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Urinary phosphate is associated with cardiovascular disease incidence

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Introduction. Elevated phosphate (P) in urine may reflect a high intake of inorganic P salts from food additives. Elevated P in plasma is linked to vascular dysfunction and calcification.

Objective. To explore associations between P in urine as well as in plasma and questionnaire-estimated P intake, and incidence of cardiovascular disease (CVD).

Methods. We used the Swedish Mammography Cohort-Clinical, a population-based cohort study. At baseline (2004–2009), P was measured in urine and plasma in 1625 women. Dietary P was estimated via a food-frequency questionnaire. Incident CVD was ascertained via register-linkage. Associations were assessed using Cox proportional hazards regression.

Results. After a median follow-up of 9.4 years, 164 composite CVD cases occurred (63 myocardial infarctions [MIs] and 101 strokes). Median P (percentiles 5–95) in urine and plasma were 2.4 (1.40–3.79) mmol/mmol creatinine and 1.13 (0.92–1.36) mmol/L, respectively, whereas dietary P intake was 1510 (1148–1918) mg/day. No correlations were observed between urinary and plasma P ($r = -0.07$) or dietary P ($r = 0.10$). Urinary P was associated with composite CVD and MI. The hazard ratio of CVD comparing extreme tertiles was 1.57 (95% confidence interval 1.05, 2.35; P trend 0.037)—independently of sodium excretion, the estimated glomerular filtration rate, both P and calcium in plasma, and diuretic use. Association with CVD for plasma P was 1.41 (0.96, 2.07; P trend 0.077).

Conclusion. Higher level of urinary P, likely reflecting a high consumption of highly processed foods, was linked to CVD. Further investigation is needed to evaluate the potential cardiovascular toxicity associated with excessive intake of P beyond nutritional requirements.

Keywords: cardiovascular disease incidence, dietary phosphate intake, phosphate-based additives, plasma phosphate, ultra-processed food, urinary phosphate

Introduction

The homeostasis of phosphorous—occurring in the body as the pentavalent form phosphate (P) with many structural and functional roles—is regulated through a complex interaction among gastrointestinal absorption, bone remodeling, and urinary excretion [1]. Dietary P intake promotes the production of hormones increasing its renal clearance [2–4], reverting a high postprandial blood P concentration to fasting state. During normal conditions, urinary P excretion reflects P absorbed in the digestive tract [5, 6]. Excess dietary P intake may disrupt the P-responsive hormones (fibroblast growth factor-23, parathyroid hormone, and

calcitriol) [7], contributing to vascular calcification [8–10]. Elevated plasma P concentrations are linked to increased oxidative stress of the endothelial cells, vascular dysfunction [11], and artery calcification [12–14].

The main dietary sources of phosphorus in its natural form (organic P) are protein-rich foods, such as dairy products, meat, fish, and legumes. In contrast, the inorganic P salts are incorporated into foods as additives, especially prevalent in ultra-processed food (UPF) [15]. UPFs [16], whose consumption has increased substantially over the last decades [17, 18], are foods that undergo intense industrial processing and may contain substances usually not found in domestic kitchens. Unintentional contamination during processing and packaging (e.g., phosphorous flame retardants) has also been observed in highly processed foods [19].

Unlike organic P, added inorganic P has high bioavailability and is almost entirely absorbed in the digestive tract [20–24]. An accurate quantification of inorganic P intake is currently not possible due to a lack of a quantitative labeling system of additives on packaged food. Roughly estimated, P from additives may contribute up to 20%–30% of the total dietary P intake [15] but could be substantially higher [25]. UPF is likely the main source of the so-called hidden P that may not be properly quantified in food composition tables and population dietary survey studies [20, 26–28].

Based on this background, we hypothesized that elevated levels of P in urine, mostly reflecting a high consumption of inorganic P from additives common in UPF, might be associated with an increased incidence of cardiovascular disease (CVD). For this purpose, we assessed P in urine, plasma, and diet in a population-based cohort of women.

Material and methods

Study population

The Swedish Mammography Cohort (SMC) is a large population-based longitudinal cohort established in 1987–1990 and part of the national research infrastructure SIMPLER (www.simpler4health.se). All women born between 1914 and 1948 residing in two counties in central Sweden (n = 90,303) were invited to complete a self-administrated questionnaire concerning diet (response rate 74%). In 1997, a more detailed questionnaire was sent to all participants who were still alive and living in the study area (response rate 70%). More details on the study design have been published elsewhere [29].

The present study was conducted in a clinical sub-cohort, the Swedish Mammography Cohort-Clinical (SMC-C). It was established in 2003–2009 by inviting women from the cohort <85-year old and living in Uppsala to participate in a health examination, comprising the baseline of the present study. Of them, 5022 (response rate 61%) completed a diet and lifestyle questionnaire, and measurements of height, weight, and a fasting blood sample were collected. Starting from 2004, 4480 of them also provided urine samples for element analyses. Element analysis in urine was performed in two analytical assessment rounds. The first, which included urine from women <70 years of age, did not include P because it was not yet a research focus. Thus, urinary P was measured only in the second round, which included urine from all SMC-C women ≥70-year old and all those who were younger and had performed their urine sampling after December 2008 (in total n = 1782). Thus, after excluding those with prevalent CVD (n = 157), our final study population included 1625 women with urinary P at baseline (of whom 21% <70 years; Fig. 1). Written informed consent was obtained from all study participants, and the study was approved by the Regional Ethical Review Board in Stockholm, Sweden.

P biomarkers

In 2004, urine sampling for element analyses was initiated, and the women received posted instructions and containers for the collection of first voided morning urine, to provide at the day of examination (n = 4480). Baseline total urinary P was assessed in first voided morning urine and measured in acid-diluted samples (1:10 in 1% nitric acid, 65% w/w, Scharlab S.L., Sentmenat, Spain), using ICP-MS (Agilent Technologies 7700×, Tokyo, Japan) with an Octopole Reaction System operated in helium mode at the Institute of Environmental Medicine, Karolinska Institutet. The limit of detection (calculated as three times the standard deviation of the blank values) for was 4.3 μg/L (0.0001 mmol/L). As quality control, two commercial reference materials were included in each run, and there was good agreement between recommended and obtained phosphorous values (Seronorm Trace Elements Urine 1011644: approximate value: 559 mg/L, obtained

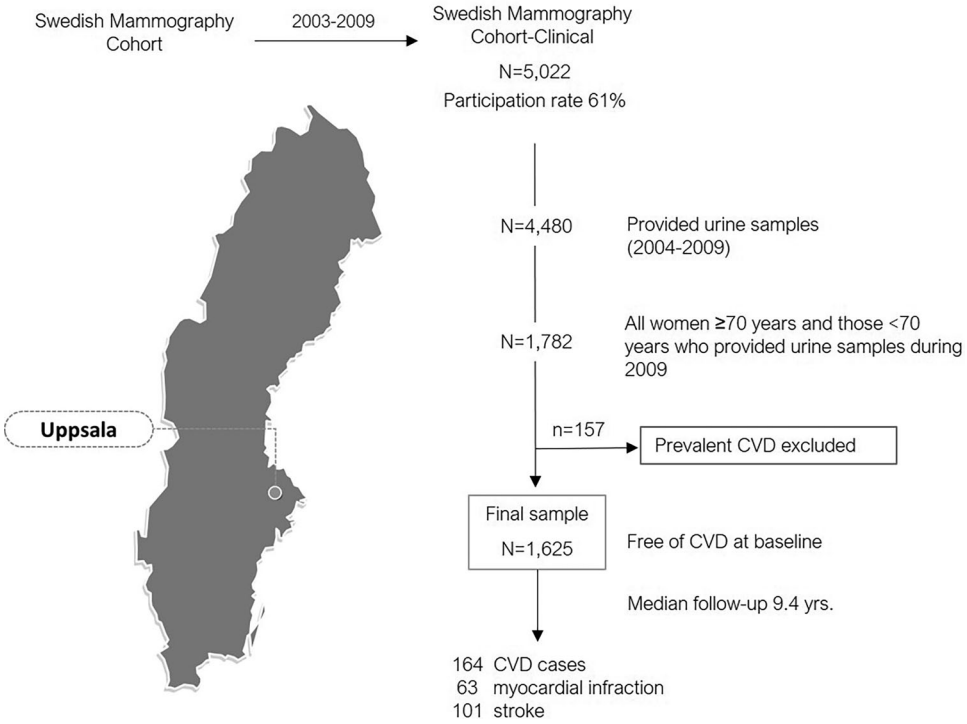

Fig. 1 *Flow chart of the study population sample from the Swedish Mammography Cohort-Clinical. Includes myocardial infarction and stroke (ischemic stroke and unspecified stroke). CVD, cardiovascular disease.*

value 504 ± 37 mg/L [n = 20]; Seronorm Trace Elements Urine 1011645: approximate value: 543 mg/L, obtained value 503 ± 40 mg/L [n = 19]). Urine P concentrations were adjusted for the variation in dilution by dividing them by the urine creatinine concentration, measured at Skåne University Hospital using an enzymatic method (Roche Diagnostics, Germany), and expressed in mmol P/mmol creatinine.

Baseline plasma P was measured in fasting samples by use of routine clinical method and an Architect C16000 (Abbott Laboratories, Abbott Park, IL, USA) [30] at the Department of Clinical Chemistry and Pharmacology (Uppsala University Hospital). The laboratory is accredited according to SS-EN-ISO/IEC 15189 and participates in external quality assessment schemes from Equalis AB (Uppsala, Sweden).

Dietary P intake and covariates

Estimated dietary intake of P was derived from the semiquantitative 124-item food-frequency questionnaire completed by the participants at baseline (2004–2009), obtained by multiplying the individual intake frequency and portion size with the content in specific foods, compiled using the Swedish Food Agency's database [31]. Dietary P intake was adjusted for total energy intake using the residual method [32].

Self-reported information on education, family history of myocardial infarction (MI), smoking, physical activity, diet, and alcohol consumption, use of vitamin D supplements and medication use were obtained from the questionnaires completed at baseline (2004–2009). From the Swedish National Prescribed Drug Register, we obtained complete information on diuretics use (the Anatomical Therapeutic Chemical classification system, ATC-code C03). Since this register was initiated in mid-2005, and some urine samples were collected prior to this date, we completed the register data with the self-reported use of diuretics.

Prevalent diabetes and hypertension at baseline were identified as a combination of information based on self-reports (including the use of antihypertensive, and antidiabetic drugs), the National

Diabetes Register, and the Patient Register. A Mediterranean diet score was created based on the eight-point scoring system (low to high adherence) reflecting the consumption of fruits and vegetables, whole grain/fiber-rich foods, legumes and nuts, fish, fermented dairy foods, olive/rapeseed oil, and alcohol (in moderation) as positive components; and red and processed meat and excess alcohol as negative components [33].

We measured cystatin C and creatinine in plasma (Uppsala University Hospital) and sodium (ICP-MS at the Institute of Environmental Medicine, Karolinska Institutet) in urine (as a proxy for dietary salt intake). Estimated glomerular filtration rate (eGFR) was calculated by using the combined creatinine–cystatin C equation [34].

Cardiovascular outcomes

CVD was defined as first incident MI (International Classification (ICD-10 code I21)), ascertained either via computerized linkage to the National Hospital Discharge Register [35] or the Cause of Death Register (verified by autopsy), or first incident ischemic stroke (National Hospital Discharge Register ICD-10 I63-I64). Participants were followed up from baseline (2004–2009) to December 31, 2017.

Statistical analyses

The correlation between P in urine and in plasma and the energy-adjusted dietary P intake was assessed by Spearman´s rank correlation. We categorized participants into tertiles of urinary P, plasma P, and dietary P at baseline. Associations of P concentrations with CVD, MI, and stroke were examined prospectively using Cox proportional hazard regression models, setting age as the underlying timescale, and calculating hazard ratios (HRs) and their 95% confidence interval (CI). Tests for linear trends (P trend) across tertiles were conducted by assigning the median value for each tertile and modeling it as a continuous variable. We verified the proportionality of hazards with a test based on Schoenfeld residuals; the nonsignificant result (p-value >0.80 in all cases) suggested that the proportionality assumption was met.

Regression models were age-adjusted (model 1) and additionally adjusted for the following baseline variables (model 2): body mass index (continuous), postsecondary education >12 years (yes/no), family history of MI <60 years (yes/no), history of diabetes (yes/no), history of hypertension (yes/no), smoking (never, former, or current smoker), walking/cycling >20 min/day (yes/no), leisure-time inactivity >5 h/day (yes/no), adherence to Mediterranean diet (0–4, 5, 6–8 score), alcohol consumption (g/day), vitamin D supplement use (yes/no), and eGFR ($\leq$ or >60 mL/min/1.73 m^2). In model 3, we additionally adjusted for urinary sodium (mmol/mmol creatinine), plasma calcium—which is linked to both the exposure and risk of CVD [36, 37]—and use of diuretics. For urinary P analyses, we also estimated an additional model 4 further adjusted for plasma P, suspecting this variable as a potential confounder of the urinary P-CVD association, assuming that alterations in fasting plasma P are consequences of P homeostasis dysregulation rather than differences in dietary P intake. Based on an a priori judgment related to the limited sample size and number of cases, no stratified analyses were performed. All probability values were two-sided. Analyses were performed with Stata 16 SE software (Stata Corporation Inc., TX, USA).

Results

The age of the women at baseline ranged from 56 to 85 years (median 73 years). Median (percentiles 5–95) P concentration in urine and plasma was 2.4 (1.40–3.79) mmol/mmol creatinine and 1.13 (0.92–1.36) mmol/L, respectively, whereas the median estimated P intake was 1510 (1148–1918) mg/day.

Participants with the highest urinary P concentrations (third tertile) had slightly more frequently a family history of MI <60 years, were more frequently current smokers, were less frequently taking vitamin D supplements, had higher levels of urinary sodium, and slightly more often users of diuretics. No relevant differences in plasma P concentrations, dietary P intake, or eGFR were observed across tertiles of urinary P concentrations (Table 1). Participants in the upper range of plasma P concentrations, on the other hand, were more educated, were more frequently current smokers, were less inactive, and used less diuretics than those with lower plasma P (Table 1). No meaningful correlation was observed between urinary and plasma P ($r = -0.07$), between urinary P and dietary P ($r = 0.10$), or between plasma P and dietary P ($r = -0.01$).

Among 1625 women who were followed up during a median period of 9.4 years (15,195 person-years),

Table 1. *Age-standardized baseline characteristics of Swedish Mammography Cohort-Clinical participants according to tertiles of phosphate concentrations (N = 1625).*

	Tertiles of urinary phosphate (mmol/mmol creatinine)			Tertiles of plasma phosphate (mmol/L)		
	T1	T2	T3	T1	T2 1.14	T3 1.26
Phosphate, median (IQR)	1.8 (0.3–2.1)	2.4 (2.1–2.7)	3.2 (2.8–12.7)	1.01 (0.95–1.05)	(1.11–1.16)	(1.23–1.32)
No. women	542	542	541	557	535	523
Urinary phosphate, mmol/mmol[a], percentile (5–95)	1.09–2.09	2.15–2.71	2.78–4.34	1.43–3.83	1.37–3.73	1.38–3.70
Plasma phosphate, mmol/L, percentile (5–95)	0.94–1.36	0.90–1.35	0.92–1.36	0.85–1.08	1.09–1.19	1.20–1.42
Dietary phosphate, mg/day, percentile (5–95)[b]	1122–1898	1157–1908	1176–1923	1140–1882	1175–1919	1147–1928
Age, years, mean (SD)	73 (7)	73 (7)	74 (6)	74 (7)	73 (7)	72 (7)
BMI, kg/m^2, mean (SD)	25.5 (4.1)	26.1 (4.3)	26.6 (4.6)	26.8 (4.2)	25.9 (4.3)	25.4 (4.4)
Postsecondary education >12 years (%)	28	30	27	21	30	34
Family history of MI before 60 years (%)	17	16	20	19	17	17
History of diabetes (%)	9	5	9	7	7	8
History of hypertension (%)	28	22	27	29	24	25
Smoking (%)						
Never	58	56	57	60	55	56
Former	37	38	34	36	38	36
Current	5	6	9	4	7	8
Walking/cycling >20 min/day, (%)	74	75	73	71	75	76
Leisure-time inactivity >5 h/day, (%)	12	14	13	15	12	12
Adherence to Mediterranean diet (score 1–9), mean (SD)	4.2 (1.6)	4.4 (1.6)	4.3 (1.6)	4.2 (1.6)	4.4 (1.6)	4.5 (1.6)
Alcohol consumption, g/day, mean (SD)	5.0 (6.3)	5.3 (6.0)	5.2 (6.1)	4.7 (5.8)	5.1 (5.4)	5.7 (7.1)
Vitamin D supplement use (%)	11	11	9	10	9	11
Urinary sodium, mmol/mmol[a], mean (SD)	14.8 (7.1)	15.0 (6.3)	17.4 (7.9)	16.1 (8.0)	15.6 (6.6)	15.4 (6.9)
Plasma calcium, mmol/L, mean (SD)	2.32 (0.12)	2.32 (0.12)	2.32 (0.11)	2.31 (0.14)	2.31 (0.10)	2.32 (0.11)
Estimated glomerular filtration rate, mL/min/1.73 m^2, mean (SD)	72 (17)	73 (16)	74 (16)	73 (16)	73 (16)	74 (17)
Use of diuretics (%)	14	14	17	16	13	14

Abbreviations: BMI, body mass index; MI, myocardial infarction; SD, standard deviation.
[a]Creatinine-adjusted.
[b]Energy-adjusted.

Table 2. *Hazard ratios (HR, 95% confidence interval [CI]) estimating the associations between categories of urinary phosphate concentrations and cardiovascular disease.*

	Tertiles of urinary phosphate			
	T1	T2	T3	P trend
Urinary phosphate, mmol/mmol, (percentile 5–95)	1.09–2.09	2.15–2.71	2.78–4.34	
Cardiovascular disease[a]				
Cases/*n*	44/542	58/542	66/541	
Person-years	5037	5032	5126	
Model 1, HR (95%CI)	1 (ref.)	1.33 (0.90, 1.97)	1.41 (0.96, 2.06)	0.094
Model 2, HR (95%CI)	1 (ref.)	1.48 (0.99, 2.21)	1.53 (1.03, 2.29)	0.047
Model 3, HR (95%CI)	1 (ref.)	1.48 (0.99, 2.22)	1.52 (1.01, 2.27)	0.056
Model 4, HR (95%CI)	1 (ref.)	1.53 (1.02, 2.28)	1.57 (1.05, 2.35)	0.037
Myocardial infarction				
Cases/*n*	17/542	29/542	29/541	
Person-years	5132	5164	5262	
Model 1, HR (95%CI)	1 (ref.)	1.72 (0.95, 3.13)	1.60 (0.88, 2.92)	0.167
Model 2, HR (95%CI)	1 (ref.)	2.00 (1.08, 3.69)	1.83 (0.98, 3.43)	0.084
Model 3, HR (95%CI)	1 (ref.)	2.00 (1.08, 3.70)	1.87 (0.99, 3.53)	0.074
Model 4, HR (95%CI)	1 (ref.)	2.04 (1.10, 3.79)	1.93 (1.02, 3.64)	0.061
Stroke				
Cases/*n*	28/542	32/542	41/541	
Person-years	5122	5134	5228	
Model 1, HR (95%CI)	1 (ref.)	1.13 (0.68, 1.88)	1.35 (0.83, 2.19)	0.211
Model 2, HR (95%CI)	1 (ref.)	1.23 (0.73, 2.07)	1.41 (0.85, 2.33)	0.187
Model 3, HR (95%CI)	1 (ref.)	1.23 (0.73, 2.07)	1.39 (0.84, 2.30)	0.212
Model 4, HR (95%CI)	1 (ref.)	1.28 (0.76, 2.15)	1.43 (0.86, 2.36)	0.178

Note: The Swedish Mammography Cohort-Clinical. Model 1: adjusted for age; model 2: further adjusted for BMI, education, family history of myocardial infarction before 60 years., history diabetes, history of hypertension, smoking, walking/cycling >20 min/day, leisure-time inactivity >5 h/day, adherence to Mediterranean diet, alcohol consumption, vitamin D supplement use and estimated glomerular filtration rate; model 3: further adjusted for urinary sodium (mmol/mmol creatinine), plasma calcium (mmol/L), and use of diuretics (ATC-codes C03); model 4: further adjusted for plasma phosphate (mmol/L).
Abbreviation: BMI, body mass index.
[a]Myocardial infarction and stroke combined.

164 incident CVD cases occurred (63 MI cases and 101 stroke cases). Urinary P concentration was dose-dependently associated with increased CVD risk, defined as composite MI and stroke events (Table 2). The multivariable-adjusted HRs (95%CI) of CVD for model 2 were 1.48 (0.99, 2.21) and 1.53 (1.03, 2.29) for the second and third tertiles, respectively, when compared with the first tertile of urinary P (P trend 0.047). Adding urinary sodium excretion, plasma calcium, and use of diuretics information (model 3) had no major impact on the association. After additional adjustment for plasma P concentrations (model 4), the HRs of CVD for the second and third tertile were 1.53 (1.02, 2.28) and 1.57 (1.05, 2.35), respectively, when compared with the lowest tertile (P trend 0.037). When cardiovascular events were differentiated, the significant association with MI was stronger—HR 1.93 (1.02, 3.64; P trend 0.061)—whereas it was weaker for stroke—HR 1.43 (0.86, 2.36; P trend 0.178) (Table 2).

High plasma P was close to being statistically significantly associated with elevated CVD risk (Table 3). The multivariable-adjusted HRs (95%CI) of CVD for the second and third tertiles of P in plasma were 1.27 (0.86, 1.86) and 1.41 (0.96, 2.07), respectively, when compared to the lowest tertile (P trend 0.075). Adding urinary sodium, plasma calcium and use of diuretics information to the model had

Table 3. *Hazard ratios (HR, 95% confidence interval [CI]) estimating the associations between categories of plasma phosphate concentrations and cardiovascular disease.*

	Tertiles of plasma phosphate			
	T1	T2	T3	P trend
Plasma phosphate, mmol/L, percentile (5–95)	0.85–1.08	1.09–1.19	1.20–1.42	
Cardiovascular disease[a]				
Cases/*n*	52/567	56/535	60/523	
Person-years	5229	5064	4902	
Model 1, HR (95%CI)	1 (ref.)	1.19 (0.82, 1.74)	1.35 (0.93, 1.95)	0.117
Model 2, HR (95%CI)	1 (ref.)	1.27 (0.86, 1.86)	1.41 (0.96, 2.07)	0.075
Model 3, HR (95%CI)	1 (ref.)	1.28 (0.87, 1.88)	1.41 (0.96, 2.07)	0.077
Myocardial infarction				
Cases/*n*	27/557	23/535	25/523	
Person-years	5347	5204	5027	
Model 1, HR (95%CI)	1 (ref.)	0.94 (0.54, 1.64)	1.08 (0.63, 1.86)	0.796
Model 2, HR (95%CI)	1 (ref.)	1.05 (0.59, 1.85)	1.16 (0.66, 2.03)	0.619
Model 3, HR (95%CI)	1 (ref.)	1.05 (0.59, 1.85)	1.15 (0.66, 2.03)	0.626
Stroke				
Cases/*n*	28/567	36/535	37/523	
Person-years	5335	5161	4988	
Model 1, HR (95%CI)	1 (ref.)	1.43 (0.87, 2.34)	1.55 (0.95, 2.53)	0.080
Model 2, HR (95%CI)	1 (ref.)	1.50 (0.91, 2.48)	1.63 (0.98, 2.69)	0.058
Model 3, HR (95%CI)	1 (ref.)	1.51 (0.91, 2.51)	1.63 (0.98, 2.71)	0.057

Note: *The Swedish Mammography Cohort-Clinical*. Model 1: adjusted for age; model 2: further adjusted for BMI, education, family history of myocardial infarction before 60 years., history diabetes, history of hypertension, smoking, walking/cycling >20 min/day, leisure-time inactivity >5 h/day, adherence to Mediterranean diet, alcohol consumption, vitamin D supplement use and estimated glomerular filtration rate; model 3: further adjusted for urinary sodium (mmol/mmol creatinine), plasma calcium (mmol/L), and use of diuretics (ATC-codes C03).
Abbreviation: BMI, body mass index.
[a]Myocardial infarction and stroke combined.

no impact on the estimates. In this case, the associations were mainly driven by stroke as a specific outcome and not MI (Table 3). Estimated dietary P intake was not associated with the incidence of CVD or stroke, but inversely associated with MI, HR of 0.55 (0.30, 1.00; P trend 0.049) (Table 4).

Discussion

In this population-based prospective cohort of upper middle-aged and elderly women, urinary P was associated with an increased incidence of composite CVD as well as with MI. Weaker associations, not reaching statistical significance, were observed when evaluating P in plasma in relation to the CVD events. As expected, dietary questionnaire estimated P intake—mainly accounting for organic P, naturally present in foods—was not correlated to P either in plasma or urine. Higher questionnaire-based P intake was not associated with composite CVD but showed an inverse association with MI.

Inorganic P in UPF, urine P concentrations, and CVD

Inorganic P-based additives are commonly used in UPF to enhance flavor, preserve, regulate acidity, make foods creamier, allow melting of foods that would not normally melt, maintain the juiciness of meat, and prevent beverages from separating into individual ingredients, among several other functional applications in food processing. Sodium phosphates (E 339), potassium phosphates (E 340), calcium phosphates (E 341), diphosphates (E 450), triphosphates (E 451), or polyphosphates (E 452) are some examples of the nearly 50 commonly used P ingredients [15]. Typical foods with significant amounts of added P-based additives are processed meat, ham, sausages, canned fish, baked

Table 4. *Hazard ratios (HR, 95% confidence interval [CI]) estimating the associations between categories of dietary phosphate and cardiovascular disease[a].*

	Tertiles of dietary energy-adjusted phosphate			
	T1	T2	T3	P trend
Energy-adjusted dietary phosphate, mg/day, percentile (5–95)	1057–1372	1391–1561	1587–2074	
Cardiovascular disease[a]				
Cases/*n*	59/473	40/473	54/472	
Person-years	4471	4582	4442	
Model 1, HR (95%CI)	1 (ref.)	0.71 (0.47, 1.06)	0.90 (0.62, 1.31)	0.657
Model 2, HR (95%CI)	1 (ref.)	0.76 (0.51, 1.15)	0.88 (0.60, 1.28)	0.546
Model 3, HR (95%CI)	1 (ref.)	0.75 (0.49, 1.12)	0.86 (0.59, 1.26)	0.498
Myocardial infarction				
Cases/*n*	29/473	20/473	19/472	
Person-years	4597	4666	4588	
Model 1, HR (95%CI)	1 (ref.)	0.73 (0.41, 1.29)	0.65 (0.37, 1.17)	0.151
Model 2, HR (95%CI)	1 (ref.)	0.80 (0.45, 1.43)	0.55 (0.30, 1.01)	0.051
Model 3, HR (95%CI)	1 (ref.)	0.78 (0.43, 1.40)	0.55 (0.30, 1.00)	0.049
Stroke				
Cases/*n*	34/473	23/473	36/472	
Person-years	4587	4653	4525	
Model 1, HR (95%CI)	1 (ref.)	0.71 (0.42, 1.21)	1.05 (0.66, 1.68)	0.757
Model 2, HR (95%CI)	1 (ref.)	0.78 (0.45, 1.33)	1.06 (0.66, 1.73)	0.737
Model 3, HR (95%CI)	1 (ref.)	0.77 (0.45, 1.32)	1.06 (0.65, 1.72)	0.811

Note: SMC ($N = 1418$ [207 subjects with missing data on dietary phosphate intake]). Model 1: adjusted for age; model 2: further adjusted for BMI, education, family history of myocardial infarction before 60 years., history diabetes, history of hypertension, smoking, walking/cycling >20 min/day, leisure-time inactivity >5 h/day, adherence to Mediterranean diet, alcohol consumption, vitamin D supplement use and estimated glomerular filtration rate; model 3: further adjusted for urinary sodium (mmol/mmol creatinine), plasma calcium (mmol/L), and use of diuretics (ATC-codes C03).
Abbreviations: BMI, body mass index; SMC, The Swedish Mammography Cohort.
[a]Myocardial infarction and stroke combined.

goods, beer, wine, cola, and other soft drinks [15]. A diet high in UPF is suggested to increase the daily P intake by 250–1000 mg as compared to a diet based on fresh and unprocessed foods [25].

The inorganic P content has been disregarded in previous attempts to estimate dietary P intake from nutrient databases [28] because the added amount of food additives is neither declared on the food labeling [20, 38] nor always considered in the standard nutrient databases. These inaccuracies lead to a gross underestimation of dietary P intake [28]. As expected, in our data, questionnaire-estimated dietary P was not correlated with urinary P ($r = 0.10$).

Intestinal absorption of P depends on its form and source. Organic P from natural sources is less digestible and hence less bioavailable (absorption ~20%–60%) than inorganic P, which has the maximum potential bioavailability (absorption greater than 80%) [15, 20–24]. Animal studies have confirmed this pattern [39, 40].

In healthy subjects, nearly 100% of plasma P is filtered via the renal glomerulus, and 80%–90% is reabsorbed via sodium-mediated facilitated cotransporters in the renal tubules. The portion not reabsorbed is excreted in the urine, and total renal P excretion is balanced to P intake [6, 24]. Therefore, in the general population with preserved renal function, urinary P is a reliable marker of the intestinal absorption of P and thus potentially useful for the assessment of dietary P intake in epidemiologic studies [23, 27, 41, 42].

Animal and human data have shown that dietary P intake and oral P loading stimulated increases in

parathyroid hormone and fibroblast growth factor-23 [7, 43, 44], whose deregulation is suggested to have a pathogenic cardiovascular effect [8, 9, 45, 46]. Our findings, with the highest tertile of urinary P being associated with increased CVD risk, suggest that a high content of inorganic P may play a role in cardiovascular damage. This finding may also add insights into the mechanisms linking UPF to CVD risk [47].

Our results indicated stronger associations for MI than for stroke. It is suggested that P affects the vascular calcification of the smaller arterial beds, especially the coronary arteries [48]. If confirmed, our results may reflect differences in risk factors between coronary disease and stroke, and in reactivity between the coronary and cerebral arteries [49]. Indeed, ischemic stroke is a complex heterogenic entity, and different stroke subtypes with possible differential risk factors have been suggested [50].

Our findings differ, however, with the limited previous literature. Among 1325 community-living elderly men from the US, urinary P was not related to CVD mortality [51]. In 880 elderly Americans—mainly male patients with established coronary artery disease—24-h urinary P excretion (median 20 mmol) was associated with lower, rather than higher, risk of cardiovascular events [52]. The reasons for inconsistent results are not known but could potentially be due to studying specific differences. Although the first study addressed mortality alone, and its mean P in urine was lower (0.45 ± 0.17 mg/mg creatinine ~1.6 mol/mol creatinine), the second included only those with established disease. Furthermore, these previous studies have been carried out in men, whereas our cohort includes women. A greater excretion of P in females than in males in response to oral challenge of P has been reported [53], but any sex-specific differences in P-homeostasis or susceptibility to large intakes of P in relation CVD risk can only be speculated.

Plasma P concentrations and CVD

Disruption of P-responsive hormone regulation has been detected at high dietary P consumption but without measurable change in plasma P concentrations in healthy adults and in animal studies [54]. This disruption promotes arterial calcification, hypertension, and left ventricular dysfunction—even with plasma P within the normal range in healthy subjects [8, 9]—as the body has the ability to correct the elevated plasma P to fasting concentrations [43, 44, 55]. Under normal physiologic conditions, a balance is achieved by complex endocrine feedback, and there is an adjustment of urine excretion that equals the net intake [1]. Accordingly, we observed no correlation between P in blood and urine in our studied women.

In a healthy population, high plasma P concentration (even within the upper-normal range) was associated with atherosclerosis, coronary calcification, impairment of the endothelial function, and microvascular dysfunction [56, 57]. Even a short time high postprandial P concentration may impair endothelial function [10, 58]. In our study, in which only 1% of the women had plasma P levels above the normal range (>1.45 mmol/L), there was a weaker association between CVD and P in plasma than with P in urine, and did not strictly reach statistical significance; if anything, this was more pronounced for stroke than for MI as a specific outcome. Although we do not have a clear explanation for this result, sustained hyperphosphatemia might imply certain kidney dysfunction, in which the compensatory mechanisms fail [49,72]. We cannot rule out that other mechanisms are involved in this association.

Strengths and limitations

The main strengths of this study are the reasonably large sample of women with three different measurement types of P, the prospective design, and the availability of data to finely adjust for potential confounding parameters—including eGFR, diuretic use, urinary sodium, plasma calcium, and vitamin D supplementation. However, the results of this study should be interpreted with some limitations taken into account. The main one is that we only conducted a single measurement of urinary P in first voided spot morning urine, which may not be enough to obtain a reliable indicator of long-term intake of P [27]. This could result in some non-differential misclassification of the exposure, and further studies with repeated measurements of urinary P are warranted to validate our findings. Moreover, we cannot rule out that other characteristics of diet or non-dietary factors contributed to the association observed between urinary P and CVD. Our premise was that a high urinary P excretion in these women was likely the result of an ample intake of inorganic P, which

in turn was due to a high UPF consumption. Yet, due to the design of the dietary questionnaire, we were unable to verify the link to UPF consumption. Additional sources of P might include organophosphates (pesticides and contamination of UPF during processing/packaging) [19, 59] and residues of P in drinking water from fertilizers. Most likely these sources are minor. The use of bisphosphonates as drug therapy for osteoporosis may also contribute. The ascertained number of cases of stroke and MI was limited, and the lack of statistical significance in some analyses was likely due to lack of statistical power. For the same reason, we could not explore different a priori assumptions involving restrictions or subgrouping. Finally, since the study was restricted to upper middle-aged and elderly women, we cannot generalize the findings to men or other age groups.

Conclusions

Higher concentrations of urinary P were associated with an increased risk of composite CVD and MI. A weaker association was observed between plasma P and CVD. These findings may propose that a diet high in P–based additives may contribute negatively to cardiovascular health and could be one mechanism underlying the evidenced link between UPF and CVD. Accurate estimates of inorganic dietary P intake are essential, for which a comprehensive labeling of P-based additives in UPF is required.

Author contributions

Carolina Donat-Vargas and Agneta Åkesson had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Concept and design*: Agneta Åkesson. *Acquisition; analysis; or interpretation of data*: All authors. *Drafting of the manuscript*: Carolina Donat-Vargas, Pilar Guallar-Castillon. *Critical revision of the manuscript for important intellectual content*: All authors. *Statistical analysis*: Carolina Donat-Vargas, Jenny Nyström.

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Conflict of interest statement

The authors declare that there is no conflicts of interest that could be perceived as prejudicing the impartiality of the research reported.

Data availability statement

Data are available from SIMPLER (www.simpler4health.se) for researchers who meet the criteria—that is, an ethical approval is required for access to SIMPLER data.

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