delayed entry on January 1, 2005 for all analyses (median follow-up 9.9 years).[26]

We used Cox proportional hazards regression models to estimate hazard ratios and 95% CI for the CVD outcomes associated with cGMP tertiles. The tertiles were based on the distribution of cGMP in the random cohort sample, and estimated hazard ratios comparing the second and third tertiles with the first tertile (reference category). To account for the case–cohort design, we used the method of Lin[27] to fit weighted proportional hazards models. In addition to modeling cGMP as categorical variable, we assessed associations of cGMP as a continuous variable with CVD outcomes, per a 1 SD increase in $\log_e$-transformed cGMP levels. We also modeled $\log_e$-transformed cGMP levels as restricted cubic splines with knots at the 5th, 50th, and 95th percentiles of its distribution in the cohort random sample.

For each outcome, we used 4 models with increasing degrees of adjustment. Model 1 adjusted for demographic factors: age, sex, and race/center groups. Model 2 further adjusted for lifestyle variables: education, physical activity, smoking, alcohol consumption, and body mass index. Model 3 further adjusted for intermediate CVD risk factors: systolic blood pressure, use of antihypertensive medications, total cholesterol, high-density lipoprotein cholesterol, use of lipid-lowering medication, diabetes mellitus, and estimated glomerular filtration rate. Finally, model 4 further adjusted for $\log_e$-transformed NT-proBNP.

We conducted subgroup analyses by sex, race, and NT-proBNP tertile. Spearman's rank-order correlation efficient was used to assess the correlation between cGMP and NT-proBNP. Finally, we performed additional analyses to evaluate the association of NT-proBNP levels with CVD outcomes adjusting for $\log_e$-transformed cGMP. All reported *P* values were 2-sided and the significance level was set at 0.05. Statistical analyses were performed using Stata version 15 (StataCorp LP, College Station, TX).

Results

The average age of participants in the random cohort sample (n=617) at baseline was 62.4 years (SD 5.6), and their median cGMP was 3.3 pmol/mL (interquartile interval 2.3–4.4). Participants with higher cGMP levels were more likely to be older, black, and nondiabetic; they were more likely to have lower levels of body mass index, triglycerides, estimated glomerular filtration rate, and higher levels of systolic blood pressure, high-density lipoprotein cholesterol, and NT-proBNP (Table 1). cGMP and NT-proBNP were correlated with a Spearman correlation coefficient of 0.37. The baseline characteristics of participants who developed HFpEF, HF, ASCVD, and CHD over follow-up are shown in Table S1.

Table 1. Baseline Characteristics by cGMP Tertiles Among Random Cohort Sample

	Overall	Tertile 1 (0.2 to <2.6 pmol/mL)	Tertile 2 (2.6 to <4.0 pmol/mL)	Tertile 3 (4.0 to 19.8 pmol/mL)	*P* Value
N	617	211	210	196	
Age, y	62.4 (5.6)	61.3 (5.0)	63.6 (5.7)	62.4 (5.8)	<0.001
Sex (% men)	272 (44.1)	91 (43.1)	101 (48.1)	80 (40.8)	0.32
Race (% black)	139 (22.5)	38 (18.0)	47 (22.4)	54 (27.6)	0.07
Education* (%)					
<High school	89 (14.5)	27 (12.9)	36 (17.1)	26 (13.4)	0.64
High school or vocational school	274 (44.6)	94 (44.8)	88 (41.9)	92 (47.4)	
College, graduate, or professional school	251 (40.9)	89 (42.4)	86 (41.0)	76 (39.2)	
BMI, kg/m^2	28.8 (5.8)	29.5 (5.8)	29.0 (5.5)	28.0 (5.9)	0.02
Physical activity index†	2.6 (0.8)	2.5 (0.9)	2.7 (0.8)	2.5 (0.8)	0.07
Smoking status, %					
Never	257 (41.9)	87 (41.6)	86 (41.1)	84 (43.1)	0.72
Former	263 (42.9)	91 (43.5)	95 (45.5)	77 (39.5)	
Current	93 (15.2)	31 (14.8)	28 (13.4)	34 (17.4)	
Alcohol consumption (%)					
Noncurrent	280 (45.7)	83 (39.7)	105 (50.2)	92 (47.2)	0.08
Current	333 (54.3)	126 (60.3)	104 (49.8)	103 (52.8)	
Systolic BP, mm Hg	127.0 (18.3)	122.6 (16.3)	127.8 (17.5)	130.7 (20.2)	<0.001
Diastolic BP, mm Hg)	71.1 (9.8)	70.2 (9.7)	71.5 (10.1)	71.6 (9.6)	0.26
Use of hypertension medications, %	200 (32.6)	58 (27.5)	67 (32.2)	75 (38.5)	0.06
Total cholesterol, mg/dL	200.7 (36.0)	205.2 (39.6)	196.8 (33.2)	200.3 (34.6)	0.06
HDL cholesterol, mg/dL	51.0 (17.5)	50.1 (16.8)	49.1 (15.8)	54.1 (19.5)	0.01
Triglycerides, mg/dL	118.0 (86.0, 166.0)	133.0 (84.0, 175.0)	120.0 (90.0, 167.0)	110.0 (83.5, 146.5)	0.04
Use of lipid-lowering medications (%)	61 (9.9)	19 (9.0)	24 (11.4)	18 (9.2)	0.65
Diabetes mellitus, %	71 (11.6)	29 (13.8)	30 (14.4)	12 (6.1)	0.02
eGFR, mL/min per 1.73 m^2	87.4 (15.1)	90.9 (14.1)	85.7 (14.7)	85.5 (16.1)	<0.001
Estradiol, pg/mL	27.5 (13.1, 39.2)	26.4 (11.7, 36.5)	27.2 (14.4, 39.1)	28.8 (13.1, 42.4)	0.18
NT-proBNP, pg/mL	59.4 (28.7, 111.6)	41.5 (22.4, 69.4)	57.2 (27.7, 110.6)	90.6 (57.7, 151.3)	<0.001

BMI indicates body mass index; BP, blood pressure; cGMP, cyclic guanosine monophosphate; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; NT-proBNP, N-terminal pro B-type natriuretic peptide.

*Values in the table are mean (SD), median (interquartile interval), or number (percentage).

†Data for physical activity are from Atherosis Risk in Communities (ARIC) visit 3.

During a median of 9.9 years of follow-up, there were 283 incident cases of HFpEF, 329 any HF, 151 ASCVD, and 125 CHD. In models adjusted for demographics, lifestyle characteristics, and CVD risk factors, higher plasma cGMP levels were associated with a higher risk of incident CVD events (Table 2, model 3). The hazard ratios (95% CI) associated with the highest tertile of cGMP compared with lowest for HFpEF, HF, ASCVD, and CHD were 1.88 (1.17–3.02), 2.18 (1.18–4.06), 2.84 (1.44–5.60), and 2.43 (1.19–5.00), respectively. In models further adjusted for NT-proBNP, the associations were attenuated and no longer significant for HFpEF and HF, but remained statistically significant for ASCVD and CHD (hazard ratio [95% CI] for HFpEF: 1.30 [0.79–2.14], HF: 1.68 [0.88–3.22], ASCVD: 2.56 [1.26–.20] and CHD: 2.25 [1.07–.71]) (Table 2, model 4).

Table 2. Hazard Ratios (95% CI) for Cardiovascular Outcome Associations With cGMP Levels

	N Events/Person-Years	IR	Model 1	Model 2	Model 3	Model 4
HFpEF						
cGMP tertiles						
First	81/33 303	4.4	1 (Ref.)	1 (Ref.)	1 (Ref.)	1 (Ref.)
Second	88/29 924	5.3	1.00 (0.68, 1.47)	1.07 (0.70, 1.64)	1.12 (0.72, 1.75)	1.02 (0.65, 1.61)
Third	114/27 789	7.5	1.49 (1.02, 2.16)*	1.82 (1.21, 2.73)*	1.88 (1.17, 3.02)*	1.30 (0.79, 2.14)
Per 1 SD increase in $\log_e$-cGMP	283/91 016	5.6	1.24 (1.05, 1.48)*	1.35 (1.10, 1.65)*	1.30 (1.04, 1.63)*	1.08 (0.86, 1.36)
Any heart failure						
cGMP tertiles						
First	90/33 252	7.9	1 (Ref.)	1 (Ref.)	1 (Ref.)	1 (Ref.)
Second	105/29 788	11.9	1.19 (0.71, 2.01)	1.38 (0.76, 2.50)	1.45 (0.80, 2.64)	1.36 (0.75, 2.45)
Third	134/27 583	16.5	1.81 (1.10, 2.97)*	2.34 (1.32, 4.14)*	2.18 (1.18, 4.06)*	1.68 (0.88, 3.22)
Per 1 SD increase in $\log_e$-cGMP	329/90 623	11.8	1.31 (1.06, 1.61)*	1.43 (1.11, 1.84)*	1.34 (1.03, 1.74)*	1.17 (0.89, 1.54)
ASCVD						
cGMP tertiles						
First	37/31 211	6.2	1 (Ref.)	1 (Ref.)	1 (Ref.)	1 (Ref.)
Second	44/27 264	9.3	1.34 (0.66, 2.72)	1.40 (0.69, 2.85)	1.36 (0.68, 2.74)	1.33 (0.67, 2.67)
Third	70/26 183	19.3	3.01 (1.63, 5.57)*	3.32 (1.81, 6.11)*	2.84 (1.44, 5.60)*	2.56 (1.26, 5.20)*
Per 1 SD increase in $\log_e$- cGMP	151/84 659	11.2	1.47 (1.10, 1.98)*	1.48 (1.10, 2.00)*	1.29 (0.94, 1.78)	1.18 (0.84, 1.65)
Coronary heart disease						
cGMP tertiles						
First	33/31 443	5.9	1 (Ref.)	1 (Ref.)	1 (Ref.)	1 (Ref.)
Second	35/27 812	6.7	1.02 (0.47, 2.22)	1.08 (0.50, 2.34)	1.05 (0.49, 2.26)	1.03 (0.48, 2.22)
Third	57/27 416	14.5	2.54 (1.32, 4.89)*	2.80 (1.45, 5.39)*	2.43 (1.19, 5.00)*	2.25 (1.07, 4.71)*
Per 1 SD increase in $\log_e$-cGMP	125/86 671	8.9	1.44 (1.01, 2.05)*	1.44 (1.01, 2.06)*	1.29 (0.90, 1.87)	1.20 (0.83, 1.75)

ASCVD indicates atherosclerotic cardiovascular disease; cGMP, cyclic guanosine monophosphate; HFpEF, heart failure with preserved ejection fraction; IR, incident rate.
Model 1: age, sex, race/center. Model 2: model 1 + education, smoking, alcohol consumption, body mass index, physical activity. Model 3: model 2 + systolic blood pressure, hypertension medication, diabetes mellitus, total and high-density lipoprotein cholesterol, lipid-lowering medication, estimated glomerular filtration rate. Model 4: model 3 + log-transformed N-terminal pro B-type natriuretic peptide. 1 SD of $\log_e$-transformed cGMP levels: 0.64.
*P<0.05.

In spline regression analyses, there was generally a positive dose–response relationship between higher cGMP levels and incident CVD outcomes (Figure 2). The P values for the nonlinear spline components of cGMP for the outcomes of HFpEF, HF, ASCVD, and CHD were 0.80, 0.27, 0.90, and 0.52, respectively, indicating that the associations between cGMP with these end points were approximately linear.

In subgroup analysis, the associations between cGMP and CVD outcomes were consistent across NT-pro-BNP levels, with P values for interaction of 0.94, 0.32, 0.56, and 0.52 for HFpEF, HF, ASCVD, and CHD, respectively (Table 3). There were no significant interactions by sex and race for the associations of cGMP and CVD outcomes, although the associations appeared to be stronger in women than men (Tables S2, S3). Finally, the associations between NT-proBNP and HFpEF/HF outcomes were slightly attenuated but remained strong after additionally adjusting for $\log_e$-transformed cGMP (Table S4). However, the association between NT-proBNP with ASCVD/CHD outcomes was no longer significant after adjusting for cGMP.

Discussion

In this community-based cohort of middle-aged to older men and women followed for 10 years, higher plasma cGMP levels were associated with an increased risk of incident HF and CVD outcomes. However, the associations for HFpEF and HF were largely explained by NT-proBNP, an upstream messenger to cGMP in the NP signaling pathway. On the other hand, the associations of cGMP with ASCVD and CHD, while attenuated, remained strong and statistically significant after adjustment

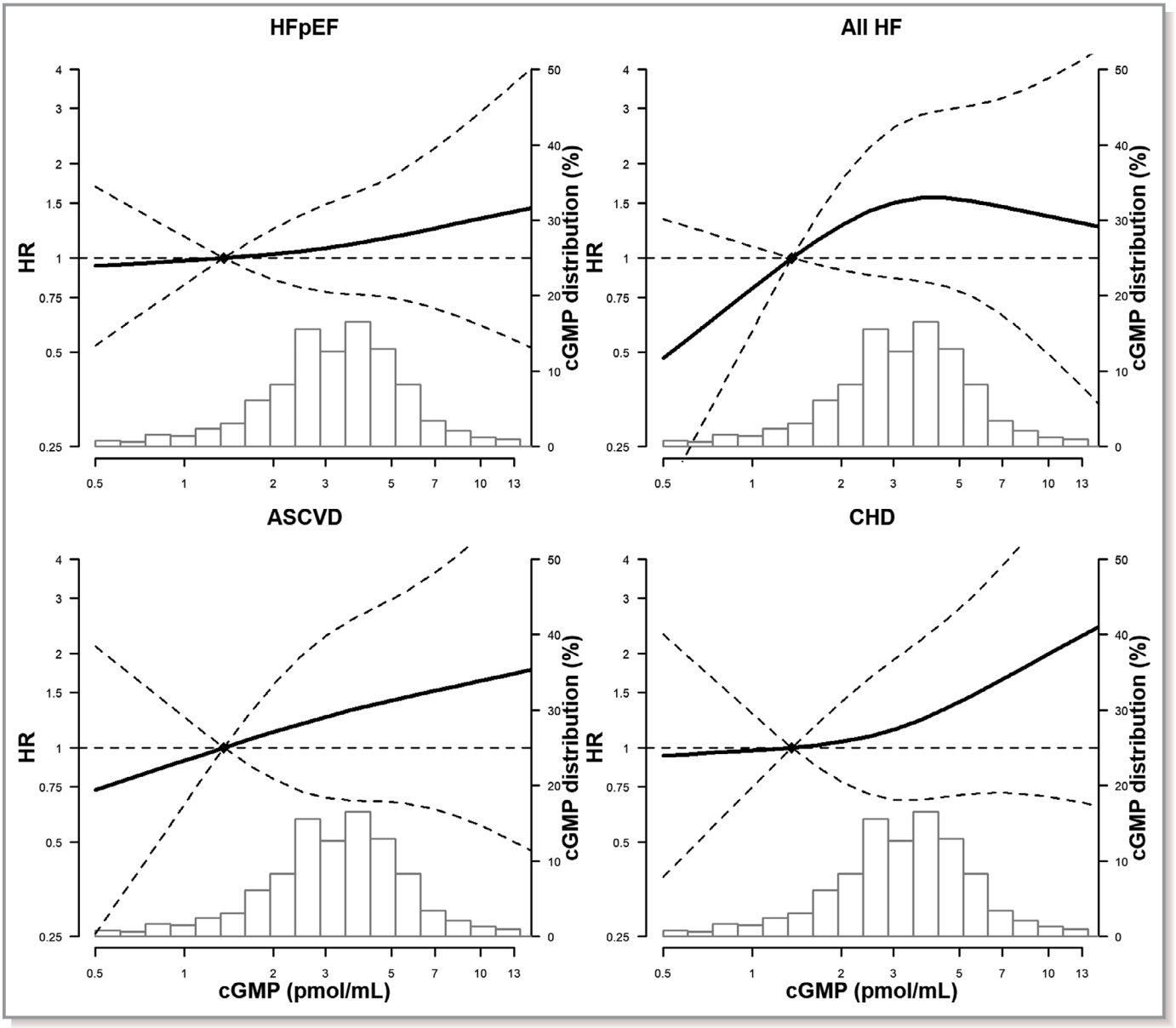

Figure 2. HR for incident cardiovascular outcomes by cGMP levels. The curves represent the adjusted hazard ratios for heart failure with preserved ejection fraction (HFpEF), heart failure (HF), atherosclerotic cardiovascular disease (ASCVD), and coronary heart disease (CHD) by log-cGMP levels. The dose–response association was estimated using linear cubic splines for log-cGMP levels in multivariable Cox regression models. The models were adjusted for age, sex, race/center, education, smoking, alcohol consumption, body mass index, physical activity, systolic blood pressure, hypertension medication, diabetes mellitus, total and high-density lipoprotein cholesterol, lipid-lowering medication, estimated glomerular filtration rate, and log-transformed NT-proBNP. Curves represent adjusted HRs (solid lines) and their 95% CI (dashed lines) based on restricted cubic splines for log-cGMP with knots at the 5th, 50th, and 95th percentiles of the distribution of cGMP in the random cohort sample. The reference values (diamond dots) were set at 10th percentile of the distribution of cGMP in the random cohort sample. The histogram represents the distribution of cGMP in the random cohort sample. ASCVD indicates atherosclerotic cardiovascular disease; cGMP, cyclic guanosine monophosphate; CHD, coronary heart disease; HFpEF, heart failure with preserved ejection fraction; HR, hazard ratio; NT-proBNP, N-terminal pro B-type natriuretic peptide.

for NT-proBNP, suggesting alternate pathways in their relationships not fully explained by the NP pathway. The associations between cGMP levels and CVD outcomes were generally consistent by sex and NT-proBNP levels, although the associations appeared to be stronger in women than in men. Our findings contradict our initial hypothesis of an inverse association between cGMP and CVD outcomes, and suggest that the association of plasma cGMP with HF outcomes reflect the underlying association between NT-proBNP and HF outcomes. In hindsight, this makes plausible biological sense given that circulating plasma cGMP is largely reflective of NP, and not NO, pools.

Table 3. Hazard Ratios (95% CI) for Cardiovascular Outcomes Associations With cGMP and NT-proBNP Levels

	NT-pro-BNP tertiles			
	First	Second	Third	
HFpEF				
cGMP tertiles				
First	1 (Ref.)	1 (Ref.)	1 (Ref.)	
Second	0.88 (0.38, 2.01)	1.41 (0.69, 2.88)	0.58 (0.25, 1.33)	
Third	1.58 (0.54, 4.60)	1.39 (0.66, 2.95)	1.21 (0.57, 2.57)	
Per 1-SD increase in $\log_e$-cGMP	1.22 (0.71, 2.08)	1.09 (0.80, 1.50)	1.11 (0.75, 1.63)	
P interaction				0.94
Any heart failure				
cGMP tertiles				
First	1 (Ref.)	1 (Ref.)	1 (Ref.)	
Second	0.99 (0.33, 2.97)	1.78 (0.69, 4.55)	1.34 (0.49, 3.71)	
Third	2.53 (0.83, 7.69)	2.71 (1.06, 6.92)†	1.36 (0.54, 3.46)	
Per 1 SD increase in $\log_e$-cGMP	1.56 (0.77, 3.17)	1.42 (0.93, 2.16)	1.03 (0.70, 1.51)	
P interaction				0.32
ASCVD				
cGMP tertiles				
First	1 (Ref.)	1 (Ref.)	1 (Ref.)	
Second	0.61 (0.15, 2.43)	1.79 (0.55, 5.86)	1.89 (0.51, 6.96)	
Third	2.80 (0.77, 10.18)	4.40 (1.43, 13.55)†	2.02 (0.65, 6.28)	
Per 1 SD increase in $\log_e$-cGMP	1.09 (0.50, 2.35)	1.53 (0.88, 2.65)	1.06 (0.71, 1.58)	
P interaction				0.56
Coronary heart disease				
cGMP tertiles				
First	1 (Ref.)	1 (Ref.)	1 (Ref.)	
Second	0.70 (0.18, 2.75)	1.27 (0.33, 4.84)	1.16 (0.31, 4.39)	
Third	1.96 (0.43, 8.90)	3.37 (1.09, 10.44)†	1.88 (0.62, 5.70)	
Per 1 SD increase in $\log_e$-cGMP	0.91 (0.48, 1.73)	1.49 (0.78, 2.85)	1.28 (0.83, 1.97)	
P interaction				0.52

ASCVD indicates atherosclerotic cardiovascular disease; cGMP, cyclic guanosine monophosphate; HFpEF, heart failure with preserved ejection fraction; NT-proBNP, N-terminal pro B-type natriuretic peptide.

1 SD of $\log_e$-transformed cGMP levels: 0.64.

*Model adjusted for age, sex, race/center, education, smoking, alcohol consumption, body mass index, physical activity, systolic blood pressure, hypertension medication, diabetes mellitus, total and high-density lipoprotein cholesterol, lipid-lowering medication, and estimated glomerular filtration rate.

†$P<0.05$.

cGMP Signaling Pathways and Mechanisms of Action

BNP is a hormone released by myocardial cells as a compensatory mechanism in response to ventricular wall stretch stemming from increased ventricular blood volume.[28] BNP-signaling binds to the particulate (membrane-bound) guanylate cyclase complex (guanylyl cyclases-A), which stimulates the synthesis of cGMP. cGMP in turn binds to regulatory domains in protein kinase G as well as modulates selective phosphodiesterases that control cGMP or cyclic adenosine monophosphate hydrolysis[7]. Prior Mendelian randomization studies have suggested a protective effect of the NP-cGMP pathway, demonstrating that genetic variants associated with increased circulating BNP were found to be associated with reduced frequency of hypertension, metabolic dysfunction, and mortality.[29,30] However, the current study found a direct association between plasma NT-proBNP and cGMP with CVD outcomes.

Rather than suggesting cGMP and its associated signaling are maladaptive, the new findings are likely explained by 2 factors. First, elevated plasma cGMP, like NT-proBNP, serves as a biomarker for subclinical CVD, its elevation being a reflection of compensations that ultimately proved inadequate. Second, cGMP-targeting phosphodiesterases could have potentially depressed cGMP levels despite higher NP-stimulated synthesis. If so, the relation of cGMP to CVD risk would not have mirrored that of NT-proBNP. That they do mirror one another indicates that phosphodiesterases regulatory differences are less important in predicting CVD evolution.

Another reason for the observed correlations between cGMP and NT-proBNP is that we assessed plasma, which reflects cGMP production via the NP pathway more than the NO pathway.[31] This is because NP-generated cGMP resides at the plasma membrane and is secreted into the extracellular space, whereas NO signaling via intracellular guanylyl cyclases-1 generates very small compartmentalized cGMP that is not as easily detected in plasma.[32] A prior study found that infusion of the endogenous NO inhibitor, asymmetrical dimethylarginine, decreased plasma cGMP, lowered cardiac output, and increased vascular resistance.[33] However, in other studies, nitrate therapy decreased[17] or did not change plasma cGMP.[34] Similarly, in the INDIE-HFpEF (Inorganic Nitrite Delivery to Improve Exercise Capacity in HFpEF) trial, inorganic nitrite also did not change plasma cGMP levels.[35] By contrast, studies of sacubitril/valsartan therapy in HFrEF subjects, the former being a neprilysin inhibitor that can augment NP-dependent signaling, have consistently found associations with elevated plasma and urinary cGMP.[10] Both plasma and urinary cGMP are derived from the NP pathway, but they are not correlated.[36] Plasma cGMP is only partially eliminated through renal clearance, whereas urinary cGMP is primarily generated by renal cells.[31]

Population-Based Studies

Based on preclinical and small clinical studies, stimulation of the cGMP pathway appeared to be a promising potential therapeutic strategy for the treatment of HFpEF.[37] Nevertheless, more understanding is needed regarding the utility of measuring plasma cGMP levels as a prognostic marker of incident disease. However, few prior population studies have evaluated the association between cGMP with incident CVD risk. A case–control study showed that plasma cGMP levels were higher in 18 HF patients compared with 15 controls.[17] Similarly, plasma and urinary cGMP levels were significantly higher in 50 congestive HF patients than in 70 randomly selected healthy participants.[36] In another study of 84 asymptomatic men, plasma cGMP was positively associated with carotid intima-media thickness and diameter, but was not associated with atherosclerotic plaques.[16] These studies were limited by cross-sectional or retrospective designs, small sample sizes, poor adjustment for covariates, and the use of highly selected samples. Importantly, our study newly adds to the literature by now presenting associations of plasma cGMP with various incident CVD outcomes in a community-based cohort free of HF and CVD at baseline, and our prospective findings of cGMP being associated with incident CVD events are consistent with prior cross-sectional studies.

Biomarker Paradox

In the PARADIGM-HF (Prospective Comparison of ARNI [Angiotensin Receptor–Neprilysin Inhibitor] with ACEI [Angiotensin-Converting–Enzyme Inhibitor] to Determine Impact on Global Mortality and Morbidity in Heart Failure) trial, both urinary cGMP and plasma BNP levels were higher with sacubitril/valsartan treatment than with enalapril, but NT-proBNP levels were lower. Since BNP is a substrate for neprilysin, the higher BNP levels likely reflect the effect of the neprilysin inhibitor sacubitril while the lower concentrations of NT-proBNP on drug treatment likely reflect the favorable effects on reducing myocardial stress.[10] This paradox highlights the complexity of interpreting these biomarkers in serum/plasma, where despite the cardioprotective effects of natriuretic peptides, plasma levels of NT-proBNP are elevated in pathological states.

We had hypothesized that cGMP would be inversely associated with CVD outcomes, but found the opposite instead. The NP pathway is a major upstream regulator of plasma cGMP,[31] and our findings here in the ARIC study, as well as in a cross-sectional analysis from the MESA study (Multi-Ethnic Study of Atherosclerosis),[38] indicate that plasma cGMP levels directly track with plasma NT-proBNP levels. Our results suggest that abnormalities in the NP-cGMP signaling pathway may precede HFpEF and other CVDs. These changes may be amenable to therapeutic interventions. However, in our study, the associations between cGMP and CVD outcomes did not differ across NT-proBNP levels, suggesting that the cardiovascular effects of cGMP did not depend on the bioavailability of NT-proBNP. A limitation of our study was the lack of information on other natriuretic peptides including BNP and atrial natriuretic peptide, as well as the lack of information on NO-dependent cGMP, and we were not able to tease out the associations of plasma cGMP derived from other signaling pathways.

Race and Sex Differences

We could not detect differences in the association of cGMP levels with CVD end points by sex and race. However,

because of limited power, the effect modifications by sex and race require evaluation in other studies. Plasma cGMP levels in our random cohort sample were slightly lower in whites than in blacks (median 3.1 versus 3.7 pmol/mL, p=0.03), whereas previous studies demonstrate that plasma NT-proBNP levels were higher in whites than in blacks in ARIC and in other cohorts,[39,40] and genetic European ancestry was associated with higher NT-proBNP levels compared with African ancestry.[40,41] The associations of NT-proBNP with all-cause and cardiovascular mortality were similar by race in the REGARDS (Reasons for Geographic and Racial Differences in Stroke) study,[39] but there may be racial differences in NP-mediated cGMP production and its association with CVD outcomes. Additionally, women have both higher cGMP levels and NT-proBNP levels when compared with men,[42] and the prevalence of HFpEF is higher in women versus men.[43] The racial and sex differences in cGMP signaling physiology and their implications for prognosis and treatment of CVD need to be further examined in population studies, including a more detailed evaluation of the differences in genetic variants that determine cGMP levels.

Strengths and Limitations

Our study has some additional limitations. First, we measured plasma cGMP and NT-proBNP at only a single point in time and did not have data on longitudinal changes that may be more informative of the underlying cardiovascular changes that determine disease risk. Single measurements may have also resulted in measurement error because of within-person variability in cGMP and NT-proBNP levels. Second, our study was designed primarily to evaluate the association of cGMP with incident HFpEF (the case status selected for our case–cohort design), and had limited power to evaluate some CVD end points such as stroke and HFrEF, as well as to perform subgroup analyses. Third, we could only identify HF and CVD cases through hospitalization or death certificate, which might miss patients who were not hospitalized presenting with less severe cases of HF managed entirely in the outpatient setting. Nevertheless, among HF diagnosed in a community-based outpatient setting, 74% are hospitalized within 1.7 years.[44] Finally, our study was observational in nature, and we were not able to evaluate whether inhibition or enhancement of cGMP by therapeutic interventions were associated with changes of risk for CVD outcomes.

The strengths of this study included the use of a well-established cohort with a rigorous study protocol, high-quality measurements of cGMP and other study variables, and detailed information on multiple potential factors, including NT-proBNP. The 10 years of follow-up also enabled us to estimate the long-term associations of cGMP with incident ASCVD and HF risk.

Conclusions

In summary, our findings suggest that abnormalities in the NP-cGMP signaling pathway precede the development of HFpEF and other CVD end points. We found that higher cGMP levels were associated with incident HFpEF and any HF, but these associations were attenuated and were no longer significant after adjusting for NT-proBNP. Since cGMP synthesis is activated by NPs, our findings suggest that the association of plasma cGMP with HF outcomes reflects the underlying association between NT-proBNP and HF outcomes. However, the associations of the highest tertile of cGMP levels (compared with lowest) with ASCVD and CHD outcomes remained statistically significant even after adjusting for NT-proBNP, suggesting other pathways explain the relationship that are not entirely mediated through NT-proBNP. Repeated measurements of biomarkers in this pathway are needed to better understand the complex changes that occur before the development of clinically overt CVD. Additional studies should further explore the potential role of cGMP as a diagnostic and prognostic biomarker for HF and other CVD outcomes.

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Disclosures

None.

References

1. Owan TE, Hodge DO, Herges RM, Jacobsen SJ, Roger VL, Redfield MM. Trends in prevalence and outcome of heart failure with preserved ejection fraction. *N Engl J Med*. 2006;355:251–259.
2. Maeder MT, Kaye DM. Heart failure with normal left ventricular ejection fraction. *J Am Coll Cardiol*. 2009;53:905–918.

3. Borlaug BA, Paulus WJ. Heart failure with preserved ejection fraction: pathophysiology, diagnosis, and treatment. *Eur Heart J*. 2011;32:670–679.
4. Montfort WR, Wales JA, Weichsel A. Structure and activation of soluble guanylyl cyclase, the nitric oxide sensor. *Antioxid Redox Signal*. 2017;26:107–121.
5. Kuhn M. Molecular physiology of membrane guanylyl cyclase receptors. *Physiol Rev*. 2016;96:751–804.
6. Lee DI, Zhu G, Sasaki T, Cho GS, Hamdani N, Holewinski R, Jo SH, Danner T, Zhang M, Rainer PP, Bedja D, Kirk JA, Ranek MJ, Dostmann WR, Kwon C, Margulies KB, Van Eyk JE, Paulus WJ, Takimoto E, Kass DA. Phosphodiesterase 9A controls nitric-oxide-independent cGMP and hypertrophic heart disease. *Nature*. 2015;519:472–476.
7. Kokkonen K, Kass DA. Nanodomain regulation of cardiac cyclic nucleotide signaling by phosphodiesterases. *Annu Rev Pharmacol Toxicol*. 2017;57:455–479.
8. Ranek MJ, Kokkonen-Simon KM, Chen A, Dunkerly-Eyring BL, Vera MP, Oeing CU, Patel CH, Nakamura T, Zhu G, Bedja D, Sasaki M, Holewinski RJ, Van Eyk JE, Powell JD, Lee DI, Kass DA. PKG1-modified TSC2 regulates mTORC1 activity to counter adverse cardiac stress. *Nature*. 2019;566:264–269.
9. Armstrong PW, Roessig L, Patel MJ, Anstrom KJ, Butler J, Voors AA, Lam CSP, Ponikowski P, Temple T, Pieske B, Ezekowitz J, Hernandez AF, Koglin J, O'Connor CM. A multicenter, randomized, double-blind, placebo-controlled trial of the efficacy and safety of the oral soluble guanylate cyclase stimulator: the VICTORIA Trial. *JACC Heart Fail*. 2018;6:96–104.
10. Packer M, McMurray JJ, Desai AS, Gong J, Lefkowitz MP, Rizkala AR, Rouleau JL, Shi VC, Solomon SD, Swedberg K, Zile M, Andersen K, Arango JL, Arnold JM, Belohlavek J, Bohm M, Boytsov S, Burgess LJ, Cabrera W, Calvo C, Chen CH, Dukat A, Duarte YC, Erglis A, Fu M, Gomez E, Gonzalez-Medina A, Hagege AA, Huang J, Katova T, Kiatchoosakun S, Kim KS, Kozan O, Llamas EB, Martinez F, Merkely B, Mendoza I, Mosterd A, Negrusz-Kawecka M, Peuhkurinen K, Ramires FJ, Refsgaard J, Rosenthal A, Senni M, Sibulo AS Jr, Silva-Cardoso J, Squire IB, Starling RC, Teerlink JR, Vanhaecke J, Vinereanu D, Wong RC. Angiotensin receptor neprilysin inhibition compared with enalapril on the risk of clinical progression in surviving patients with heart failure. *Circulation*. 2015;131:54–61.
11. Solomon SD, Rizkala AR, Lefkowitz MP, Shi VC, Gong J, Anavekar N, Anker SD, Arango JL, Arenas JL, Atar D, Ben-Gal T, Boytsov SA, Chen CH, Chopra VK, Cleland J, Comin-Colet J, Duengen HD, Echeverria Correa LE, Filippatos G, Flammer AJ, Galinier M, Godoy A, Goncalvesova E, Janssens S, Katova T, Kober L, Lelonek M, Linssen G, Lund LH, O'Meara E, Merkely B, Milicic D, Oh BH, Perrone SV, Ranjith N, Saito Y, Saraiva JF, Shah S, Seferovic PM, Senni M, Sibulo AS Jr, Sim D, Sweitzer NK, Taurio J, Vinereanu D, Vrtovec B, Widimsky J Jr, Yilmaz MB, Zhou J, Zweiker R, Anand IS, Ge J, Lam CSP, Maggioni AP, Martinez F, Packer M, Pfeffer MA, Pieske B, Redfield MM, Rouleau JL, Van Veldhuisen DJ, Zannad F, Zile MR, McMurray JJV. Baseline characteristics of patients with heart failure and preserved ejection fraction in the PARAGON-HF trial. *Circ Heart Fail*. 2018;11:e004962.
12. Tsai EJ, Kass DA. Cyclic GMP signaling in cardiovascular pathophysiology and therapeutics. *Pharmacol Ther*. 2009;122:216–238.
13. Cui R, Iso H, Pi J, Kumagai Y, Yamagishi K, Tanigawa T, Shimamoto T. Metabolic syndrome and urinary cGMP excretion in general population. *Atherosclerosis*. 2007;190:423–428.
14. Cui R, Iso H, Pi J, Kumagai Y, Yamagishi K, Tanigawa T, Shimamoto T. Relationship between urinary cGMP excretion and serum total cholesterol levels in a general population. *Atherosclerosis*. 2005;179:379–386.
15. Jafari B, Mohsenin V. Activation of heme oxygenase and suppression of cGMP are associated with impaired endothelial function in obstructive sleep apnea with hypertension. *Am J Hypertens*. 2012;25:854–861.
16. Devynck MA, Simon A, Pernollet MG, Chironi G, Gariepy J, Rendu F, Levenson J. Plasma cGMP and large artery remodeling in asymptomatic men. *Hypertension*. 2004;44:919–923.
17. Shotan A, Mehra A, Ostrzega E, Hsueh W, Do YS, Fisher DA, Hurst A, Johnson JV, Elkayam U. Plasma cyclic guanosine monophosphate in chronic heart failure: hemodynamic and neurohormonal correlations and response to nitrate therapy. *Clin Pharmacol Ther*. 1993;54:638–644.
18. The atherosclerosis risk in communities (ARIC) study: design and objectives. The ARIC investigators. *Am J Epidemiol*. 1989;129:687–702.
19. White AD, Folsom AR, Chambless LE, Sharret AR, Yang K, Conwill D, Higgins M, Williams OD, Tyroler HA. Community surveillance of coronary heart disease in the Atherosclerosis Risk in Communities (ARIC) Study: methods and initial two years' experience. *J Clin Epidemiol*. 1996;49:223–233.
20. Florido R, Kwak L, Lazo M, Nambi V, Ahmed HM, Hegde SM, Gerstenblith G, Blumenthal RS, Ballantyne CM, Selvin E, Folsom AR, Coresh J, Ndumele CE. Six-year changes in physical activity and the risk of incident heart failure: ARIC study. *Circulation*. 2018;137:2142–2151.
21. Chang PP, Wruck LM, Shahar E, Rossi JS, Loehr LR, Russell SD, Agarwal SK, Konety SH, Rodriguez CJ, Rosamond WD. Trends in hospitalizations and survival of acute decompensated heart failure in four US communities (2005–2014): ARIC study community surveillance. *Circulation*. 2018;138:12–24.
22. Baecke JA, Burema J, Frijters JE. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. *Am J Clin Nutr*. 1982;36:936–942.
23. Gress TW, Nieto FJ, Shahar E, Wofford MR, Brancati FL. Hypertension and antihypertensive therapy as risk factors for type 2 diabetes mellitus. Atherosclerosis Risk in Communities Study. *N Engl J Med*. 2000;342:905–912.
24. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF III, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150:604–612.
25. Michos ED, Selvin E, Misialek JR, McEvoy JW, Ndumele CE, Folsom AR, Ballantyne CM, Lutsey PL. 25-Hydroxyvitamin D levels and markers of subclinical myocardial damage and wall stress: the atherosclerosis risk in communities study. *J Am Heart Assoc*. 2016;5:e003575. DOI: 10.1161/JAHA.116.003575.
26. Hanley JA, Foster BJ. Avoiding blunders involving 'immortal time'. *Int J Epidemiol*. 2014;43:949–961.
27. Lin DY. On fitting Cox's proportional hazards models to survey data. *Biometrika*. 2000;87:37–47.
28. Kinnunen P, Vuolteenaho O, Ruskoaho H. Mechanisms of atrial and brain natriuretic peptide release from rat ventricular myocardium: effect of stretching. *Endocrinology*. 1993;132:1961–1970.
29. Seidelmann SB, Vardeny O, Claggett B, Yu B, Shah AM, Ballantyne CM, Selvin E, MacRae CA, Boerwinkle E, Solomon SD. An NPPB promoter polymorphism associated with elevated N-terminal pro-B-type natriuretic peptide and lower blood pressure. Hypertension, and mortality. *J Am Heart Assoc*. 2017;6:e005257. DOI: 10.1161/JAHA.116.005257.
30. Cannone V, Boerrigter G, Cataliotti A, Costello-Boerrigter LC, Olson TM, McKie PM, Heublein DM, Lahr BD, Bailey KR, Averna M, Redfield MM, Rodeheffer RJ, Burnett JC Jr. A genetic variant of the atrial natriuretic peptide gene is associated with cardiometabolic protection in the general community. *J Am Coll Cardiol*. 2011;58:629–636.
31. Mair J, Puschendorf B. Is measurement of cyclic guanosine monophosphate in plasma or urine suitable for assessing in vivo nitric oxide production? *Circulation*. 1998;97:1209–1210.
32. Murad F. Shattuck Lecture. Nitric oxide and cyclic GMP in cell signaling and drug development. *N Engl J Med*. 2006;355:2003–2011.
33. Kielstein JT, Impraim B, Simmel S, Bode-Boger SM, Tsikas D, Frolich JC, Hoeper MM, Haller H, Fliser D. Cardiovascular effects of systemic nitric oxide synthase inhibition with asymmetrical dimethylarginine in humans. *Circulation*. 2004;109:172–177.
34. Vorderwinkler KP, Artner-Dworzak E, Jakob G, Mair J, Diensti F, Pichler M, Puschendorf B. Release of cyclic guanosine monophosphate evaluated as a diagnostic tool in cardiac diseases. *Clin Chem*. 1991;37:186–190.
35. Borlaug BA, Anstrom KJ, Lewis GD, Shah SJ, Levine JA, Koepp GA, Givertz MM, Felker GM, LeWinter MM, Mann DL, Margulies KB, Smith AL, Tang WHW, Whellan DJ, Chen HH, Davila-Roman VG, McNulty S, Desvigne-Nickens P, Hernandez AF, Braunwald E, Redfield MM; National Heart, Lung, and Blood Institute Heart Failure Clinical Research Network. Effect of inorganic nitrite vs placebo on exercise capacity among patients with heart failure with preserved ejection fraction: the INDIE-HFpEF randomized clinical trial. *JAMA*. 2018;320:1764–1773.
36. Jakob G, Mair J, Vorderwinkler KP, Judmaier G, Konig P, Zwierzina H, Pichler M, Puschendorf B. Clinical significance of urinary cyclic guanosine monophosphate in diagnosis of heart failure. *Clin Chem*. 1994;40:96–100.
37. Greene SJ, Gheorghiade M, Borlaug BA, Pieske B, Vaduganathan M, Burnett JC Jr, Roessig L, Stasch JP, Solomon SD, Paulus WJ, Butler J. The cGMP signaling pathway as a therapeutic target in heart failure with preserved ejection fraction. *J Am Heart Assoc*. 2013;2:e000536DOI: 10.1161/JAHA.113.000536.
38. Ying W, Zhao D, Ouyang P, Subramanya V, Vaidya D, Ndumele CE, Guallar E, Sharma K, Shah SJ, Kass DA, Hoogeveen RC, Lima JA, Heckbert SR, deFilippi CR, Post WS, Michos ED. Associations Between the Cyclic Guanosine Monophosphate (cGMP) Pathway and Cardiovascular Risk Factors: MESA. *J Am Heart Assoc*. 2019;8:e013149. DOI:10.1161/JAHA.119.013149.
39. Bajaj NS, Gutierrez OM, Arora G, Judd SE, Patel N, Bennett A, Prabhu SD, Howard G, Howard VJ, Cushman M, Arora P. Racial differences in plasma levels

of N-terminal pro-B-type natriuretic peptide and outcomes: the reasons for geographic and racial differences in stroke (REGARDS) study. *JAMA Cardiol*. 2018;3:11–17.

40. Gupta DK, Daniels LB, Cheng S, deFilippi CR, Criqui MH, Maisel AS, Lima JA, Bahrami H, Greenland P, Cushman M, Tracy R, Siscovick D, Bertoni AG, Cannone V, Burnett JC, Carr JJ, Wang TJ. Differences in natriuretic peptide levels by race/ethnicity (from the multi-ethnic study of atherosclerosis). *Am J Cardiol*. 2017;120:1008–1015.
41. Wang TJ, Larson MG, Levy D, Benjamin EJ, Corey D, Leip EP, Vasan RS. Heritability and genetic linkage of plasma natriuretic peptide levels. *Circulation*. 2003;108:13–16.
42. Ying W, Zhao D, Ouyang P, Subramanya V, Vaidya D, Ndumele CE, Sharma K, Shah SJ, Heckbert SR, Lima JA, deFilippi CR, Budoff MJ, Post WS, Michos ED. 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.
43. Sharma K, Kass DA. Heart failure with preserved ejection fraction: mechanisms, clinical features, and therapies. *Circ Res*. 2014;115:79–96.
44. Roger VL, Weston SA, Redfield MM, Hellermann-Homan JP, Killian J, Yawn BP, Jacobsen SJ. Trends in heart failure incidence and survival in a community-based population. *JAMA*. 2004;292:344–350.

SUPPLEMENTAL MATERIAL

Table S1. Baseline characteristics of participants who developed HFpEF, HF, ASCVD, or CHD over follow-up.

	No events‡	HFpEF	HF	ASCVD	CHD
N	487	283	329	151	125
cGMP (pmol/mL)	3.1 (2.3, 4.3)	3.7 (2.5, 5.3)	3.7 (2.6, 5.2)	3.8 (2.7, 5.0)	3.9 (2.6, 4.9)
Age (years)	62.0 (5.6)	64.7 (5.3)	64.9 (5.2)	64.0 (5.5)	64.0 (5.4)
Sex (% men)	206 (42.3)	83 (29.3)	112 (34.0)	56 (37.1)	51 (40.8)
Race (% Black)	106 (21.8)	75 (26.5)	87 (26.4)	41 (27.2)	31 (24.8)
Education (%)**					
<High school	63 (13.0)	72 (25.4)	83 (25.2)	32 (21.3)	27 (21.8)
High school or vocational school	215 (44.3)	108 (38.2)	133 (40.4)	66 (44.0)	54 (43.5)
College, graduate, or professional school	207 (42.7)	103 (36.4)	113 (34.3)	52 (34.7)	43 (34.7)
BMI (kg/m^2)	28.6 (5.6)	30.7 (6.6)	30.4 (6.6)	29.9 (5.4)	29.7 (5.1)
Physical activity index†	2.6 (0.8)	2.5 (0.8)	2.4 (0.8)	2.5 (0.9)	2.6 (0.8)
Smoking Status (%)					
Never	214 (44.3)	109 (38.8)	120 (36.6)	59 (39.3)	47 (37.6)
Former	203 (42.0)	112 (39.9)	141 (43.0)	64 (42.7)	54 (43.2)
Current	66 (13.7)	60 (21.4)	67 (20.4)	27 (18.0)	24 (19.2)
Alcohol consumption (%)					
Non-current	222 (46.0)	155 (55.2)	173 (52.7)	85 (56.7)	67 (53.6)
Current	261 (54.0)	126 (44.8)	155 (47.3)	65 (43.3)	58 (46.4)
Systolic BP (mm Hg)	125.3 (17.5)	134.1 (20.8)	133.8 (20.3)	134.9 (20.8)	132.5 (18.0)
Diastolic BP (mm Hg)	71.3 (9.7)	70.8 (10.5)	70.5 (10.4)	70.5 (11.5)	69.8 (10.7)
Use of hypertension meds (%)	137 (28.2)	147 (51.9)	170 (51.8)	71 (47.3)	55 (44.4)
Total cholesterol (mg/dl)	200.8 (35.1)	204.8 (38.5)	204.5 (38.4)	203.1 (39.2)	203.8 (40.7)
HDL cholesterol (mg/dl)	51.4 (18.0)	50.5 (17.5)	50.6 (16.9)	49.1 (16.1)	48.4 (15.6)
Triglycerides (mg/dl)	117.0 (85.0, 170.0)	124.0 (97.0, 178.0)	122.0 (96.0, 169.0)	126.0 (95.0, 163.0)	131.0 (97.0, 174.0)
Use of lipid lowering medications (%)	48 (9.9)	48 (17.1)	50 (15.3)	25 (16.8)	20 (16.1)
Diabetes (%)	49 (10.1)	84 (29.8)	89 (27.1)	37 (24.7)	33 (26.4)
eGFR (ml/min/1.73 m^2)	88.2 (14.7)	84.4 (17.9)	84.4 (17.7)	84.2 (15.9)	84.1 (16.0)
Estradiol (pg/mL)	26.5 (12.0, 38.7)	22.1 (9.6, 39.2)	24.7 (12.1, 39.8)	27.2 (10.6, 40.9)	27.7 (12.4, 41.9)

NT-proBNP (pg/mL)	57.3 (27.6, 102.1)	98.2 (53.3, 185.0)	96.6 (49.9, 178.1)	91.1 (49.9, 151.3)	85.8 (46.7, 152.1)

* Values in the table are mean (SD), median (interquartile interval), or number (percentage).
† Data are from ARIC visit 3.
‡ The "no event" group refers to participants who did not develop any heart failure (HF), atherosclerosis cardiovascular disease (ASCVD), or coronary heart disease (CHD) during follow-up (between January 1, 2005 and December 31, 2016).

Table S2. Hazard ratio (and 95% CI) for CVD outcomes associated with cGMP levels, by sex.

	Women	Men	*p*-interaction
Heart failure with preserved ejection fraction			0.70
cGMP tertiles			
First	1 (Ref.)	1 (Ref.)	
Second	1.01 (0.59, 1.73)	1.05 (0.49, 2.24)	
Third	1.34 (0.75, 2.39)	1.20 (0.52, 2.79)	
Per 1-SD increase in log-cGMP	1.11 (0.86, 1.44)	1.02 (0.68, 1.51)	
Any heart failure			0.24
cGMP tertiles			
First	1 (Ref.)	1 (Ref.)	
Second	1.30 (0.64, 2.67)	1.39 (0.54, 3.61)	
Third	2.08 (1.01, 4.28)	1.20 (0.44, 3.24)	
Per 1-SD increase in log-cGMP	1.30 (0.92, 1.83)	1.00 (0.69, 1.44)	
Atherosclerotic cardiovascular disease			0.16
cGMP tertiles			
First	1 (Ref.)	1 (Ref.)	
Second	2.31 (0.91, 5.84)	0.80 (0.29, 2.19)	
Third	3.93 (1.59, 9.71)	1.81 (0.67, 4.85)	
Per 1-SD increase in log-cGMP	1.43 (0.93, 2.20)	0.96 (0.60, 1.51)	
Coronary heart disease			0.37
cGMP tertiles			
First	1 (Ref.)	1 (Ref.)	
Second	2.05 (0.73, 5.72)	0.55 (0.18, 1.67)	
Third	3.06 (1.11, 8.39)	1.95 (0.72, 5.28)	
Per 1-SD increase in log-cGMP	1.41 (0.85, 2.33)	1.03 (0.61, 1.75)	

*Model adjusted for age, race/center, education, smoking, alcohol consumption, BMI, physical activity, systolic blood pressure, hypertension medication, diabetes, total and HDL cholesterol, lipid-lowering medication, eGFR, log-transformed NT-pro-BNP.
1 SD of $\log_e$-transformed cGMP levels: 0.64.

Table S3. Hazard ratio (and 95% CI) for CVD outcomes associated with cGMP levels, by race.

	Whites	Blacks	*p*-interaction
Heart failure with preserved ejection fraction			0.84
cGMP tertiles			
First	1 (Ref.)	1 (Ref.)	
Second	1.02 (0.61, 1.71)	0.94 (0.38, 2.36)	
Third	1.27 (0.74, 2.21)	1.14 (0.47, 2.76)	
Per 1-SD increase in $\log_e$-cGMP	1.08 (0.85, 1.38)	1.03 (0.70, 1.53)	
Any heart failure			0.82
cGMP tertiles			
First	1 (Ref.)	1 (Ref.)	
Second	1.27 (0.66, 2.45)	1.60 (0.45, 5.72)	
Third	1.51 (0.77, 2.95)	1.54 (0.45, 5.30)	
Per 1-SD increase in $\log_e$-cGMP	1.14 (0.86, 1.51)	1.08 (0.72, 1.62)	
Atherosclerotic cardiovascular disease			0.97
cGMP tertiles			
First	1 (Ref.)	1 (Ref.)	
Second	1.40 (0.65, 2.99)	0.74 (0.15, 3.53)	
Third	2.27 (1.03, 5.03)	1.78 (0.49, 6.44)	
Per 1-SD increase in $\log_e$-cGMP	1.13 (0.79, 1.61)	1.11 (0.62, 2.01)	
Coronary heart disease			0.90
cGMP tertiles			
First	1 (Ref.)	1 (Ref.)	
Second	1.17 (0.53, 2.58)	0.22 (0.05, 0.96)	
Third	2.05 (0.92, 4.57)	1.17 (0.27, 5.15)	
Per 1-SD increase in $\log_e$-cGMP	1.16 (0.80, 1.68)	1.09 (0.46, 2.58)	

*Model adjusted for age, sex, education, smoking, alcohol consumption, BMI, physical activity, systolic blood pressure, hypertension medication, diabetes, total and HDL cholesterol, lipid-lowering medication, eGFR, log-transformed NT-pro-BNP.
1 SD of $\log_e$-transformed cGMP levels: 0.64.

Table S4. Hazard ratio (and 95% CI) for CVD outcomes associated with NT-pro-BNP levels.

	Model 1	Model 2
Heart failure with preserved ejection fraction		
NT-pro-BNP tertiles		
First	1 (Ref.)	1 (Ref.)
Second	**2.38 (1.47, 3.88)**	**2.28 (1.38, 3.77)**
Third	**3.87 (2.24, 6.69)**	**3.57 (2.00, 6.37)**
Per 1 SD increase in log_e-NT-proBNP	**2.00 (1.55, 2.57)**	**1.94 (1.49, 2.53)**
Any heart failure		
NT-pro-BNP tertiles		
First	1 (Ref.)	1 (Ref.)
Second	1.41 (0.78, 2.55)	1.28 (0.70, 2.34)
Third	**3.13 (1.64, 5.94)**	**2.65 (1.34, 5.22)**
Per 1 SD increase in log_e-NT-proBNP	**1.71 (1.28, 2.28)**	**1.57 (1.16, 2.13)**
Atherosclerotic cardiovascular disease		
NT-pro-BNP tertiles		
First	1 (Ref.)	1 (Ref.)
Second	**2.10 (1.08, 4.08)**	**1.96 (1.02, 3.80)**
Third	**2.53 (1.15, 5.55)**	2.13 (0.94, 4.85)
Per 1 SD increase in log_e-NT-proBNP	**1.46 (1.05, 2.02)**	1.33 (0.93, 1.90)
Coronary heart disease		
NT-pro-BNP tertiles		
First	1 (Ref.)	1 (Ref.)
Second	2.00 (0.97, 4.12)	1.84 (0.90, 3.74)
Third	**2.60 (1.12, 6.04)**	2.17 (0.94, 5.02)
Per 1 SD increase in log_e-NT-proBNP	**1.40 (1.00, 1.96)**	1.26 (0.88, 1.79)

*Model 1 adjusted for age, race/center, education, smoking, alcohol consumption, BMI, physical activity, systolic blood pressure, hypertension medication, diabetes, total and HDL cholesterol, lipid-lowering medication, eGFR.
Model 2: Model 1 + log_e-cGMP.
1 SD of log_e-transformed NT-proBNP levels: 1.14.