GlycA, a novel proinflammatory glycoprotein biomarker, and high-sensitivity C-reactive protein are inversely associated with sodium intake after controlling for adiposity: the Prevention of Renal and Vascular End-Stage Disease study[1,2]

Eke G Gruppen,[3,4] Margery A Connelly,[5] Priya Vart,[6] James D Otvos,[5] Stephan JL Bakker,[3] and Robin PF Dullaart[4]*

Departments of [3]Nephrology and [4]Endocrinology, University of Groningen and University Medical Center Groningen, Groningen, Netherlands; [5]LipoScience, Laboratory Corporation of America Holdings, Raleigh, NC; and [6]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD

ABSTRACT

Background: The extent to which dietary sodium intake may confer alterations in the inflammatory status is unclear. GlycA is a novel proton nuclear magnetic resonance spectroscopy-measured biomarker of systemic inflammation, which is associated with the development of cardiovascular disease and diabetes.

Objective: We determined associations of the inflammatory markers GlycA and high-sensitivity C-reactive protein (hsCRP) with 24-h sodium excretion.

Design: A cross-sectional, population-based study was performed in 3935 subjects who were not using an antihypertensive medication, lipid-lowering drugs, or a glucose-lowering treatment. Urinary sodium excretion was calculated as the mean of two 24-h urine excretions. Linear regression models were used, with 24-h sodium excretion as an independent variable and GlycA or ln hsCRP as a dependent variable.

Results: The mean ± SD sodium excretion was 143.0 ± 53.4 mmol/24 h. The GlycA concentration was 343.6 ± 58.7 μmol/L, and the geometric mean of the hsCRP concentration was 1.20 mg/L (95% CI: 1.16, 1.25 mg/L). In age- and sex-adjusted analyses, GlycA and ln hsCRP were not significantly associated with 24-h sodium excretion [*B*: 1.23 (95% CI: −0.67, 3.13; $P = 0.21$) and 0.03 (95% CI: −0.004, 0.07; $P = 0.08$), respectively, per 1-SD increase]. After additional adjustment for body mass index (BMI), both GlycA (*B*: −2.76; 95% CI: −4.65, −0.86; $P = 0.004$) and ln hsCRP (*B*: −0.07; 95% CI: −0.11, −0.04; $P < 0.001$) were inversely associated with 24-h sodium excretion. These associations were similar if adjustment was performed for waist circumference instead of BMI or if additional adjustment was performed for relevant clinical and laboratory variables and were particularly present in men.

Conclusions: The proinflammatory biomarkers GlycA and hsCRP are inversely related to higher 24-h sodium excretion when taking into account the variation in adiposity. These inverse relations remain present after taking into account other covariates. *Am J Clin Nutr* 2016;104:415–22.

Keywords: C-reactive protein, diet, GlycA, glycoproteins, inflammation, nuclear magnetic resonance spectroscopy, sodium excretion

INTRODUCTION

High sodium intake has been linked to several major health issues. Lower sodium intake decreases blood pressure (BP)[7] in both hypertensive and normotensive subjects (1, 2). Therefore, reducing sodium intake has been proposed as a target for cardiovascular disease (CVD) prevention (3, 4). In addition, high dietary salt intake may cause BP-independent organ damage such as left ventricular hypertrophy and microalbuminuria (4–6). The WHO recommends that all adults should consume ≤86 mmol Na/d irrespective of the presence of hypertension (7). Nonetheless, evidence has been inconclusive regarding the link between reduced sodium intake and lower cardiovascular disease risk (1, 8).

Besides beneficial effects of reduced sodium intake on BP, low dietary sodium could also unfavorably influence pathways that are conceivably involved in cardiometabolic risk. Low sodium intake <50 mmol/d may even be associated with increased risk of cardiovascular death (9). A Cochrane review has shown that low sodium intake significantly increased plasma renin, aldosterone, and catecholamines as well as plasma total cholesterol and triglycerides (10).

Inflammatory processes play a role in the pathogenesis of atherosclerosis and hypertension (11, 12). However, details of the mechanism and the precise inflammatory mediators are not

[1] Supported by the LipoScience/LabCorp, where the GlycA measurements were performed at no cost.

[2] Supplemental Figures 1 and 2 and Supplemental Tables 1 and 2 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

*To whom correspondence should be addressed. E-mail: e.g.gruppen@umcg.nl.

[7] Abbreviations used: BP, blood pressure; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; hsCRP, high-sensitivity C-reactive protein; Lp-PLA_2, lipoprotein-associated phospholipase A_2; PREVEND, Prevention of Renal and Vascular End-Stage Disease; T2DM, type 2 diabetes mellitus; UAE, urinary albumin excretion; WC, waist circumference.

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completely understood. High-sensitivity C-reactive protein (hsCRP) has been most widely studied as a marker of low-grade systemic inflammation. Indeed, several studies reported an independent association of hsCRP with risks of CVD and hypertension (13–15). GlycA is a novel nuclear magnetic resonance signal derived from mobile *N*-acetyl methyl groups, specifically from the *N*-acetylglucosamine and *N*-acetylgalactosamine moieties, on the carbohydrate side chains of glycosylated proteins (16). The main contributors to the GlycA signal are α1-acid glycoprotein, α1-antitrypsin, haptoglobin, α1-antichymotrypsin, and transferrin (16). GlycA is regarded as a marker of low-grade systemic inflammation (16–18). Evidence has been accumulating that GlycA may predict CVD as well as incident type 2 diabetes mellitus (T2DM) (17, 19–21).

Of interest, dietary salt restriction may relate to alterations in the concentrations of inflammatory markers. An intervention study showed that a reduction of sodium intake was accompanied by an increase in hsCRP (22). In contrast, a cross-sectional study showed that higher amounts of 24-h sodium excretion were associated with increased concentrations of serum hsCRP (23). Remarkably, this association was lost after adjustment for BMI. This finding would raise the possibility that adiposity is an intermediate factor between the sodium balance and low-grade systemic inflammation.

Therefore, the aim of the current study was to investigate the association of 24-h sodium excretion with the 2 inflammatory markers GlycA and hsCRP within a large population-based cohort of men and women. Second, we aimed to assess the role of adiposity in the association between sodium intake and inflammatory markers.

METHODS

Study design and population

The PREVEND (Prevention of Renal and Vascular End-Stage Disease) study is a prospective investigation of albuminuria, renal disease, and CVD in a large cohort drawn from the general population. In summary, in 1997 through 1998, all inhabitants of the city of Groningen, Netherlands, were asked to send in a morning urine sample and to fill out a short questionnaire. Pregnant women and subjects with type 1 diabetes mellitus were excluded. The urinary albumin concentration was assessed in 40,856 responders. Subjects with a urinary albumin concentration $\geq$10 mg/L (n = 7768) were invited to participate of whom 6000 individuals agreed. Furthermore, 3394 randomly selected subjects with urinary albumin concentrations $<$10 mg/L were invited, and 2592 individuals agreed to participate. These 8592 individuals constitute the PREVEND cohort. For the current study, data were used from the second screening round (2001–2003). Of the 6894 subjects who participated in the second screening round, GlycA and hsCRP were measured in 5526 subjects. We excluded subjects with missing values for sodium excretion (n = 121) and subjects who used lipid-lowering drugs (n = 563), an antihypertensive medication (n = 858), or a glucose-lowering treatment (n = 49). Thus, 3935 subjects were included in the analysis (**Supplemental Figure 1**). The PREVEND study has been approved by the Medical Ethics Committee of the University Medical Center Groningen and was conducted in accordance with the guidelines of the Declaration of Helsinki. All participants provided written informed consent.

Data collection

The procedures at each examination in the PREVEND study have been described in detail previously (24, 25). In summary, before the outpatient clinic visit, all participants completed a questionnaire regarding demographics, cardiovascular and renal disease history, smoking habits, alcohol consumption, and medication use. Information on medication use was combined with information from a pharmacy-dispensing registry, which had complete information on the drugs of $>$95% of subjects in the PREVEND study. BMI (in kg/m^2) was calculated as weight divided by height squared. Waist circumference (WC) was measured on bare skin at the natural indentation between the 10th rib and iliac crest. Smoking status was categorized as never, former, and current. Alcohol intake was categorized as almost never, 1–4 drinks/mo, 2–7 drinks/wk, and $\geq$1 drink/d. During each examination and during each visit, BP was measured on the right arm every minute for 10 min with the use of an automatic Dinamap XL Model 9300 series device (Johnson-Johnson Medical). Hypertension was defined as systolic blood pressure $>$140 mm Hg, diastolic blood pressure $>$90 mm Hg, or the use of BP-lowering drugs. T2DM was defined as a fasting serum glucose concentration $>$7.0 mmol/L, a nonfasting plasma glucose concentration $>$11.1 mmol/L, a self-report of a physician diagnosis, or the use of glucose-lowering drugs, which was retrieved from a central pharmacy registry. The estimated glomerular filtration rate (eGFR) was calculated with the use of the combined creatinine cystatin C-based Chronic Kidney Disease Epidemiology Collaboration equation (26).

Subjects collected two 24-h urine samples for 2 consecutive days after having received oral and written instructions. Urinary sodium is given as the mean of the two 24-h urine excretions.

Laboratory measurements

Plasma samples were sent frozen to LipoScience/LabCorp for testing on a Vantera Clinical Analyzer. NMR LipoProfile Test (LipoScience/LabCorp) spectra were collected, and GlycA values were quantified as previously described (16). Briefly, the GlycA nuclear magnetic resonance signal originates from the *N*-acetyl methyl group protons of the *N*-acetylglucosamine moieties located on the biantennary, triantennary, and tetra-antennary branches of plasma glycoproteins, predominantly of α1-acid glycoprotein, haptoglobin, α1-antitrypsin, α1-antichymotrypsin, and transferrin. The amplitude of the combined signals was used to calculate the GlycA concentration (μmol/L of *N*-acetylmethyl groups) (16). CVs for the GlycA assay ranged from 1.3% to 2.3% (19). hsCRP was measured with the use of nephelometry with a threshold of 0.18 mg/L (BNII; Dade Behring). Plasma glucose was measured as described (6). Serum total cholesterol was assayed on an automatic analyzer type MEGA system (Merck) with the use of the CHOD-PAP method. The measurement of serum creatinine was performed with the use of an enzymatic method on a RocheModular analyzer (Roche Diagnostics). Serum cystatin C concentrations were measured with the use of a Gentian Cystatin C Immunoassay (Gentian AS) on a Modular analyzer (Roche Diagnostics). Urinary albumin concentrations were measured with the use of nephelometry with a threshold of 2.3 mg/L and intra-assay and interassay CVs of 2.2% and 2.6%, respectively, (Dade Behring Diagnostic). Urinary sodium was determined in urine with an MEGA clinical chemistry analyzer (Merck).

Statistical analysis

SPSS (version 22.0; IBM Corp) and STATA (version 13.1; StataCorp LP) software programs were used for the data analysis. A 2-sided $P < 0.05$ was considered statistically significant except for interaction terms for which the level of significance was set at $P < 0.10$ (27). Results are presented as means ± SDs, medians (IQRs), and percentages. Skewed data were normalized by ln transformation before analyses, which was the case for urinary albumin excretion (UAE) and hsCRP. Univariable relations between different clinical variables and 24-h sodium excretion were assessed with the use of linear regression. Univariable and multivariable linear regression analyses were carried out to analyze the association of GlycA and hsCRP with 24-h sodium excretion. Because the associations were linear, on the basis of the results of the residual plots, regression coefficients (unstandardized βs) per 1-SD difference in urinary sodium excretion were calculated. Interactions were tested between 24-h sodium excretion and age, sex, menopause, and BMI. A mediation analysis was carried out to assess whether BMI (or WC) was a potential mediator between urinary sodium excretion and inflammatory markers. A mediation analysis was performed in line with the procedures outlined by Baron and Kenny (28). The Sobel test was used to test the significance of a mediating effect (29, 30).

RESULTS

Clinical and laboratory characteristics of the total 3935 subjects who were included in the analysis are shown in **Table 1**. The mean urinary sodium excretion was 143.0 ± 53.4 mmol/24 h in

TABLE 1
Baseline characteristics and correlates of GlycA and hsCRP (n = 3935)[1]

		GlycA			hsCRP		
	Total cohort	Crude	Adjusted for age, sex, and BMI	Adjusted for age, sex, and waist circumference	Crude	Adjusted for age, sex, and BMI	Adjusted for age, sex, and waist circumference
Sex, *n* (%)							
M	1797 (45.7)	Reference	Reference	Reference	Reference	Reference	Reference
F	2138 (54.3)	0.11***	0.13***,[2]	0.24***	0.05***	0.07***,[2]	0.22***
Age, y	50.4 ± 11.0[3]	0.17***	0.08***,[4]	0.10***	0.19***	0.002***,[4]	0.10***
BMI, kg/m^2	25.9 ± 4.01	0.27***	0.26***,[5]	0.07*	0.36***	0.34***,[5]	0.17***
Waist circumference, cm	89.5 ± 11.8	0.24***	0.24***	—	0.33***	0.23***	—
Smoking status, *n* (%)							
Never	1240 (31.5)	Reference	Reference	Reference	Reference	Reference	Reference
Former	1552 (39.4)	0.06**	0.02	0.02	0.07***	0.03	0.02
Current	1102 (28.0)	0.21***	0.24***	0.22***	0.13***	0.16***	0.14***
Alcohol intake, *n* (%)							
Almost never	854 (21.7)	Reference	Reference	Reference	Reference	Reference	Reference
1–4 drinks/mo	679 (17.7)	−0.08***	−0.06***	−0.07***	−0.06**	−0.03	−0.04*
2–7 drinks/wk	1321 (33.6)	−0.14***	−0.07***	−0.08***	−0.11***	−0.03	−0.04*
≥1 drink/d	1047 (26.6)	−0.11***	−0.06*	−0.07***	−0.09***	−0.04*	−0.06**
Hypertension, *n* (%)							
No	3407 (86.6)	Reference	Reference	Reference	Reference	Reference	Reference
Yes	528 (13.4)	0.13***	0.06***	0.06**	0.14***	0.08*	0.04*
CVD, *n* (%)							
No	3870 (98.3)	Reference	Reference	Reference	Reference	Reference	Reference
Yes	65 (1.7)	0.01	0.003	0.006	0.04*	0.03	0.03
Systolic blood pressure, mm Hg	121.7 ± 16.2	0.19***	0.12***	0.11***	0.20***	0.08***	0.07***
Diastolic blood pressure, mm Hg	71.7 ± 8.6	0.14***	0.10***	0.09***	0.16***	0.07***	0.06***
T2DM, *n* (%)							
No	3870 (98.3)	Reference	Reference	Reference	Reference	Reference	Reference
Yes	65 (1.7)	0.05***	0.02	0.02	0.06***	0.02	0.02
Total cholesterol, mmol/L	5.4 ± 1.06	0.20***	0.13***	0.13***	0.15***	0.05***	0.05**
eGFRcrea-cysC, mL · min^{-1} · 1.73 m^{-2}	95.4 ± 14.7	−0.18***	−0.08***	−0.08***	−0.21***	−0.10***	−0.10***
UAE,[2] mg/24 h	8.85 (8.58, 9.03)[6]	0.13***	0.10***	0.09***	0.13***	0.12***	0.12***
hsCRP,[2] mg/L	1.20 (1.16, 1.25)	0.66***	0.63***	0.62***	—	—	—
GlycA, μmol/L	343.6 ± 58.7	—	—	—	0.66***	0.59***	0.59***

[1]Data were obtained with the use of a linear regression analysis. Pearson's correlation coefficients are given. hsCRP and UAE were logarithmically transformed for correlation analysis. *$P < 0.05$, **$P < 0.01$, ***$P \leq 0.001$. CVD, cardiovascular disease; eGFRcrea-cysC, estimated glomerular filtration rate on the basis of the creatinine-cystatin C equation; hsCRP, high-sensitivity C-reactive protein; T2DM, type 2 diabetes mellitus; UAE, urinary albumin excretion.

[2]Adjusted for age and BMI.

[3]Mean ± SD (all such values).

[4]Adjusted for sex and BMI.

[5]Adjusted for sex and age.

[6]Geometric mean; 95% CI in parentheses (all such values).

the whole cohort and amounted to 161.4 ± 57.1 mmol/24 h in men and to 127.5 ± 44.6 mmol/24 h in women. The mean GlycA was 343.6 ± 58.7 μmol/L in all subjects combined and 336.8 ± 57.3 μmol/L in men and 349.4 ± 59.3 μmol/L in women. The geometric mean of hsCRP was 1.20 mg/L (95% CI: 1.16, 1.25 mg/L) in the total cohort and 1.13 mg/L (1.07, 1.19 mg/L) in men and 1.27 (1.21, 1.33 mg/L) in women. In crude analyses, both GlycA and ln hsCRP were positively related to age, sex, BMI, smoking status, hypertension, BP, T2DM, total cholesterol, and UAE. GlycA and ln hsCRP were inversely related to alcohol intake and eGFR (Table 1). The associations of GlycA and ln hsCRP with T2DM were no longer significant after adjustment for age, sex, and BMI or WC (Table 1).

Relations between different clinical variables and 24-h sodium excretion are presented in **Table 2**. The 24-h sodium excretion was positively correlated with BMI, WC, former smoking, hypertension, BP, T2DM, total cholesterol, eGFR, and UAE. The 24-h sodium excretion was inversely related to age and female sex.

In univariable analyses, GlycA and ln hsCRP were not significantly related to 24-h sodium excretion (**Table 3**). There were also no significant associations after adjustment for age and sex. Note that these associations became significant after adjustment for BMI alone [*B*: −5.72 (95% CI: −7.53, −3.91; $P < 0.001$) for GlycA; *B*: −0.11 (95% CI: −0.14, −0.08; $P < 0.001$) for hsCRP] and when adjustment for BMI was added to the existing multivariable adjustment for age and sex (Table 3). These relations remained significant after further adjustment for relevant clinical and laboratory covariates (Table 3). Similar relations were shown when adjustment for BMI was replaced with adjustment for WC (**Supplemental Table 1**). As derived from the age-, sex-, and BMI-adjusted analyses, an increase in 24-h urinary sodium excretion of 53.4 mmol/24 h above the mean (corresponding to a 1-SD increase) was associated with a decrease of 2.8 μmol/L (95% CI: −4.65, −0.86 μmol/L;, $P = 0.004$) in the GlycA concentration from 343.6 to 340.8 μmol/L and a decrease of 0.07 mg/L (95% CI: −0.11, −0.04 mg/L; $P < 0.001$) in the hsCRP concentration from geometric mean values from 1.20 to 1.12 mg/L. **Figure 1** illustrates the age-, sex-, and BMI-adjusted associations of GlycA and ln hsCRP with 24-h sodium excretion. **Supplemental Figure 2** shows the age-, sex- and WC-adjusted associations of GlycA and ln hsCRP with 24-h sodium excretion.

There were no significant interactions by either age, BMI, or menopause (*P*-interaction > 0.10 for all; data not shown). However, there was a significant interaction between sex and 24-h sodium excretion that affected both GlycA and hsCRP (in multivariable adjusted analyses: *P*-interaction = 0.001 and 0.06, respectively). Therefore, we also performed sex-stratified analyses. Baseline characteristics for men and women separately are presented in **Supplemental Table 2**. Table 3 shows the sex-stratified linear regression analyses. Similar to the nonstratified analyses, in crude stratified analyses, there was not a significant association of GlycA with 24-h sodium excretion in men (*B*: −1.95; 95% CI: −4.60, 0.70; $P = 0.15$) or in women (*B*: 2.74; 95% CI: −0.04, 4.99; $P = 0.05$) (Table 3). However, after adjustment for age and BMI, the association between 24-h sodium excretion and GlycA became significant in men (*B*: −3.74; 95% CI: −6.45, −1.02; $P = 0.007$) but not in women (*B*: −0.62; 95% CI: −3.03, 1.79; $P = 0.61$) (Table 3, model 1). Results for ln hsCRP were comparable with the association being significant in men after adjustment for age and BMI (*B*: −0.09; 95% CI: −0.14, −0.04; $P < 0.001$) (Table 3, model 1). Furthermore, adjustment for smoking status, alcohol consumption, systolic blood pressure, eGFR, UAE, cholesterol, CVD, and T2DM did not materially alter the results in both the analyses with GlycA and ln hsCRP as outcomes (Table 3, model 5).

A mediation analysis suggested that BMI could represent a possible contributor that is involved in both the association of GlycA and ln hsCRP with 24-h sodium excretion (**Figure 2**). The analysis of the mediating role of WC in the association between GlycA and ln hsCRP with 24-h sodium excretion showed similar results.

TABLE 2
Univariable linear regression analysis between various clinical and laboratory variables and urinary sodium excretion (mmol/24 h) ($n = 3935$)[1]

	Pearson's correlation coefficient
Sex	
M	Reference
F	−0.32***
Age, y	−0.08***
BMI, kg/m^2	0.23***
Waist circumference, cm	0.30***
Smoking status	
Never	Reference
Former	0.06**
Current	−0.03
Alcohol intake	
Almost never	Reference
1–4 drinks/mo	−0.008
2–7 drinks/wk	0.06**
≥1 drink/d	0.05*
Hypertension	
No	Reference
Yes	0.07***
Systolic blood pressure, mm Hg	0.14***
Diastolic blood pressure, mm Hg	0.14***
T2DM	
No	Reference
Yes	0.03*
Total cholesterol, mmol/L	0.03*
eGFRcrea-cysC, mL · min^{-1} · 1.73 m^{-2}	0.12***
ln UAE, mg/24 h	0.18***

[1]*$P < 0.05$, **$P < 0.01$, ***$P \leq 0.001$. eGFRcrea-cysC, estimated glomerular filtration rate on the basis of the creatinine-cystatin C equation; T2DM, type 2 diabetes mellitus; UAE, urinary albumin excretion.

DISCUSSION

In a large cohort of men and women, lower concentrations of GlycA and hsCRP were associated with higher amounts of 24-h sodium excretion if measures of adiposity were taken into account. BMI is an appropriate measure for overweight and obesity but cannot be used to distinguish between lean body mass, adipose tissue, or body fat distribution (31). Note that adjustment for WC gave essentially similar results, which suggested that it is indeed (visceral) fat accumulation tissue that modifies the association.

The results of our study are in line with those of an intervention study by Nakandakare et al. (22) who studied the effects of 1 wk

TABLE 3
Univariable and multivariable associations of GlycA and hsCRP (ln transformed) with sodium excretion (per 1-SD increase) in the total cohort (n = 3935) and in men (n = 1797) and women (n = 2138) separately[1]

	GlycA (dependent variable)		hsCRP (dependent variable)	
	Value	*P*	Value	*P*
Total cohort				
Crude	−1.81 (−3.65, 0.02)	0.05	−0.009 (−0.05, 0.03)	0.62
Model 1	1.23 (−0.67, 3.13)	0.21	0.03 (−0.004, 0.07)	0.08
Model 2	−2.76 (−4.65, −0.86)	0.004	−0.07 (−0.11, −0.04)	<0.001
Model 3	−2.30 (−4.16, −0.44)	0.02	−0.07 (−0.10, −0.03)	<0.001
Model 4	−2.74 (−4.65, −0.84)	0.005	−0.08 (−0.11, −0.04)	<0.001
Model 5	−2.86 (−4.76, −0.96)	0.003	−0.08 (−0.12, −0.04)	<0.001
Model 6	−2.86 (−4.76, −0.96)	0.003	−0.08 (−0.11, −0.04)	<0.001
Men				
Crude	−1.95 (−4.60, 0.70)	0.15	−0.02 (−0.07, 0.04)	0.56
Model 1[2]	−1.11 (−3.73, 1.50)	0.40	0.007 (−0.04, 0.06)	0.78
Model 2	−3.74 (−6.45, −1.02)	0.007	−0.09 (−0.14, −0.04)	<0.001
Model 3	−3.19 (−5.84, −0.55)	0.02	−0.09 (−0.14, −0.04)	0.001
Model 4	−3.54 (−6.21, −0.87)	0.01	−0.09 (−0.14, −0.04)	0.001
Model 5	−3.68 (−6.33, −1.02)	0.007	−0.09 (−0.14, −0.04)	<0.001
Model 6	−3.72 (−6.37, −1.06)	0.006	−0.09 (−0.14, 0.04)	<0.001
Women				
Crude	2.47 (−0.04, 4.99)	0.05	0.04 (−0.01, 0.09)	0.15
Model 1[2]	3.66 (1.17, 6.15)	0.004	0.06 (0.008, 0.11)	0.02
Model 2	−0.62 (−3.03, 1.79)	0.61	−0.04 (−0.09, 0.004)	0.08
Model 3	−0.24 (−2.60, 2.13)	0.84	−0.04 (−0.09, 0.006)	0.09
Model 4	−0.28 (−2.67, 2.12)	0.82	−0.04 (−0.08, 0.01)	0.14
Model 5	−0.21 (−2.60, 2.18)	0.86	−0.04 (−0.08, 0.01)	0.15
Model 6	−0.19 (−2.58, 2.20)	0.88	−0.03 (−0.08, 0.01)	0.16

[1]All values are unstandardized βs (*B*s) per 1-SD increase in sodium excretion; 95% CIs in parentheses. A 1-SD change in urinary sodium excretion corresponds to 54.3 mmol/24 h (57.1 mmol/24 h in men and 44.6 mmol/24 h in women). Data were obtained with the use of a linear regression analysis. Unless otherwise indicated, model 1 was adjusted for the crude analysis and for age and sex. Model 2 was adjusted as for model 1 and for BMI. Model 3 was adjusted as for model 2 and for smoking status (never, former, or current) and alcohol consumption (almost never, 1–4 drinks/mo, 2–7 drinks/wk, or ≥1 drink/d). Model 4 was adjusted as for model 3 and for systolic blood pressure, the estimated glomerular filtration rate on the basis of the creatinine-cystatin C equation, and urinary albumin excretion. Model 5 was adjusted as for model 4 and for total cholesterol. Model 6 was adjusted as for model 5 and for cardiovascular disease and type 2 diabetes mellitus. There was a significant interaction of sex with 24-h sodium excretion that affected both GlycA and hsCRP (multivariable adjusted analyses): *P*-interaction = 0.001 and 0.06, respectively. hsCRP, high-sensitivity C-reactive protein.
[2]Adjusted for age in the sex-stratified analysis.

of consumption of a control diet (160 mmol Na/d) followed by 3 wk of low sodium intake (60 mmol Na/d). Nonpharmacologically treated hypertensive adult patients were included in the study, whereas subjects with severe hypertriglyceridemia, obesity, diabetes, alcohol abuse, and the use of any drug that could interfere with lipid metabolism were excluded. hsCRP, IL-6, and TNF-α concentrations were all elevated by the low dietary sodium intake. In contrast, a cross-sectional study showed that higher hsCRP concentrations were positively associated with 24-h sodium excretion (23). The difference in results between the previous observational study and our current study may, at least in part, be explained by differences in the populations studied. In the earlier study, there were slightly more men, and the mean age of the investigated population was lower than that of our population. Even more importantly, in the previous observational study, no information was available on the use of medication such as statins (23), which are known to reduce systemic inflammation (32).

The mean 24-h sodium excretion was 143 mmol in our population, which was higher than the current daily recommendation of 86 mmol Na as currently proposed by the WHO (7) and of 103 mmol Na as proposed by the Health Council of the Netherlands (33). However, when data for the current study were collected, the daily recommendation in the Netherlands was 155 mmol Na (34). The age-, sex-, and BMI-adjusted calculated changes in GlycA and hsCRP in relation to differences in urinary sodium excretion as determined with the use of linear modeling were rather small and should be placed in the context of the mean 24-h sodium excretion in the population studied. For reasons that are not clear at present, the associations of GlycA and hsCRP with 24-h sodium excretion were only significant in men.

As expected, urinary sodium excretion was lower in women and in older subjects and was positively related to BP (6, 10, 35). Urinary sodium excretion was also positively related to BMI and WC (6). These anticipated relations with adiposity indexes required adjustment for these variables. However, note that the associations of GlycA and hsCRP were uncovered after adjustment for either BMI or WC. In comparison, the Nottingham survey showed a positive relation of hsCRP with urinary sodium excretion in a crude analysis that lost significance after adjustment for BMI (23). The reasons that are responsible for this

adiposity-mediated shift in the association of inflammation markers with sodium intake deserve further study. The mediation analysis in our study suggested that BMI or, alternatively, WC could influence the associations between GlycA and hsCRP and 24-h sodium excretion. However, it should be emphasized that this analysis did not provide evidence of causation. It can be envisaged that unmeasured dietary factors that may coincide with an increase in sodium intake could also predispose individuals to adiposity, but our findings should be regarded as hypothesis generating.

Why may a lower sodium intake be associated with a higher burden of circulating inflammation markers? Of the other possibilities, a lower sodium-intake activity activates the renin-angiotensin-aldosterone system (2, 10). Both a low-sodium diet and the infusion of angiotensin I resulted in lower plasma concentrations of adiponectin, which is an adipokine with well-delineated anti-inflammatory properties (36). The angiotensin II type I receptor is expressed in adipose tissue, and the blockade of this receptor attenuates in part the decrease in circulating adiponectin that is consequent to obesity and aging (37). In addition, it may be relevant that lipoprotein-associated phospholipase A_2 (Lp-PLA_2), which is a proinflammatory biomarker enzyme that is predominantly complexed to apolipoprotein B–containing lipoproteins, decreased in response to a short-term

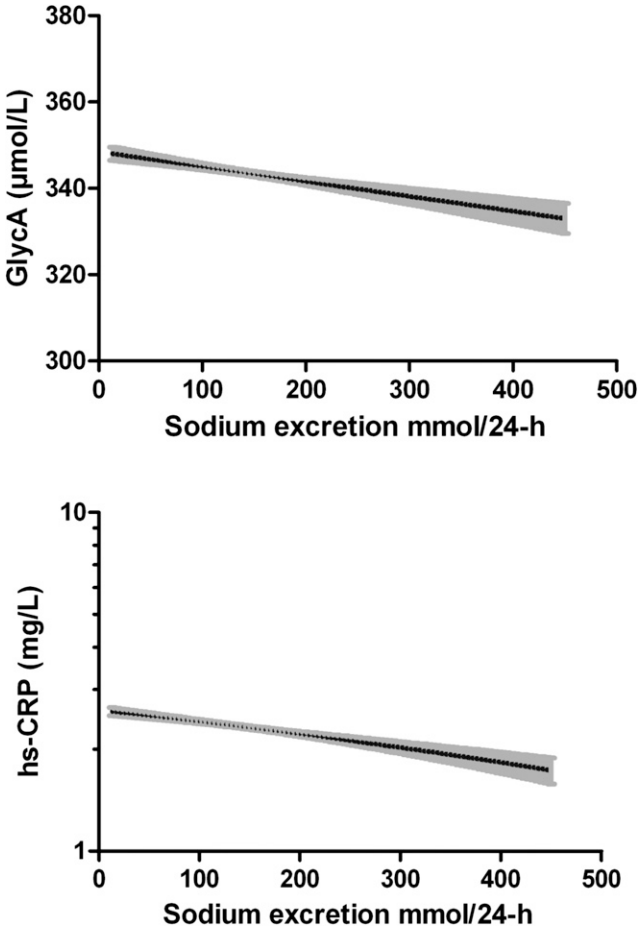

FIGURE 1 Association between sodium excretion (mmol/24-h) and GlycA and hsCRP adjusted for age, sex, and BMI in 3935 subjects. Data were fit with the use of a linear regression analysis. Gray areas indicate 95% CIs. hsCRP, high-sensitivity C-reactive protein.

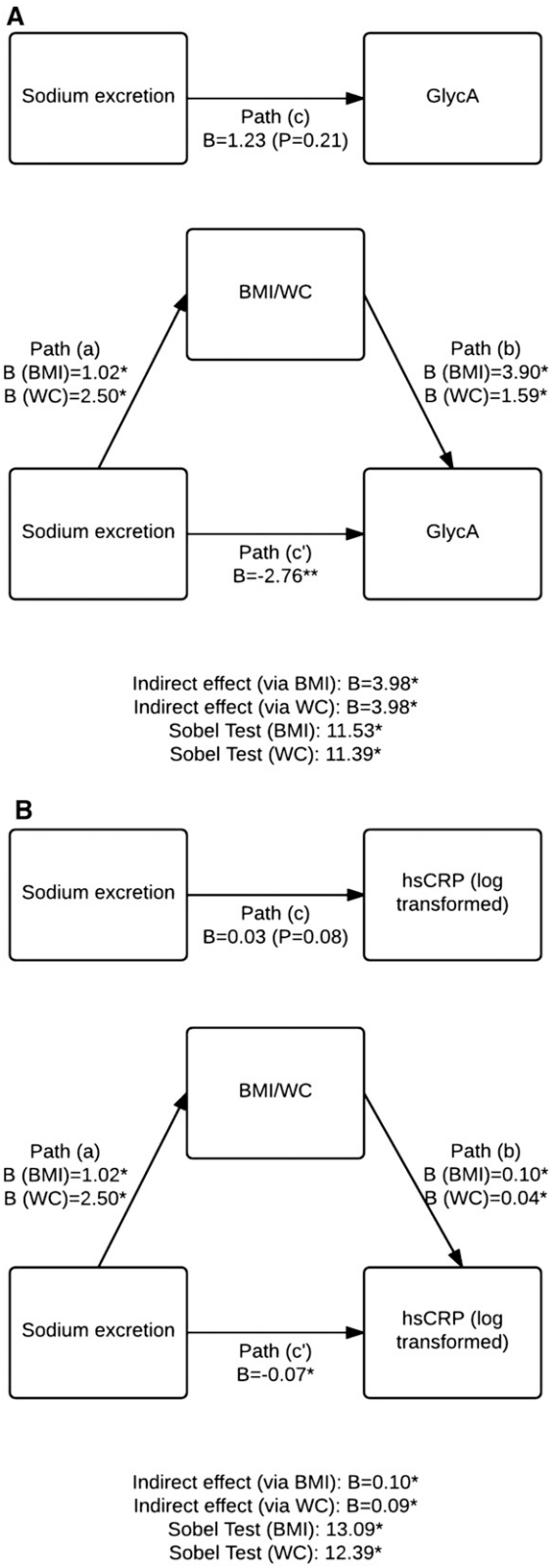

FIGURE 2 Mediation from BMI and WC in the association between sodium excretion (per 1-SD increase) and GlycA (A) and hsCRP (log transformed) (B) with age and sex controlled for. The Sobel test was used to test the significance of the mediating effect. The indirect effect (through BMI and WC) was calculated as a × b. *$P < 0.001$, **$P = 0.004$. hsCRP, high-sensitivity C-reactive protein; WC, waist circumference.

dietary sodium challenge (38). In this regard, note that the novel proinflammatory biomarker GlycA has been shown to be positively related to Lp-PLA_2 in subjects without T2DM or the metabolic syndrome (39). In view of the effect of Lp-PLA_2 to increase ILs, which are able to enhance protein glycosylation (40–43), it is conceivable that Lp-PLA_2 could be involved in the association of sodium intake with plasma GlycA concentrations.

Several limitations of our study warrant consideration. First, the cross-sectional nature of our study limited our ability to conclude a causal relation between 24-h sodium excretion and inflammation. Also, it is unknown whether changes in urinary sodium excretion are related to changes in circulating inflammation markers overtime. Second, subjects of the PREVEND cohort were predominantly white. Therefore, our results cannot be easily extrapolated to other ethnicities. This limitation may be important because some data have suggested that black and Asian individuals are more sensitive to a sodium reduction with respect to BP changes than are white individuals (10). Third, subjects with a urinary albumin concentration $\geq$10 mg/L were overrepresented in the PREVEND study. However, adjustment for UAE did not alter the results, which made it unlikely that this overrepresentation influenced the observed findings. Fourth, no information about other dietary habits that could coincide with higher sodium intake was available.

Strengths of our study include the relatively large cohort of predominantly healthy men and women and the extensive information about possible confounding factors such as the presence of CVD and the use of medications that could alter the degree of low-grade chronic inflammation. In addition, two 24-h urine collections were obtained from each participant. Repeated 24-h sodium excretion is considered to be the reference standard for sodium-intake estimation (44). Because 90–95% of the sodium ingested is excreted in the urine, 24-h sodium excretion is a good approach to estimate sodium intake (44). Although 24-h sodium overcomes the limitations of a recall bias, which is a major problem when estimating dietary sodium intake from dietary questionnaires, the limitation of a 24-h urine collection is the high frequency of incomplete sample collection.

In conclusion, in this large population-based cohort of men and women, GlycA and hsCRP were not significantly related to 24-h sodium excretion in crude analyses. Notably, in age- and sex-adjusted analyses that took into account BMI or, alternatively, WC, lower GlycA and hsCRP concentrations were both associated with higher 24-h sodium excretion, and these relations remained present after other potential covariates were taken into account.

The authors' responsibilities were as follows—EGG, PV, SJLB, and RPFD: interpreted the data analysis; EGG, PV, and RPFD: analyzed the data and performed the statistical analysis; EGG and RPFD: had primary responsibility for the final content of the manuscript; and all authors: wrote the manuscript and read and approved the final manuscript. MAC and JDO are employees of LabCorp. The remaining authors reported no conflicts of interest related to the study.

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