Journal of the American Heart Association

ORIGINAL RESEARCH

Urinary Prostaglandin E2 Excretion and the Risk of Cardiovascular and Kidney Disease

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BACKGROUND: Inhibition of prostaglandin synthesis by nonsteroidal anti-inflammatory drugs is associated with cardiovascular mortality and kidney disease. This study hypothesizes that urinary prostaglandin E2 (PGE2) and PGE2 metabolite (PGEM) excretions are markers of cardiovascular and kidney health, because they reflect both systemic and kidney-derived PGE2 production.

METHODS AND RESULTS: PGE2 and PGEM were measured in spot urine samples from 2291 participants (≥55 years old) of the population-based Rotterdam Study. Urinary PGE2 and PGEM excretions were analyzed using linear regression analyses to identify cross-sectional associations with cardiovascular risk factors and baseline estimated glomerular filtration rate (eGFR). Longitudinal associations with cardiovascular mortality and kidney outcomes (eGFR <60 or <45 mL/min per 1.73 m^2 and the composite outcome 40% eGFR loss or kidney failure) were assessed with Cox regression. Urinary PGE2 and PGEM excretions were higher with increasing age, lower eGFR, smoking, diabetes, and albuminuria. A 2-fold higher urinary PGE2 and PGEM excretion was associated with a higher risk of cardiovascular mortality (28 825 patient-years; 160 events; PGE2 hazard ratio [HR], 1.27, [95% CI, 1.06–1.54]; PGEM HR, 1.36 [95% CI, 1.10–1.67]). Higher PGE2 excretions were also associated with a higher risk of incident eGFR <60 mL/min per 1.73 m^2 (31 530 person-years; 691 events; HR, 1.13 [95% CI, 1.02–1.25]) with similar HRs for the other kidney outcomes.

CONCLUSIONS: Urinary PGE2 and PGEM excretions are novel markers for the presence and progression of cardiovascular and kidney disease. Future studies should address whether these associations are causal and can be targeted to improve cardiovascular and kidney outcomes.

Key Words: cardiovascular disease ■ chronic kidney disease ■ prostaglandin E2

Prostaglandin E2 (PGE2) is the most abundant prostaglandin in the microvasculature and the kidney and plays an important role in the regulation of both vascular and kidney function. PGE2 generally has beneficial effects on the cardiovascular system because it is an important vasodilator.[1–3] Similarly, PGE2 promotes natriuresis and is essential to prevent salt-sensitive hypertension.[4–6] In agreement, inhibition of prostaglandin production by nonsteroidal anti-inflammatory drugs (NSAIDs), which inhibit the PGE2-producing enzymes COX-1 and 2 (cyclooxygenase 1 and 2), increases the risk of cardiovascular mortality and kidney injury.[7,8]

In a pathophysiological context, however, PGE2 predominantly has negative effects.[9] For example, PGE2 production is increased in atherosclerotic plaques,[10] where it is involved in plaque instability.[11] Furthermore, COX-2 activity is higher in monocytes from people with more cardiovascular risk factors.[12] With regard to effects on the kidney, PGE2 may contribute to hyperfiltration and fibrosis.[4,13,14] In 2 small cohort studies (including <30 patients), people with chronic kidney disease

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This article was sent to Meng Lee, MD, Guest Editor, for review by expert referees, editorial decision, and final disposition.

Supplemental Material is available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.123.032835

For Sources of Funding and Disclosures, see page 11.

JAHA is available at: www.ahajournals.org/journal/jaha

CLINICAL PERSPECTIVE

What Is New?

- Urinary prostaglandin E2 (PGE2) and PGE2 metabolite excretions increase with age and are higher in people who smoke or have diabetes or albuminuria.
- Higher urinary PGE2 or PGE2 metabolite excretions are associated with an increased risk of cardiovascular mortality, all-cause mortality, and incident chronic kidney disease.

What Are the Clinical Implications?

- Urinary PGE2 and PGE2 metabolite excretions are promising markers for future cardiovascular and kidney disease.
- PGE2 may play a pathophysiological role in the early development of cardiovascular disease and chronic kidney disease; future studies should address whether this is a causal relationship.

Nonstandard Abbreviations and Acronyms

CKD-EPI	Chronic Kidney Disease-Epidemiology Collaboration
COX	cyclooxygenase
PGE2	prostaglandin E2
PGEM	prostaglandin E2 metabolite

(CKD) had increased kidney COX-2 and urinary PGE2 excretion compared with people with normal kidney function.[15,16] Kidney-derived PGE2 might also link CKD to cardiac damage,[17] as COX-2 inhibition in mice with reduced kidney function was associated with increased levels of methylarginines, which are potential cardiotoxic substances.[18] Therefore, an increase in PGE2 production could be associated with cardiovascular risk factors as well as cardiovascular and kidney outcomes.

PGE2 has a very short half-life in blood and is rapidly excreted in urine both as PGE2 and PGE metabolite (PGEM).[19] This suggests that urinary PGE2 and PGEM excretions rather than circulating PGE2 are able to reflect both systemic and kidney-derived PGE2 production in cardiovascular and kidney disease. In support of this, urinary PGE2 excretion has been linked to COX-2 expression in the kidney,[20] whereas urinary PGEM has mainly been used as a marker of systemic PGE2 production.[21–23] To date, all studies that analyzed urinary PGE2 or PGEM excretions were in patient cohorts and focused on noncardiovascular conditions such as cancer.[13,15,16,21–27] Therefore, it is unknown what factors determine urinary PGE2 and PGEM excretions in the general population and whether urinary PGE2 or PGEM can be used as a marker for cardiovascular and kidney health.

Here, we hypothesized that higher urinary PGE2 and PGEM excretions are associated with cardiovascular and kidney disease. To address this, we measured urinary PGE2 and PGEM excretions in a large prospective population-based cohort to (1) identify determinants of urinary PGE2 and PGEM excretions, (2) determine the cross-sectional association of urinary PGE2 and PGEM excretions with cardiovascular risk factors and kidney function, and (3) investigate the longitudinal association of urinary PGE2 and PGEM excretions with cardiovascular mortality and kidney function decline.

METHODS

Detailed methods are available in Data S1. The data used in this study can be obtained upon request. Requests should be directed to the management team of the Rotterdam Study (datamanagement.ergo@erasmusmc.nl), which has a protocol for approving data requests. Because of restrictions based on privacy regulations and informed consent of the participants, data cannot be made freely available in a public repository.

Study Population

The current study was embedded in the RS (Rotterdam Study), an ongoing prospective population-based cohort designed to examine risk factors for disease in older adults.[28] For the current study, participants of cohort RS-II (starting in 2000) in whom a baseline spot urine sample was available and who were not using NSAIDs (based on prescription data from collaborating pharmacies) were included. Participants were ≥55 years and followed from the date of urine sample collection until the date of death or end of data collection (January 1, 2015 for cause-specific mortality, and January 1, 2018 for all-cause mortality). The RS has been approved by the Medical Ethics Committee of the Erasmus Medical Center (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). All participants provided written informed consent to participate in the study and to have their information obtained from treating physicians.

PGE2, PGEM, and Kidney Function

PGE2 and PGEM were measured in baseline spot urine samples using commercially available competitive

enzyme-linked immunosorbent assays (Cayman Chemical item no. 514010 and 514 531, Ann Arbor, MI). Serum creatinine and cystatin C were measured using an enzymatic essay and a particle enhanced immunophelometric assay, respectively. Follow-up serum creatinine measurements were obtained from visits within the Rotterdam Study and from the Star-MDC database, a database with laboratory measurements from general practitioners. The Star-MDC serum creatinine values closely corresponded with the values from follow-up visits in the Rotterdam study and were usually obtained as part of a standard test panel for cardiovascular risk management by general practitioners.[29] Estimated glomerular filtration rate (eGFR) was calculated using the CKD-Epidemiology Collaboration (CKD-EPI) 2009 equation for creatinine and the CKD-EPI 2012 equation for cystatin C.[30] We used the 2009 CKD-EPI equation rather than the CKD-EPI 2021 equation because recent data suggest that the former performs better in European populations.[31] Start dates of dialysis or kidney transplantation were extracted from the Renine database, a national database including all Dutch patients undergoing kidney replacement therapy.[32]

Covariates

Potential determinants and confounders of the association between PGE2 and PGEM excretions and cardiovascular mortality or kidney function were selected based on previous literature and biological plausibility.[25,26] For the analyses investigating potential determinants of PGE2 and PGEM, we included baseline age, body mass index, sex, and smoking (obtained through baseline interviews and classified as current, past, or never smokers). As additional cardiovascular risk factors we used hypertension (defined as blood pressure >140/90 mm Hg or use of blood pressure lowering medication), hypercholesterolemia (defined as a total cholesterol >240 mg/dL or use of lipid-lowering medication), presence of diabetes (defined as a fasting serum glucose level ≥126 mg/dL, a nonfasting serum glucose level ≥200 mg/dL or the use of blood glucose-lowering medication, or a previous diagnosis of diabetes), baseline eGFR, and subgroups of albuminuria. Use of antiplatelet drugs and lipid-lowering medication was obtained from prescription data through linkage with participating pharmacies. All covariates had <5% missing data. Missing variables were multiply imputed 20 times using chained equations from the mice R package.

Outcomes

For cardiovascular and overall survival outcomes, information on the vital status of all participants was obtained on a weekly basis from the central registry of the municipality of Rotterdam and through linkage with records of general practitioners working in the study area. The cause of death was determined from medical records from general practitioners, nursing home physicians and hospital discharge letters, coded using *International Classification of Diseases, Tenth Revision (ICD-10)* codes by 2 independent research physicians and reviewed by a medical expert in the relevant field. Cardiovascular mortality was defined using the Systematic Coronary Risk Evaluation project definition using *ICD-10* codes I10 to 25, I44 to 51, I61 to 73, and R96.[33] We defined our main kidney survival end point as a single eGFR below 60 mL/min per 1.73 m^2 because this outcome is least influenced by survival bias.[34] In addition, we performed additional analyses using 3 alternative kidney outcomes: (1) 2 consecutive eGFR measurements below 60 mL/min per 1.73 m^2, (2) a single eGFR <45 mL/min per 1.73 m^2, and (3) the composite outcome of 40% loss of eGFR or kidney failure. For this composite outcome all participants with an available baseline eGFR were used, whereas for the other incident kidney outcomes participants with a baseline eGFR <60 mL/min per 1.73 m^2 were excluded. As an alternative to kidney outcome analyses, we performed an eGFR slope analysis. For all kidney outcome measures we used creatinine-based eGFR because no follow-up cystatin C measurements were available.

Statistical Analysis

PGE2 and PGEM were normalized to urinary creatinine and log2 transformed before analysis. Because lower urinary creatinine concentration by itself is associated with adverse kidney and cardiovascular outcomes,[35–37] log2 transformed urinary concentrations of PGE2 and PGEM with 1/urine creatinine concentration as covariate were used in sensitivity analyses for all longitudinal outcomes. Potential determinants and the association with cardiovascular risk factors of urinary PGE2 and PGEM excretions were explored using multivariable regression while adjusting for the other determinants and cardiovascular risk factors. To assess the association of urinary PGE2 excretion with cardiovascular mortality and kidney outcomes, we used Kaplan–Meier plots with tertiles of urinary PGE2 and PGEM excretions and multivariable Cox proportional-hazards models. Additionally, for cardiovascular mortality, cumulative incidence plots by tertiles of PGE2 and PGEM excretions were made and subdistribution hazards were calculated using Fine–Gray models to account for competing risks. The association of urinary PGE excretion and eGFR slope was analyzed using linear mixed models. We constructed directed acyclic graphs to investigate the potential confounders

for the association between PGE2 and cardiovascular mortality or kidney disease (Figure S1). All analyses were performed using 3 different models including the unadjusted association (model 1); model 2, adjusted for potential confounders of the associations between PGE2 and the outcomes (sex, age, body mass index, smoking status, baseline eGFR, cardiovascular mortality outcomes, and use of antiplatelet drugs); and model 3, adjusted for confounders that may also act as potential mediators (diabetes status, log of albumin-to-creatinine ratio, history of cardiovascular disease, and presence of hypertension). For the cardiovascular mortality outcome, we also added total cholesterol, high-density lipoprotein cholesterol, and use of lipid-lowering medication. Nonlinear associations for important predictors were tested using restricted cubic splines with 4 knots. For all survival analyses nonproportional hazards were excluded by examination of the Schoenfeld residuals. To assess the bias of potential unmeasured confounders we calculated E-values.[38] *P* values <0.05 were considered statistically significant, except for the interaction analyses where <0.1 was used. Statistical analyses were performed using R (version 4.1).

RESULTS

Higher Urinary PGE2 and PGEM Excretions Are Associated With Cardiovascular Risk Factors

The study cohort consisted of 2291 participants with a mean±SD age of 64.3±7.8 years and including 54.3% women (Table 1; Table S1; Figure S2). The median urinary excretions of PGE2 and PGEM were 81.4 pg/mmol creatinine (interquartile range, 60.5–115.1 pg/mmol) and 47.7 pg/mmol creatinine (interquartile range, 33.4–66.5), respectively and their excretions were correlated ($r=0.46$, $P<0.001$; Figure S3A and S3B). Both urinary PGE2 and PGEM excretions were significantly higher in older participants, those with a lower body mass index, smokers, participants with diabetes, and participants with albuminuria (Figure 1; Figure S4; Table 2). The difference in urinary PGE2 and PGEM excretions between men and women, participants with and without hypertension, and participants with and without hypercholesterolemia were not statistically significant after adjusting for the other determinants and cardiovascular risk factors (Figure S4; Table 2). Urinary PGE2 and PGEM excretions showed a nonlinear association with baseline eGFR, with the highest PGE2 excretion in participants with a high eGFR. When using cystatin C-based eGFR, the higher urinary excretion in participants with an eGFR >90 mL/min per 1.73 m^2 was less pronounced (Figure 1B; Table 2).

Urinary PGE2 and PGEM Excretions Are Associated With Cardiovascular Mortality

A total of 2291 participants were included for the cardiovascular survival analysis, with a median of 13.9 years of follow-up (interquartile range, 13.5–14.4, total 28 825 patient-years; Figure S2). Both the high tertile of urinary PGE2 (160 events of cardiovascular mortality in 2239 participants) and PGEM excretion (161 events in 2241 participants) were associated with an increased incidence of cardiovascular and noncardiovascular mortality ($P<0.001$ for both outcomes; Figure 2). This association was also present in the fully adjusted model (hazard ratio [HR] for high versus low tertile of urinary PGE2 excretion, 1.62 [95% CI, 1.04–2.25], HR for urinary PGEM excretion, 2.04 [95% CI, 1.24–3.34]; Table 3). Furthermore, each doubling of urinary excretion of PGE2 or PGEM was associated with an increased risk of cardiovascular mortality in the adjusted models (PGE2 HR, 1.27 [95% CI, 1.06–1.54]; PGEM HR, 1.36 [95% CI, 1.10–1.67]; Table 3). Subdistribution HRs showed a similar association (Table S2) and the HRs were also similar when using urinary concentrations of PGE2 and PGEM with 1/creatinine as a covariate (Table S3). Urinary PGE2 and PGEM excretions were also associated with overall mortality (Table S4). The association of urinary PGE2 excretion with the risk of cardiovascular mortality was stronger in men (HR per doubling of PGE2 excretion, 1.54 [95% CI, 1.23–1.93]) compared with women (HR, 0.88 [95% CI, 0.65–1.20], *P* for interaction 0.03) and differed according to smoking status ($P=0.06$). The association between urinary PGEM excretion and cardiovascular mortality was stronger in participants without diabetes (HR, 1.41 [95% CI, 1.13–1.76]) compared with participants with diabetes (HR, 1.14 [95% CI, 0.69–1.88], *P* for interaction 0.09) (Figure S5). The E-values for urinary PGE2 and PGEM excretions on cardiovascular mortality were 1.86 (lower CI, 1.31) and 2.06 (lower CI, 1.43), respectively, making it unlikely that unmeasured confounding explains these associations.

Urinary PGE2 and PGEM Excretions Are Associated With Kidney Outcomes

A total of 2223 participants had an available baseline eGFR and were included in the analyses investigating kidney outcomes (Figure S2). In total, 18 586 eGFR assessments were available with a median of 7 per participant (range 1–65 eGFRs) for a median of 13.0 years (interquartile range, 10.1–14.3). During follow-up (31 530 person-years), 691 of the 2055 participants with a baseline eGFR >60 mL/min per 1.73 m^2 reached incident eGFR <60 mL/min per 1.73 m^2. Participants in the high tertile of urinary PGE2 and PGEM excretions had a higher risk of incident eGFR <60 (Figure 3A). In the adjusted models, higher urinary PGE2 excretion was

Table 1. Baseline Characteristics Per Tertile of Urinary PGE2 Excretion

	Total (N=2291)	Low PGE2 (N=747)	Medium PGE2 (N=746)	High PGE2 (N=746)	*P* value
Female sex, n (%)	1243 (54.3%)	398 (53.3%)	424 (56.8%)	407 (54.6%)	0.4
Age, y	64.33 (7.75)	62.3 (6.0)	63.8 (7.1)	67.0 (9.1)	<0.001
BMI groups*					<0.001
Underweight, n (%)	13 (0.6%)	1 (0.1%)	2 (0.3%)	10 (1.3%)	
Healthy weight, n (%)	701 (30.7%)	184 (24.7%)	217 (29.2%)	280 (37.7%)	
Overweight, n (%)	1120 (49.0%)	394 (52.9%)	368 (49.5%)	333 (44.8%)	
Obese, n (%)	450 (19.7%)	166 (22.3%)	157 (21.1%)	120 (16.2%)	
Hypertension, n (%)	1425 (62.3%)	446 (59.8%)	467 (62.6%)	477 (64.0%)	0.2
Systolic blood pressure, mm Hg	142.79 (21.73)	141.7 (20.4)	142.5 (22.2)	143.8 (22.6)	0.2
Diastolic blood pressure, mm Hg	78.51 (10.72)	79.3 (10.7)	78.6 (10.7)	77.5 (10.7)	0.006
Total cholesterol, mg/dL	224 (38)	224 (38)	227 (37)	222 (38)	0.03
Low-density lipoprotein cholesterol, mg/dL	171 (38)	172 (38)	172 (38)	169 (38)	0.2
Lipid-lowering agents, n (%)	291 (12.7%)	94 (12.6%)	87 (11.7%)	101 (13.5%)	0.6
Smoking status					0.009
Never smoker, n (%)	707 (31.0%)	250 (33.6%)	233 (31.3%)	212 (28.6%)	
Former smoker, n (%)	1130 (49.5%)	371 (49.8%)	372 (50.0%)	354 (47.7%)	
Current smoker, n (%)	446 (19.5%)	124 (16.6%)	139 (18.7%)	176 (23.7%)	
Diabetes, n (%)	301 (13.1%)	90 (12.0%)	86 (11.5%)	118 (15.8%)	0.03
History of cardiovascular disease, n (%)	236 (10.3%)	61 (8.2%)	73 (9.8%)	92 (12.3%)	0.03
Platelet inhibition, n (%)	288 (12.6%)	87 (11.6%)	80 (10.7%)	111 (14.9%)	0.04
Baseline eGFR, mL/min per 1.73 m^2	80.80 (13.54)	81.22 (12.26)	81.36 (13.03)	79.87 (15.00)	0.07
eGFR groups					<0.001
eGFR <60, n (%)	168 (7.6%)	32 (4.5%)	50 (6.8%)	82 (11.3%)	
eGFR 60–90, n (%)	1411 (63.5%)	495 (69.0%)	465 (63.7%)	421 (58.0%)	
eGFR >90, n (%)	644 (29.0%)	190 (26.5%)	215 (29.5%)	223 (30.7%)	
Albuminuria†					<0.001
No albuminuria, n (%)	2134 (93.1%)	713 (95.4%)	713 (95.6%)	664 (89.0%)	
Microalbuminuria, n (%)	138 (6.0%)	32 (4.3%)	29 (3.9%)	72 (9.7%)	
Macroalbuminuria, n (%)	19 (0.8%)	2 (0.3%)	4 (0.5%)	10 (1.3%)	
PGE2, pg/mmol, median (IQR)	81.4 (60.5–114.8)	53.1 (43.5–60.5)	81.4 (73.8–90.1)	137.7 (114.9–180.6).	Not tested
PGE2 metabolite, pg/mmol, median (IQR)	48.2 (33.7–66.9)	37.2 (25.3–5.4)	47.1 (34.8–61.4)	63.4 (45.6–90.9)	<0.001

Data are presented as mean (SD), unless otherwise specified. Conversion factor for total cholesterol and high-density lipoprotein cholesterol mg/dL to mmol/L, ×0.02586. BMI indicates body mass index; eGFR, estimated glomerular filtration rate; IQR, interquartile range; and PGE2, prostaglandin E2.

*Body mass index groups: underweight: BMI <18.5 kg/m^2, normal weight: BMI ≥18.5 and <25 kg/m^2, overweight: BMI ≥25 and <30 kg/m^2, obese: BMI ≥30 kg/m^2.

†Albuminuria groups are defined as: no albuminuria (<22 mg/g for men, <30 mg/g for women), microalbuminuria (22–221 mg/g for men, 30–310 mg/g for women), macroalbuminuria (>221 mg/g for men >310 mg/g for women).

associated with incident eGFR <60 mL/min per 1.73 m^2 (HR per 2-fold higher PGE2, 1.13 [95% CI, 1.02–1.25], high versus low tertile of PGE2 HR, 1.26 [95% CI, 1.03–1.53]). For PGEM there was no significant linear association (HR per 2-fold higher PGEM, 1.09 [95% CI, 0.99–1.21], high versus low tertile HR, 1.02 [95% CI, 0.84–1.23]). However, after modeling PGEM as a cubic spline with 4 knots, we did find an association, mainly in the higher ranges of urinary PGEM excretion (*P*=0.007 for association, *P*=0.014 for nonlinearity; Figure 3B). Both higher urinary PGE2 and urinary PGEM excretions were also associated with the other kidney outcomes, and the associations did not markedly change when using the urine concentration as determinant with 1/creatinine as covariate (Figure 4; Tables S5 and S6; Figures S6 through S8). 2117 participants had at least 2 eGFR assessments available and were used for the eGFR slope analysis. The mean rate of decline in eGFR in all participants was −0.65 mL/min/1.73 m^2 per year (95% CI, −0.69 to −0.60). Higher urinary PGE2 excretion was associated with a faster decline in eGFR (−0.11 mL/min per 1.73 m^2 per year [95% CI, −0.17 to −0.05]). For PGEM this association was not significant in the adjusted model (−0.06 mL/min per 1.73 m^2 per year [95% CI, −0.14 to −0.02]; Table 4). The associations between urinary PGE2 and PGEM excretions

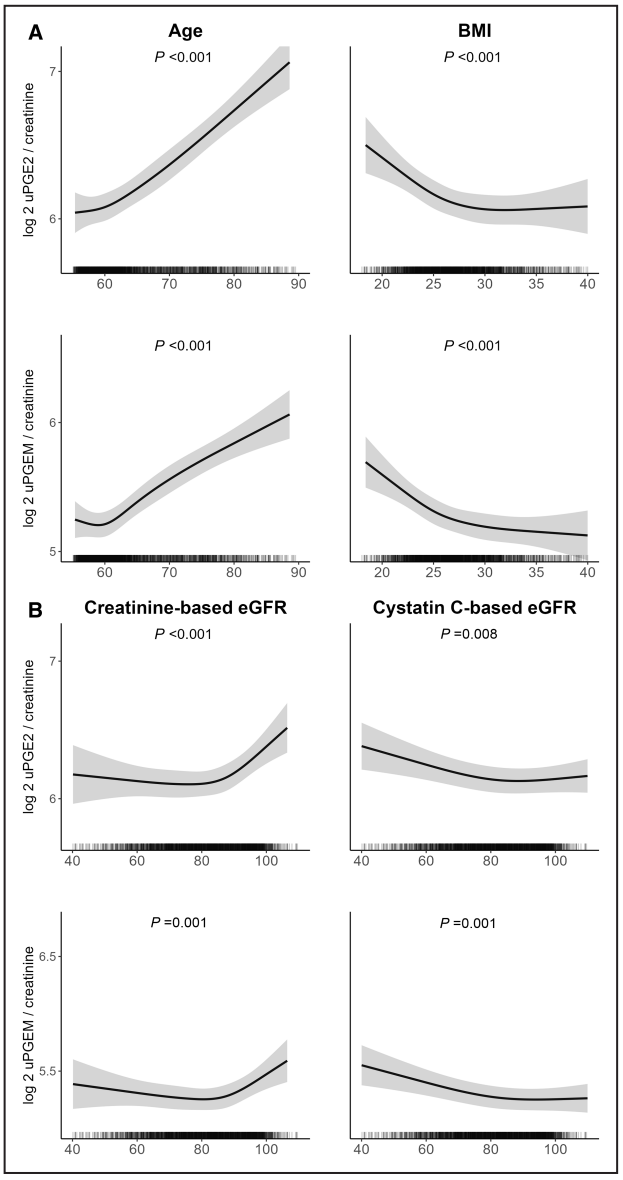

Figure 1. Determinants of urinary PGE2 and PGEM excretions. Effect plots showing the nonlinear associations between urinary prostaglandin E2 (PGE2) (top row) and prostaglandin E2 metabolite (PGEM) (bottom row) excretion and age, body mass index (BMI) (**A**) and creatinine-based estimated glomerular filtration rate (eGFR) and cystatin C-based eGFR (**B**). These plots are prediction plots from multivariable models corrected for age, sex, BMI, creatinine-based eGFR, smoking status, presence of diabetes, albuminuria, and hypercholesterolemia; for the plot with cystatin C-based eGFR, creatinine-based eGFR is not used as a covariate. All covariates are set at either their median (age 61.7 years, BMI 26.6 kg/m^2, eGFR 82.1 mL/min per 1.73 m^2) or most common value (female sex, never smoker, no diabetes, no albuminuria, no hypercholesterolemia). *P* values are from Wald tests for the overall effect of adding the nonlinear term. On the *x* axis a rug plot depicting data point density is shown.

and the rate of eGFR decline were not different when using concentrations and 1/creatinine as covariate (Table S7). The HR for the association between PGE2 and incident eGFR <60 mL/min per 1.73 m^2 in participants with diabetes was lower (*P*=0.06), which was also observed with some but not all other kidney outcomes (Figures S9 through S12). For urinary PGEM excretion there were no consistent differences between subgroups (Figures S9 through S12). The E-value for urinary PGE2 excretions on incident eGFR <60 mL/min per 1.73 m^2 was 1.40 (lower CI, 1.13) (Table S8), making it unlikely that unmeasured confounding explains this association.

DISCUSSION

In this study we hypothesized that urinary PGE2 and PGEM excretions are indicators of cardiovascular and kidney health and tested this using a prospective population-based cohort. We show that older people and people with cardiovascular risk factors, lower eGFR, and higher albuminuria have higher urinary PGE2 and PGEM excretions. Furthermore, we show that higher urinary PGE2 and PGEM excretions are independently associated with higher cardiovascular mortality and risk of kidney function decline. Our results indicate that urinary PGE2 and PGEM excretions are markers for the presence and progression of cardiovascular and kidney disease.

The observation that urinary PGE2 and PGEM excretions increase with age was independent of other determinants and cardiovascular risk factors. Previous work showed that COX-2 and PGE2 production are higher in macrophages, lung tissue, and kidneys of older individuals compared with younger individuals.[39] Our findings therefore suggest that urinary PGE2 and PGEM excretions could be markers of vascular health with age. Participants with cardiovascular risk factors also had higher urinary excretions of PGE2 and PGEM. The associations between urinary PGE2 and PGEM excretions with smoking and diabetes have been reported previously.[13,25,27,40] Nicotine and other constituents of cigarette smoke directly upregulate COX-2 expression in epithelial cells, and diabetes is associated with increased COX-2 activity in the kidney and vasculature.[41–43]

Further support for the involvement of the PGE2 system in vascular health and disease comes from the finding that higher urinary PGE2 and PGEM excretions are strongly associated with cardiovascular mortality. This is in line with the previously reported role of PGE2 in atherosclerosis and recent studies showing that single nucleotide polymorphisms in genes encoding proteins in PGE2 signaling pathways are associated with cardiovascular disease.[10,11,44] Urinary PGEM excretion showed a stronger association with cardiovascular mortality compared with urinary PGE2 excretion, consistent with the proposed systemic origin of urinary

Table 2. Association of Urinary PGE2 and PGEM Excretion With Cardiovascular Risk Factors and Kidney Function

	PGE2				PGEM			
	Uncorrected		Multivariable		Uncorrected		Multivariable	
Characteristic	β (95% CI)	*P* value	β (95% CI)	*P* value	β (95% CI)	*P* value	β (95% CI)	*P* value
Sex, male vs female	0.03 (−0.03 to 0.1)	0.36	0.03 (−0.04 to 0.09)	0.41	0.07 (0.00 to 0.14)	0.047	0.07 (0 to 0.13)	0.058
Age, y*	0.21 (0.12 to 0.29)	<0.001	0.25 (0.16 to 0.34)	<0.001	0.26 (0.17 to 0.35)	<0.001	0.3 (0.21 to 0.39)	<0.001
Body mass index, kg/m^2*	−0.11 (−0.19 to −0.03)	<0.001	−0.12 (−0.2 to −0.04)	<0.001	−0.14 (−0.23 to −0.06)	<0.001	−0.14 (−0.22 to −0.06)	<0.001
Smoking status (overall effect)		0.004		0.003		<0.001		<0.001
Current smoker vs former, now nonsmoker	0.16 (0.06 to 0.25)	0.001	0.16 (0.07 to 0.25)	0.001	0.22 (0.13 to 0.32)	<0.001	0.22 (0.13 to 0.32)	<0.001
Current smoker vs never smoker	0.09 (0.0004 to 0.17)	0.049	0.11 (0.02 to 0.19)	0.013	0.19 (0.10 to 0.27)	<0.001	0.21 (0.12 to 0.29)	<0.001
Hypertension	0.10 (0.03 to 0.17)	0.003	0.01 (−0.06 to 0.08)	0.78	0.03 (−0.03 to 0.10)	0.33	−0.05 (−0.12 to 0.02)	0.16
Diabetes	0.17 (0.08 to 0.27)	<0.001	0.11 (0.01 to 0.21)	0.028	0.21 (0.11 to 0.31)	<0.001	0.16 (0.06 to 0.26)	0.001
Hypercholesterolemia	−0.01 (−0.08 to 0.06)	0.77	0.02 (−0.04 to 0.08)	0.55	−0.05 (−0.12 to 0.02)	0.138	−0.02 (−0.09 to 0.04)	0.50
Estimated glomerular filtration rate, mL/min per 1.73 m^2*	0.00 (−0.09 to 0.09)	<0.001	0.10 (0.02 to 0.19)	<0.001	−0.03 (−0.12 to 0.06)	<0.001	0.06 (−0.03 to 0.15)	0.19
Albuminuria (overall effect)		<0.001		<0.001		<0.001		<0.001
Microalbuminuria vs no albuminuria	0.43 (0.29–0.56)	<0.001	0.26 (0.12 to 0.39)	<0.001	0.27 (0.13 to 0.41)	<0.001	0.09 (−0.04 to 0.23)	0.18
Macroalbuminura vs no albuminuria	0.61 (0.23 to 0.99)	0.002	0.3 (−0.09 to 0.68)	0.13	1.2 (0.82 to 1.57)	<0.001	0.86 (0.48 to 1.24)	<0.001

β from univariable and multivariable regression models for cardiovascular risk factors for log2-transformed creatinine corrected PGE2 and PGEM excretions. The multivariable analysis is adjusted for all included cardiovascular risk factors and the determinants of PGE2 and PGEM. Nonlinear effects were fitted with restricted cubic splines with 4 knots. The multivariate models are adjusted for the other variables depicted in the table. PGE2 indicates prostaglandin E2; and PGEM, prostaglandin E2 metabolite.

*Difference in log2-transformed PGE2 or PGEM from low (25%) and high (75%) quartile with other covariates at their median or standard value.

PGEM.[21–23] Of interest, we observed a stronger association between PGE2 and cardiovascular mortality in men than in women, whereas we did not identify a sex difference regarding the kidney outcomes. Sex differences in the prostaglandin system have been reported previously, including a lack of effect of acetylsalicylic acid for primary prevention in women.[45] The stronger association between PGE2 and cardiovascular mortality in men could possibly be explained by differences in inflammation in atherosclerosis between men and women.[46] Future studies should investigate if urinary PGE2 and PGEM excretions are also associated with other cardiovascular end points. Besides the association with cardiovascular mortality, we also found an association with noncardiovascular and overall mortality. Because higher urinary PGEM excretion has been observed in patients with cancer, shared risk factors for cancer and cardiovascular disease such as smoking, diabetes, and aging may explain this association.[21–23,47] Whether the associations between PGE2 and cardiovascular mortality are also present in younger populations remains to be explored. We did not observe consistent differences in the associations in the younger half of our population compared with the older half.

In addition to cardiovascular outcomes, urinary PGE2 and PGEM excretions were associated with kidney function and the risk of kidney function decline. Higher urinary PGE2 and PGEM excretions have been reported previously in people with CKD and early diabetic kidney disease[15,16] but not yet in the general population. These associations could be causal, because recent animal studies show that pharmacological inhibition of PGE2 receptors attenuates CKD progression.[4] In a randomized clinical trial COX inhibition reduced proteinuria without a reduction in systemic blood pressure.[48] How PGE2 accelerates kidney injury is incompletely understood, but injury may be mediated by PGE2-induced hyperfiltration,[13] inflammation, and fibrosis.[4] An unexpected finding was higher urinary PGE2 and PGEM excretions in participants with eGFR >90 mL/min per 1.73 m^2. This is likely explained

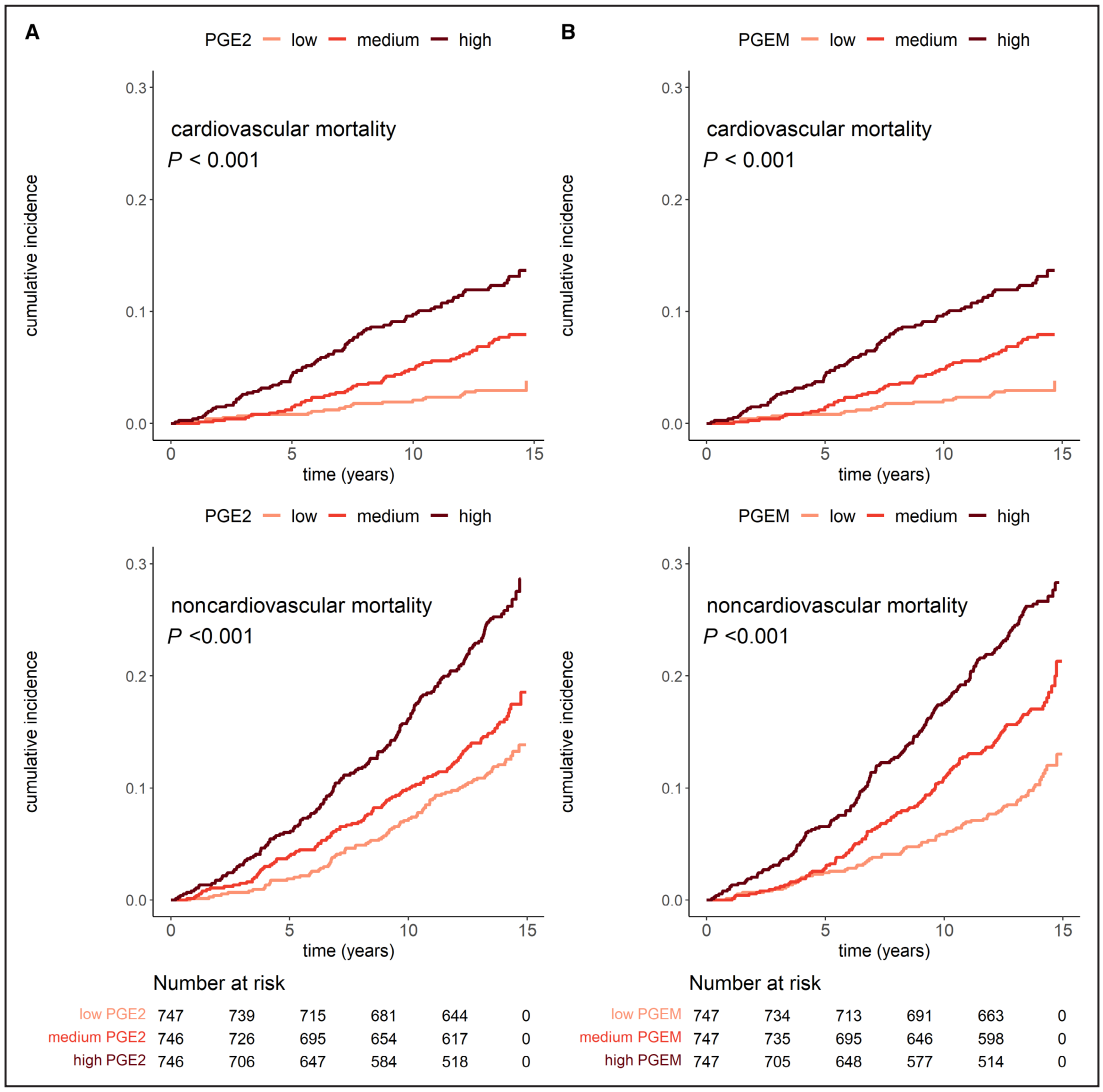

Figure 2. Cardiovascular mortality.
Cumulative incidence curves for cardiovascular and noncardiovascular mortality for tertiles of urinary prostaglandin E2 (PGE2) (**A**) and prostaglandin E2 metabolite (PGEM), (**B**) excretions, respectively. *P* values are from log-rank tests.

Table 3. Association of Urinary PGE2 and PGEM Excretion With Cardiovascular Mortality

	Unadjusted	Model 2	Model 3
Urinary PGE2 excretion			
Per 2-fold higher PGE2	1.95 (1.66–2.30)	1.38 (1.14–1.66)	1.27 (1.06–1.54)
PGE2 low tertile	Reference	Reference	Reference
PGE2 medium tertile	1.52 (0.94–2.44)	1.29 (0.80–2.08)	1.41 (0.87–2.27)
PGE2 high tertile	3.57 (2.34–5.44)	1.80 (1.15–2.80)	1.62 (1.04–2.52)
Urinary PGEM excretion			
Per 2-fold higher PGEM	2.14 (1.77–2.58)	1.52 (1.24–1.86)	1.36 (1.10–1.67)
PGEM low tertile	Reference	Reference	Reference
PGEM medium tertile	2.56 (1.56–2.41)	1.83 (1.10–3.03)	1.87 (1.13–3.10)
PGEM high tertile	4.53 (2.83–7.24)	2.23 (1.37–3.64)	2.04 (1.24–3.34)

Hazard ratios (95% CI) for 2-fold PGE2, PGEM, and tertiles of urinary PGE2 or PGEM excretions. Model 2: corrected for potential confounders: age, sex, body mass index, smoking status, baseline estimated glomerular filtration rate, and use of antiplatelet drugs. Model 3: model 2+albumin-to-creatinine ratio, hypertension, diabetes, history of cardiovascular disease, total cholesterol, high-density lipoprotein cholesterol, and use of lipid-lowering agents. PGE2 indicates prostaglandin E2; and PGEM, prostaglandin E2 metabolite.

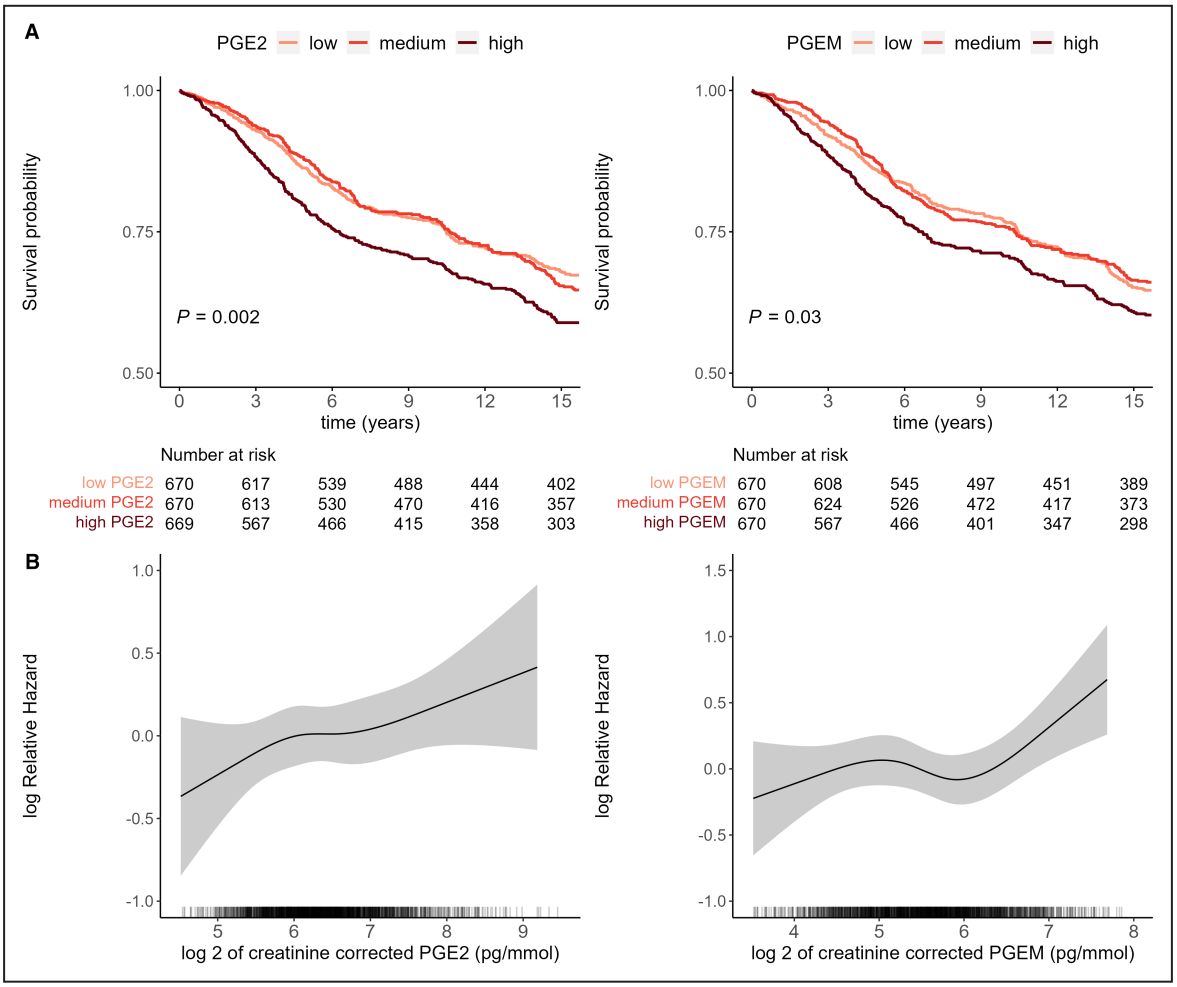

Figure 3. Survival outcomes for incident estimated glomerular filtration rate <60 mL/min per 1.73 m^2.
A, Kaplan–Meier survival curves and risk tables for incident estimated glomerular filtration rate (eGFR) <60 mL/min per 1.73 m^2 stratified to tertiles of prostaglandin E2 (PGE2, n=2009; events, n=677) and prostaglandin E metabolite (PGEM, n=2010; events, n=678). *P* value from log-rank test. **B**, Hazard plots from the fully corrected Cox proportional hazard model (model 3) using a restricted cubic spline with 4 knots, showing nonlinearity for urinary PGEM excretion (*P*=0.014) but not for urinary PGE2 excretion (*P*=0.7). On the *x* axis a rug plot with the density of PGE2 and PGEM measurements is shown.

by non-eGFR determinants of creatinine such as muscle mass, because this was less pronounced when using cystatin C-based eGFR. Other possible explanations include hyperfiltration or lower precision of the CKD-EPI equation at higher eGFRs.[13,30]

Based on our data, it is tempting to speculate that PGE2 is involved in the close relationship between cardiovascular and kidney disease. The association of urinary PGE2 and PGEM excretions with shared risk factors for cardiovascular and kidney disease, such as smoking and hypertension, could explain our findings. Our findings could also be explained by the role of PGE2 in atherosclerosis,[10,11] as even minor reductions in kidney function are associated with atherosclerosis and atherosclerosis contribute to CKD.[49,50] Beyond the traditional cardiovascular risk factors, urinary PGE2 and PGEM excretions could be markers of low-grade inflammation, another possible mediator in both early cardiovascular disease and kidney damage.[51,52] Alternatively, kidney-derived PGE2 might directly influence the cardiovascular system. The kidney is an important source of PGE2 production, as in mice global COX-2 knockout causes >1000 gene expression changes in the kidney but only a single change in the vasculature.[53] Furthermore, kidney PGE2 production is increased in experimental CKD and was implicated in the associated cardiac hypertrophy.[54]

The strength of this study is the large and well-characterized population of middle-aged and elderly participants, which allowed us to gain insight into early disease mechanisms. Also, the cohort is set up prospectively with a high participation rate and we used urine samples collected at study entry, reducing selection and information bias.[28] We also acknowledge

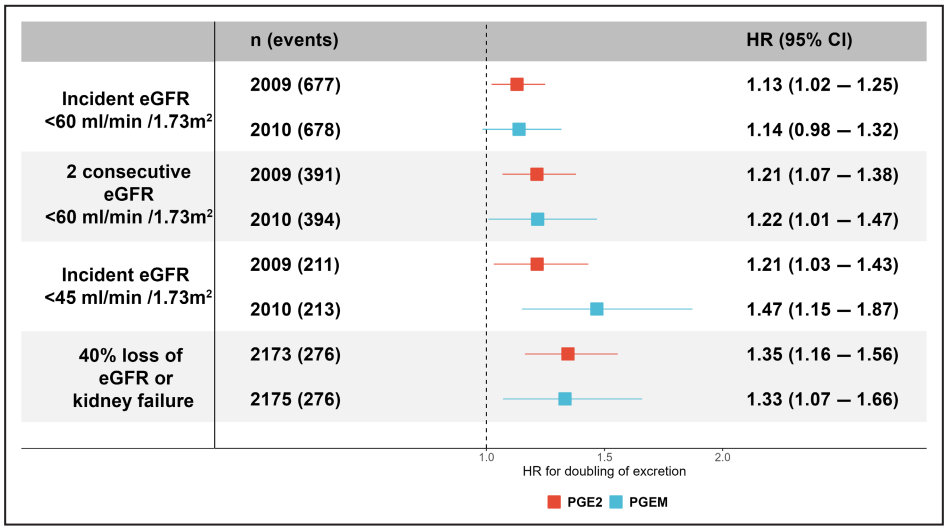

Figure 4. Forest plot of the associations between urinary PGE2 and PGEM excretions and kidney outcomes.
Hazard ratios (HRs) for the association of urinary prostaglandin E2 (PGE2, blue) and prostaglandin E2 metabolite (PGEM, red) and the 4 different kidney outcomes. The HRs are corrected for the confounders age, sex, body mass index, smoking status, and baseline estimated glomerular filtration rate (eGFR).

a number of limitations. First, the reference standard for prostaglandin quantification is mass spectrometry.[55] Because this method is less applicable for high-throughput analysis in large cohorts, we measured PGE2 and PGEM using an immunoassay, which is widely used for urine samples in clinical research and confirmed previous associations obtained using mass spectrometry.[13,24–26] Second, the lack of serial measurements of albuminuria precluded the use of the Kidney Disease: Improving Global Outcomes CKD classification as an outcome. This was circumvented by using different kidney outcomes, which all showed the same associations. Third, data regarding over-the-counter use of NSAIDs were not available in our study, potentially leading to confounding for the association between urinary PGE2 and PGEM excretions on our outcomes. The prevalence of over-the-counter NSAID use in our elderly population is unknown but 1 study showed that incidental NSAID use was lower in high-risk patients from the general population >70 years of age (~7%) with the majority of use being nonchronic use of low-dose ibuprofen or high-dose acetylsalicylic acid.[56] Fourth, participants of the RS are mainly of European ancestry limiting generalizability to other ethnicities. Finally, we cannot exclude residual confounding, although we were able to adjust for many well-defined confounders and our E-value analyses suggested a low risk of unmeasured confounding.

Table 4. Association of Urinary PGE2 and PGEM Excretion With Change in eGFR Over Time

	Difference in eGFR in mL/min per 1.73 m² per year (95% CI)		
	Unadjusted	Model 2	Model 3
Urinary PGE2 excretion			
Per 2-fold higher PGE2	−0.18 (−0.24 to −0.12)	−0.14 (−0.20 to −0.07)	−0.11 (−0.17 to −0.05)
PGE2 low tertile	Reference	Reference	Reference
PGE2 medium tertile	−0.21 (−0.33 to −0.09)	−0.19 (−0.31 to −0.08)	−0.19 (−0.31 to −0.08)
PGE2 high tertile	−0.29 (−0.41 to −0.17)	−0.22 (−0.34 to −0.10)	−0.19 (−0.31 to −0.07)
Urinary PGEM excretion			
Per 2-fold higher PGEM	−0.13 (−0.20 to −0.07)	−0.08 (−0.14 to −0.02)	−0.06 (−0.12 to 0.002)
PGEM low tertile	Reference	Reference	Reference
PGEM medium tertile	−0.05 (−0.17 to 0.07)	−0.02 (−0.13 to 0.10)	−0.03 (−0.14 to 0.09)
PGEM high tertile	−0.21 (−0.34 to −0.09)	−0.11 (−0.23 to 0.02)	−0.08 (−0.20 to 0.04)

Results from linear mixed models. Model 2: corrected for potential confounders: age, sex, body mass index, smoking status, and baseline eGFR. Model 3: model 2+diabetes status, albumin-to-creatinine ratio, history of cardiovascular disease, and presence of hypertension. eGFR indicates estimated glomerular filtration rate; PGE2, prostaglandin E2; and PGEM, prostaglandin E2 metabolite.

CONCLUSIONS

In conclusion, our study shows that higher urinary PGE2 and PGEM excretions are associated with increased risk of cardiovascular mortality and kidney function decline. Future studies should investigate if urinary PGE2 and PGEM excretions are also associated with other cardiovascular end points and compare their utility with other cardiovascular prediction models. These findings link previous experimental data on the role of PGE2 in cardiovascular and kidney disease to the general population, and suggest that urinary PGE2 and PGEM excretions are early markers of cardiovascular and kidney disease.

ARTICLE INFORMATION

Received September 29, 2023; accepted January 23, 2024.

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Acknowledgments

The authors are grateful to the study participants, the staff from the Rotterdam Study, and the participating general practitioners and pharmacists.

Sources of Funding

The Rotterdam Study is funded by Erasmus Medical Center and Erasmus University, Rotterdam, ZonMw – The Netherlands Organization for Health Research and Development, the Dutch Ministry of Education, Culture and Science, the Dutch Ministry for Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam. Frank Geurts, Robert A. Fenton, and Ewout J. Hoorn are funded by the Novo Nordisk Foundation (NFF18OC0031686). Ewout J. Hoornis also funded by the European Research Council (ERC-COG 101125504).

Disclosures

None.

Supplemental Material

Data S1.
Tables S1–S8.
Figures S1–S12.

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