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Serum Phosphorus and Mortality in the Third National Health and Nutrition Examination Survey (NHANES III): Effect Modification by Fasting

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Abstract

Background—Serum phosphorus levels have been associated with mortality in some but not all studies. Since dietary intake prior to measurement can affect serum phosphorus levels, we hypothesized that the association between serum phosphorus and mortality is strongest in those who have fasted for longer duration.

Study Design—Prospective cohort study.

Setting & Participants—Nationally representative sample of 12,984 participants aged 20 years or older in the Third National Health and Nutrition Examination Survey (1988–1994).

Factors—Serum phosphorus, fasting duration (dichotomized as ≥12 or <12 hours)

Outcomes—All-cause and cardiovascular mortality determined by death certificate data from the National Death Index.

Measurements—Serum phosphorus measured in a central laboratory and fasting duration recorded as amount of time since food or drink other than water was consumed.

Results—Individuals fasting ≥12 hours had lower serum phosphorus levels than those fasting <12 hours (3.34 vs. 3.55 mg/dL; $p<0.001$) and higher correlation with repeated measurement (0.66 vs. 0.53; $p=0.002$). In multivariable-adjusted Cox regression models, the highest quartile of serum

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phosphorus was associated with increased mortality in participants fasting ≥12 hours (adjusted HR, 1.74; 95% CI, 1.38–2.20; reference, lowest quartile) but not in participants fasting <12 hours (adjusted HR, 1.08; 95% CI, 0.89–1.32; p for interaction=0.002). Relationships were consistent using 8 hours as the fasting cutpoint or cardiovascular mortality as the outcome.

Limitations—Observational study, lack of fibroblast growth factor 23 or intact parathyroid hormone measurements.

Conclusions—Fasting but not non-fasting serum phosphorus levels were associated with increased mortality. Risk prognostication based on serum phosphorus may be improved using fasting levels.

Keywords

phosphorus; phosphate; mortality; death; fasting; cardiovascular; fast

There is strong observational evidence that higher fasting serum phosphorus levels are associated with increased risk of death, even in individuals with normal kidney function. (1–3) Postulated mechanisms by which elevated serum phosphorus levels could increase mortality risk are through vascular calcification (4, 5) and endothelial dysfunction. (6) On the other hand, studies examining the association between randomly measured serum phosphorus and death have not been conclusive, with some showing associations with mortality (7–9) and others finding no association (10, 11). Heterogeneity in these studies' findings may be due to variable number of measurements used to ascertain the exposure of serum phosphorus or random variability introduced by dietary intake on serum phosphorus throughout the course of the day.

Serum phosphorus levels exhibit a diurnal variation with a mid-morning trough, an early afternoon peak followed by a plateau, and a higher peak in the early morning hours. (13) Diet may amplify this variation: higher phosphorus intake results in higher mean 24-hour serum phosphorus levels, with the early afternoon rise particularly exaggerated by dietary phosphorus intake and diminished by phosphorus restriction. Similarly, fasting may decrease the circadian variation in phosphorus levels. (14, 15)

Because dietary intake may introduce random variability in serum phosphorus measurements, we hypothesized that the association between serum phosphorus and mortality would be stronger with longer fasting duration and tested this in the Third National Health and Nutrition Examination Survey (NHANES III).

Methods

Study Population

Participants were drawn from NHANES III (1988–1994), a nationally representative sample of the civilian, non-institutionalized US population. Details of the survey design have been reported previously. (16) A total of 13,165 non-pregnant adults aged 20 years or older participated in the mobile examination center and had complete data on mortality, fasting duration, estimated GFR, urine albumin-creatinine ratio, 25-hydroxyvitamin D and other relevant covariates. After excluding individuals who fasted ≥24 hours (n=44) and

individuals with venipuncture times that did not match scheduled examination sessions (n=137), we had 12,984 participants in our study sample.

Measurement of Serum Phosphorus

Serum phosphorus was measured using a Hitachi model 737 multichannel analyzer (Boehringer Mannheim Diagnostics, Indianapolis, IN) by reacting inorganic phosphorus with ammonium molybdate in an acidic solution to form ammonium phosphomolybdate, which was quantified in the UV range (340 nm) through the use of a sample-blanked end point method.

Fasting Duration and Examination Session

All participants interviewed in NHANES were invited to mobile examination centers, where questionnaires were administered and laboratory testing was performed. Participants in morning sessions were instructed to fast for at least 12 hours whereas participants in afternoon/evening sessions were instructed to fast for at least 6 hours. Time (hour and minute) of venipuncture was also recorded in NHANES, and we used this data to verify morning (7:30 am-11:59 am), afternoon (12:00 pm-3:59 pm), and evening (4:00 pm-8:00 pm) sessions. Fasting time was recorded as the amount of time since food or drink other than water was consumed. Afternoon and evening sessions were combined into one group for our analysis, as there were very few in the evening group who fasted 12 hours. Participants were dichotomized into two groups by whether they fasted $\geq$ or $<$ 12 hours, which corresponds to the approximate overall median fasting duration.

Assessment of Covariates

Age, gender, race, ethnicity, cigarette smoking (never, former, or current), physical activity, and family income were self-reported. Physical activity was assessed by the number of moderate to vigorous intensity activities (e.g., walking, jogging, running, bicycling, swimming, dancing, or yard work) per week, and physical inactivity was defined as performing 0 moderate to vigorous intensity activities. Socioeconomic status was captured as the poverty income ratio, or the ratio of family income to the federal poverty threshold (specific to the year of the interview); poverty was defined as a poverty income ratio $\leq$ 1.

Height and weight were measured using standardized methods, and body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Hypertension was defined as self-reported history of hypertension, measured systolic blood pressure $\geq$ 140 mmHg, measured diastolic blood pressure $\geq$ 90 mmHg, or the self-reported use of blood pressure medications. Serum creatinine, phosphorus, and 25-hydroxyvitamin D levels were measured as previously described. (16) Low vitamin D was defined by the lowest quintile of 25-hydroxyvitamin D level (<16.2 ng/dL). Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) creatinine equation (17) after calibrating serum creatinine values to Cleveland Clinic reference values. Urine albumin-creatinine ratio (ACR) was assessed from random urine samples, and log-transformed in regression models due to its skewed distribution.

Outcomes

All-cause and cardiovascular mortality data were abstracted from the NHANES III Mortality File, which captures the vital status and cause of death of survey participants from survey participation (1988–1994) until December 31, 2006. (18) Cardiovascular mortality was defined by *International Classification of Diseases, 10th Edition, Clinical Modification* (ICD-10-CM) codes I00–178 derived from death certificate data. (19)

Statistical Analysis

Analyses incorporated appropriate sample weights to account for the complex survey design according to NHANES analytic guidelines. Means and proportions of baseline characteristics were compared across quartiles using linear or logistic regression for continuous and categorical variables, respectively. Standard errors were estimated using Taylor Series Linearization as recommended. (16) The variability of serum phosphorus measurements was examined in a subset of individuals with repeated serum phosphorus measurements who underwent venipuncture in the same examination session using Pearson correlation coefficients. Comparison of correlation coefficients by fasting duration was made using the Fisher r-to-z transformation. (20)

Cox proportional hazards models were used to investigate associations between serum phosphorus and all-cause and cardiovascular mortality adjusted for examination session (morning vs. afternoon/evening), age, gender, African-American race, Mexican ethnicity, poverty, inactivity, BMI (knot at 20 kg/m²), smoking status, systolic blood pressure, diabetes, non-high density lipoprotein cholesterol (knot at 100 mg/dL), ln(ACR), eGFR, and vitamin D status. To better characterize the shape of the association between serum phosphorus and mortality, serum phosphorus was modeled using linear splines with a knot at 3.5 mg/dL (determined empirically using locally-weighted smoothing plots), and categorically by quartiles.

To examine whether the relationship between serum phosphorus and mortality differed by fasting duration, we formally tested interaction terms and conducted subgroup analyses stratified by fasting duration. In sensitivity analyses, regression models were fit in subgroups of examination session (morning vs. afternoon/evening) as well as using 8 hours as the cutpoint for fasting duration, and in a sub-group of patients with CKD defined by eGFR <60 ml/min/1.73m² or urine ACR ≥ 30 mg/g. All analyses were performed using Stata version 11.2 (StataCorp LP, College Station, TX).

Results

Baseline Characteristics of NHANES Participants, by Fasting Duration

Of the 12,984 participants in our study sample, 6,633 fasted ≥ 12 hours, and 6,351 fasted < 12 hours (Table 1). A total of 1,453 and 1,540 deaths occurred in those who fasted ≥ 12 hours and <12 hours, respectively, during a median of 14.3 years of follow-up. Individuals who fasted ≥ 12 hours were younger, less likely to have diabetes, and less likely to have eGFR <60 ml/min/1.73m² (Table 1). They were more likely to attend a morning session than afternoon/evening sessions (82.1% vs. 11.8%; p<0.001), and had lower serum

phosphorus levels, both in the morning session (3.32 vs. 3.42 mg/dL; $p=0.01$) and afternoon/evening sessions (3.42 vs. 3.57 mg/dL; $p<0.001$) (Figure 1). Median number of hours fasting was higher in the morning than the afternoon/evening sessions (13.5 vs. 7.0 hours; $p<0.001$).

Variability of Serum Phosphorus

To investigate whether the repeatability of serum phosphorus levels differed by fasting duration, we examined 998 participants who returned for repeat serum phosphorus measurements at the same examination session time (morning, afternoon, or evening). Mean time between measurements was 17.4 ± 7.7 (standard deviation) days. Correlations between serum phosphorus levels were 0.66 for participants who fasted ≥ 12 hours ($n=599$) and 0.53 for participants who fasted < 12 hours ($n=399$; p for comparison= 0.002).

Serum Phosphorus and Mortality

Continuous Analysis—Higher serum phosphorus was associated with both all-cause and cardiovascular mortality in adjusted analyses (Figure 2A and B). Above 3.5 mg/dL, every 1-mg/dL increase was associated with a 35% increased risk of death (adjusted hazard ratio [aHR], 1.35; 95% confidence interval [CI], 1.05–1.74; $p=0.02$); below 3.5 mg/dL, every 1-mg/dL increase was associated with a 19% increased risk of death (aHR, 1.19; 95% CI, 1.00–1.41; $p=0.05$). Similarly, above 3.5 mg/dL, every 1-mg/dL increase was associated with a 45% increased risk of cardiovascular death (aHR, 1.45; 95% CI, 1.05–2.00; $p=0.03$); below 3.5 mg/dL, every 1-mg/dL increase was associated with a 38% increased risk of cardiovascular death (aHR, 1.38; 95% CI, 1.07–1.78; $p=0.02$).

When analyzed by fasting duration, the association of higher serum phosphorus levels in the interval above 3.5 mg/dL with mortality was significantly stronger for those fasting longer (≥ 12 vs. < 12 hours; p for interaction= 0.04). Among those fasting ≥ 12 hours, every 1-mg/dL increase above 3.5 mg/dL was associated with an 84% increased risk of death (aHR, 1.84; 95% CI, 1.04–3.24; $p=0.04$); among those fasting < 12 hours, there was no association between serum phosphorus levels above 3.5 mg/dL and all-cause mortality (Figure 3A and C). Serum phosphorus below 3.5 mg/dL was not associated with all-cause mortality in either subgroup.

A similar pattern was observed with cardiovascular mortality whereby higher serum phosphorus above 3.5 mg/dL conferred higher risk in those fasting longer, although the interaction term between serum phosphorus level and fasting duration was not significant (p for interaction= 0.2) (Figure 3B and D).

Categorical Analysis—Among participants fasting ≥ 12 hours, the highest quartile (Q) of serum phosphorus was associated with a 74% increased risk of all-cause mortality compared to the lowest quartile (Q4 vs. Q1: aHR, 1.74; 95% CI, 1.38–2.20; $p<0.001$) (Table 2).

Among participants who fasted < 12 hours, there was no association between serum phosphorus and death (Q4 vs. Q1: aHR, 1.08; 95% CI, 0.89–1.32; $p=0.4$) (Table 2).

Results were similar in analyses using cardiovascular mortality as the outcome (Table 2). The highest quartile of serum phosphorus was associated with a two-fold higher risk of cardiovascular death in those who fasted ≥ 12 hours (Q4 vs. Q1: aHR, 2.00; 95% CI, 1.36–

2.96; $p=0.001$), whereas the phosphorus–cardiovascular mortality association was not significant in those who fasted < 12 hours (Q4 vs. Q1: aHR, 1.21; 95% CI, 0.88–1.67; $p=0.2$).

Sensitivity Analyses

Results were similar by subgroup of examination session (morning vs. afternoon/evening) with no significant interaction by examination session. In morning participants, the highest quartile of serum phosphorus was associated with a 75% increased risk of death in those who fasted ≥ 12 hours (Q4 vs. Q1: aHR, 1.75; 95% CI, 1.34–2.29; $p<0.001$), but there was no association between serum phosphorus and death in those who fasted < 12 hours (Table 3). In the afternoon/evening subgroup, the highest quartile of serum phosphorus was associated with a 99% increased risk of death compared to Q2, which had the lowest mortality risk (Q4 vs. Q2: aHR, 1.99; 95% CI, 1.21–3.27; $p=0.008$). There was no statistically significant association between serum phosphorus and death when comparing Q4 with Q1, or among those who fasted < 12 hours (Table 3).

Findings were also consistent when a cutpoint of $\geq$ or < 8 hours fasting was used: mortality risk associated with serum phosphorus above 3.5mg/dL was higher among those who fasted ≥ 8 hours than those who fasted < 8 hours (p for interaction= 0.09). In the subgroup of 2,012 patients with CKD (eGFR < 60 ml/min/1.73m² or ACR ≥ 30 mg/g), results were consistent in magnitude, but the interaction by fasting duration was not significant, likely due to limited sample size.

Discussion

In a nationally representative study population, we found that fasting duration modified the association between serum phosphorus and mortality. Serum phosphorus levels were associated with all-cause and cardiovascular mortality only among those fasting for longer duration. Our findings were consistent in morning and afternoon/evening sessions, and in sensitivity analyses using a different cutpoint for fasting duration. These results suggest the importance of accounting for fasting duration when examining associations between serum phosphorus and adverse outcomes.

A possible explanation for the observed interaction between fasting duration, serum phosphorus, and mortality is that dietary intake introduces “noise” into the data by increasing variability in serum phosphorus measures. Serum phosphorus may increase or decrease after a meal. An oral glucose load decreases serum phosphorus due to intracellular shifts, (21, 22) whereas high phosphorus loads can increase serum phosphorus. (6) Older literature suggests that prolonged fasting may diminish variability in serum phosphorus measurements. (14, 15) Further, we found that serum phosphorus concentrations were more strongly correlated with repeat measurement in those who fasted more hours.

Higher serum phosphorus levels could lead to increased risk of death by promoting vascular calcification (4, 5) or endothelial dysfunction. (6) In addition, higher serum phosphorus could reflect higher levels of parathyroid hormone or fibroblast growth factor 23, (23) a hormone produced by osteocytes that regulates phosphorus and vitamin D metabolism. (23,

24) Fibroblast growth factor 23 has been shown to induce left ventricular hypertrophy in a rat model (25) and is associated with increased risk of heart failure, (26, 27) CKD progression, (28) and death in observational studies. It is also possible that serum phosphorus concentrations could reflect subtle alterations in bone mineral metabolism as the vast majority of phosphorus is stored in bone; markers of bone turnover, such as alkaline phosphatase, have been associated with vascular calcification and death. (29–33)

Whether a reduction in serum phosphorus levels through dietary changes or medications diminishes mortality risk is uncertain. Observational studies examining dietary phosphorus intake have found that high phosphorus intake is associated with left ventricular hypertrophy (34) and increased risk of death (35), while use of phosphorus-binding agents is associated with decreased risk of death in hemodialysis patients. (36) Studies aiming to lower serum phosphorus have been small and generally compare phosphorus-binding agents, (37–39) which may themselves promote coronary calcification. (40) Considering the high prevalence of phosphorus additives in the food supply and current trends in phosphorus consumption, (41, 42) more research is needed to determine whether serum phosphorus is a modifiable risk factor through dietary or medication interventions.

There are undoubtedly non-dietary determinants of serum phosphorus concentrations, and it is possible that the relationship between fasting serum phosphorus and mortality reflects not the diet but other physiologic processes. Serum phosphorus concentrations vary considerably between individuals even at similar levels of kidney function, (7) and dietary phosphorus intake only weakly associates with serum phosphorus. (43) Beyond renal excretion, which is regulated by parathyroid hormone (44) and fibroblast growth factor 23, (23) phosphorus balance depends on shifts into and out of bone, and gastrointestinal absorption of dietary phosphorus, which is enhanced by 1,25-dihydroxyvitamin D. (45) The intestine may also play an important role in modulating renal excretion as infusion of phosphorus into the duodenum rapidly induces phosphaturia through an unknown mechanism. (46) Other factors such as menopause and estrogen use affect serum phosphorus concentrations (47–49). Additional research is needed on the mechanisms and possible adverse consequences of dysregulated phosphorus metabolism.

Strengths of this study included a nationally-representative population; further, results were robust to multiple sensitivity analyses. However, some limitations should be noted. We relied on a single serum phosphorus measurement in our main analyses as few patients had repeated measures of serum phosphorus. It is possible that repeated measures of random serum phosphorus could improve estimation of the exposure and the strength of association with mortality, as other studies have suggested. (9) Fasting duration was self-reported, and morning participants were instructed to fast for at least 12 hours whereas afternoon/evening participants were instructed to fast for at least 6 hours. Finally, NHANES III lacks measurements of parathyroid hormone, 1,25-hydroxyvitamin D or fibroblast growth factor 23, which may be important mediators or confounders of the relationship between serum phosphorus and mortality.

In conclusion, fasting duration modified the association between serum phosphorus and mortality in a population representative of the general US civilian population. The

association was much stronger among individuals fasting for longer duration, suggesting that risk prediction may be enhanced with the use of fasting phosphorus levels. More research is needed to understand the mechanisms underlying the association between fasting serum phosphorus levels and mortality.

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Figure 1.
Estimated Density Function of Serum Phosphorus by Examination Session and Fasting Duration

Figure 2.

Adjusted HRs of All-cause and CVD Mortality associated with Serum Phosphorus Level in the Overall Sample*

*Serum phosphorus is modeled by linear splines with a knot at 3.5 mg/dl. Regressions are adjusted for examination session (morning vs. afternoon/evening) age, sex, race, ethnicity, poverty-income ratio, body mass index, systolic blood pressure, diabetes, smoking status, physical activity, non-HDL cholesterol, log ACR, eGFR, and vitamin D status. Abbreviations: HR, hazard ratio; CVD, cardiovascular disease; HDL, high density lipoprotein; ACR, albumin/creatinine ratio; eGFR, estimated glomerular filtration rate

Figure 3.

Adjusted HRs of All-cause and CVD Mortality associated with Serum Phosphorus Level, by Fasting Duration*

*Serum phosphorus is modeled by linear splines with a knot at 3.5 mg/dl. Regressions are adjusted for examination session (morning vs. afternoon/evening) age, sex, race, ethnicity, poverty-income ratio, body mass index, systolic blood pressure, diabetes, smoking status, physical activity, non-HDL cholesterol, log ACR, eGFR, and vitamin D status. Abbreviations: HR, hazard ratio; CVD, cardiovascular disease; HDL, high density lipoprotein; ACR, albumin/creatinine ratio; eGFR, estimated glomerular filtration rate

Table 1

Baseline Characteristics by Fasting Duration

	<12 h (n=6351)	12 h (n=6633)	P value
Age (y)	45.1 ± 16.9	43.6 ± 16.6	0.004
Male sex	47.5%	48.2%	0.6
African-American	10.4%	9.6%	0.09
Mexican-American	4.7%	4.9%	0.4
Poverty *	12.3%	12.0%	0.8
<High school education	24.0%	22.8%	0.3
No. of moderate-vigorous activities/wk	4.0 ± 5.2	4.2 ± 5.2	0.2
Body mass index (kg/m ²)	26.6 ± 5.8	26.5 ± 5.5	0.7
Current smoker	29.1%	28.0%	0.2
Hypertension	24.0%	22.7%	0.3
Diabetes	7.8%	6.3%	0.03
Urine ACR ≥ 30 mg/g	8.7%	7.7%	0.1
eGFR < 60 ml/min/1.73m ²	5.3%	3.5%	<0.001
CKD **	12.5%	10.1%	0.01
Serum phosphorus (mg/dL)	3.55 ± 0.48	3.34 ± 0.46	<0.001
Low vitamin D #	11.7%	10.8%	0.2
Time fasted (h)	6.8 [6.0–7.7]	14.0 [13.1–15.4]	<0.001
Participated in morning session	11.8%	82.1%	<0.001

Note: Values for categorical variables are given as percentages; values for continuous variables are given as mean ± standard deviation or median [interquartile range]. Conversion factor for phosphorus in mg/dL to mmol/L, ×0.3229;

ACR, albumin-creatinine ratio; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate;

* Defined as a poverty income ratio ≥ 1

** Defined as eGFR < 60 ml/min/1.73m², ACR ≥ 30 mg/g, or both.

Defined as 25-hydroxyvitamin D level <16.2 ng/dL.

Table 2

Adjusted Hazard Ratios of Mortality by Serum Phosphorus Stratified by Fasting Duration

	<12 h (n=6351)		12 h (n=6633)	
	HR (95% CI)	P value	HR (95% CI)	P value
All-cause mortality				
Q1	1.00 (reference)		1.00 (reference)	
Q2	1.12 (0.96–1.31)	0.1	0.98 (0.82–1.18)	0.8
Q3	1.16 (0.93–1.46)	0.2	1.22 (0.95–1.57)	0.1
Q4	1.08 (0.89–1.32)	0.4	1.74 (1.38–2.20)	<0.001
CV Mortality				
Q1	1.00 (reference)		1.00 (reference)	
Q2	1.24 (0.99–1.57)	0.1	1.08 (0.80–1.45)	0.6
Q3	1.30 (0.94–1.79)	0.1	1.42 (1.03–1.98)	0.04
Q4	1.21 (0.88–1.67)	0.2	2.00 (1.36–2.96)	0.001

Note: Regressions are adjusted for examination session (morning vs. afternoon/evening), age, sex, race, ethnicity, poverty-income ratio, body mass index, systolic blood pressure, diabetes, smoking status, physical activity, non-high-density lipoprotein cholesterol, log albumin-creatinine ratio, estimated glomerular filtration rate, and vitamin D status

Abbreviations: HR, hazard ratio; CI, confidence interval; CV, cardiovascular; Q, quartile

Table 3
Adjusted HRs of All-Cause Mortality by Serum Phosphorus Stratified by Fasting Duration and Examination Session

	Morning Participants				Afternoon/Evening Participants			
	Fasted<12 h (n=839)		Fasted 12 h (n=5350)		Fasted<12 h (n=5204)		Fasted 12 h (n=1116)	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Q2	1.26 (0.75–2.11)	0.4	1.01 (0.82–1.24)	0.9	1.07 (0.89–1.28)	0.5	0.69 (0.36–1.34)	0.3
Q3	0.74 (0.37–1.47)	0.4	1.28 (0.97–1.69)	0.08	1.19 (0.95–1.50)	0.1	0.86 (0.41–1.80)	0.7
Q4	0.83 (0.46–1.50)	0.5	1.75 (1.34–2.29)	<0.001	1.07 (0.85–1.35)	0.5	1.38 (0.73–2.60)*	0.3

Note: Regressions are adjusted for age, sex, race, ethnicity, poverty-income ratio, body mass index, systolic blood pressure, diabetes, smoking status, physical activity, non-high-density lipoprotein cholesterol, log albumin-creatinine ratio, estimated glomerular filtration rate, and vitamin D status. Reference group is Q1.

* When compared to Q2: adjusted HR, 1.99; 95% CI, 1.21–3.27; p=0.008

Abbreviations: CI, confidence interval; HR, hazard ratio; Q, quartile