Epidemiology and Prevention

Relation Between Alkaline Phosphatase, Serum Phosphate, and All-Cause or Cardiovascular Mortality

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Background—Higher levels of serum alkaline phosphatase (AlkP) are associated with excess mortality in dialysis patients, but whether AlkP is associated with adverse outcomes among people without kidney failure is unknown.

Methods and Results—We first analyzed the association between AlkP and cardiovascular outcomes among 4115 participants with a previous myocardial infarction (the Cholesterol And Recurrent Events [CARE] study). Results were validated by analyzing the association between AlkP and mortality in an independent sample of 14716 adults from the general US population (the Third National Health and Nutrition Examination Survey). A graded, independent association was noted between baseline tertile of AlkP and the adjusted hazard ratio of all-cause mortality in CARE participants ($P_{\text{trend}}=0.02$). After adjustment for serum phosphate, hepatic enzymes, and other potential confounders, participants with AlkP in the highest tertile had an adjusted hazard ratio of 1.43 (95% confidence interval 1.08 to 1.89) compared with those in the lowest tertile. Multivariable-adjusted associations between higher AlkP and all-cause and cardiovascular mortality were present in the Third National Health and Nutrition Examination Survey ($P_{\text{trend}}$ across tertiles of AlkP=0.006 and 0.038, respectively). Findings from both CARE and the Third National Health and Nutrition Examination Survey were similar among individuals with and without evidence of kidney disease, defined by estimated glomerular filtration rate <60 mL · min$^{-1} · 1.73$ m$^{-2}$.

Conclusions—We found an independent relation between higher levels of AlkP and adverse outcomes among survivors of myocardial infarction and in a general population sample. The excess risk of death was present in people without evidence of kidney disease and was particularly high among people with higher levels of both AlkP and serum phosphate. (Circulation. 2009;120:1784-1792.)

Key Words: kidney failure, chronic ▪ cardiovascular diseases ▪ myocardial infarction ▪ cohort studies ▪ metabolism

Patients with kidney failure have an increased risk of cardiovascular mortality that may be due in part to vascular calcification.1-3 Intense attention has focused on the potential link between bone metabolism, vascular calcification, and cardiovascular events in patients with kidney failure.4,5 Recent reports have shown that vascular calcification and markers of mineral metabolism such as higher levels of serum phosphate are associated with adverse outcomes among people with normal kidney function.6-10 These findings have prompted examination of the link between vascular calcification, mineral metabolism, and symptomatic vascular disease in the general population.11

Clinical Perspective on p 1792

Alkaline phosphatase (AlkP) is an enzyme that catalyzes the hydrolysis of organic pyrophosphate,12 an inhibitor of vascular calcification.13 Although AlkP is expressed in a variety of tissues, its concentrations are highest in bone, liver, and the kidneys.12 Accordingly, serum levels of AlkP are primarily used in clinical practice as a marker of hepatic or bony disease. Two recent papers reported a strong independent association between AlkP and the risk of adverse outcomes in patients with kidney failure.14,15 The authors speculated that this association was due to abnormal bone metabolism, perhaps mediated by vascular calcification, which is common among dialysis patients.16 Vascular calcification may also contribute to cardiovascular risk in people with normal kidney function, but whether AlkP is associated with adverse outcomes in persons without kidney failure is unknown. The purpose of the present study was to test the hypothesis that higher levels of AlkP are associated with increased risk of all-cause mortality and
adverse cardiovascular outcomes. We first studied people with a prior history of myocardial infarction who were free of kidney failure at baseline. We then validated findings from this first data set in an independent, nationally representative sample of the general US population.

Methods

Study Design and Participants: CARE Study

The institutional review board at the University of Alberta approved this post hoc analysis of data from the previously conducted Cholesterol And Recurrent Events (CARE) trial. CARE was a randomized trial of pravastatin versus placebo in 4159 individuals with hyperlipidemia and a history of myocardial infarction, and it has been described in detail elsewhere. Briefly, men and postmenopausal women were eligible if they had had an acute myocardial infarction between 3 and 20 months before randomization, were 21 to 75 years of age, and had low-density lipoprotein cholesterol levels of 115 to 174 mg/dL (3.0 to 4.5 mmol/L), fasting glucose levels of no more than 220 mg/dL (12.2 mmol/L), left ventricular ejection fractions of no less than 25%, no clinical evidence of heart failure, and serum creatinine of no more than 2.2 mg/dL (197 μmol/L). After stratification according to clinical center, eligible and consenting participants were assigned by computer-generated random order in a double-blinded fashion to receive either 40 mg of pravastatin (Pravachol, Bristol-Myers Squibb, New York, NY) once daily or placebo. Treatment allocation was concealed by use of a centrally maintained code.

Study Design and Participants: NHANES III Study

The Third National Health and Nutrition Examination Survey (NHANES III) was a stratified, multistage probability survey designed to select a representative sample of the civilian noninstitutionalized US population. Overall, 18,825 adults 20 years of age and older completed the NHANES III interview and examination between 1988 and 1994. Mortality follow-up was obtained by linking NHANES III participants to the US National Death Index. After the exclusion of 1819 participants without follow-up data, 1166 with missing data on AlkP, and 1124 with missing data on relevant covariates, a total of 14,716 NHANES III participants were included in the present analyses. The protocol for NHANES III was approved by the National Center for Health Statistics of the Centers for Disease Control and Prevention Institutional Review Board. All participants gave informed consent. Methods for collecting data in NHANES III have been described elsewhere.

Measurement of AlkP and Kidney Function

In CARE, baseline levels of AlkP were measured with the Olympus AU1000 analyzer (Olympus, Center Valley, PA) at the CARE core study laboratory (normal adult range 38 to 126 U). In NHANES III, baseline levels of AlkP were measured with a Hitachi model 737 multichannel analyzer (Roche Diagnostics, Indianapolis, Ind; normal adult range 39 to 117 U). To increase comparability between studies, we classified AlkP into study-specific tertiles. For both studies, we estimated glomerular filtration rate (GFR) using the 4-item Modification of Diet in Renal Disease formula. Serum creatinine from NHANES III was calibrated to the assay used in developing this formula. We defined normal kidney function as estimated GFR ≥60 mL·min⁻¹·1.73 m⁻² and chronic kidney disease (CKD) as estimated GFR <60 mL·min⁻¹·1.73 m⁻².

Study Outcomes

The primary outcome for the present analysis was all-cause mortality, which was available for both data sets. For CARE participants, we also considered several secondary outcomes, including death due to coronary disease, the development of symptomatic heart failure, fatal or nonfatal myocardial infarction confirmed by serum creatine kinase measurements, and the composite of death due to coronary disease, nonfatal myocardial infarction, new congestive heart failure, or stroke. For NHANES III participants, we also considered death due to cardiovascular causes. The cause of death for CARE participants was determined by the study outcomes committee without knowledge of the individual’s treatment assignment or laboratory values. Cause of death for NHANES III participants was determined with the underlying cause listed on death certificates. Methods for matching NHANES III participants to the National Death Index and obtaining International Statistical Classification of Diseases, Injuries, and Causes of Death (ICD) revision 9 (ICD-9) and ICD revision 10 (ICD-10) codes for cause of death have been described previously.

Identical matching methodology applied to the NHANES I Epidemiologic Follow-up Study for validation purposes found that 96.1% of deceased participants and 99.4% of living participants were classified correctly. The ICD-9 was used for deaths that occurred between 1988 and 1998 and ICD-10 for deaths that occurred during 1999 and 2000. Cardiovascular mortality was defined by any 1 of ICD-9 codes 390 to 434 and 436 to 459 or ICD-10 codes 100 through 199.

Statistical Analysis

Participant characteristics were calculated for CARE and NHANES III, separately, by tertile of AlkP. χ² Tests, 1-way ANOVA tests, or Kruskal-Wallis tests were used to test for differences in categorical or continuous factors, respectively, between different categories of AlkP. Stepwise forward-selection logistic regression was used to determine factors that were associated with baseline AlkP levels in the highest tertile among CARE participants. On the basis of our previous work, a panel of additional variables that were associated with adverse clinical outcomes or that might be associated with higher AlkP levels were selected a priori for consideration together with age, sex, race, and serum phosphate in the logistic regression models: smoking status; alcohol consumption; diabetes; use of angiotensin-converting enzyme inhibitors, β-adrenergic blockers, and aspirin; glomerular filtration rate; presence or absence of proteinuria on dipstick urinalysis; systolic and diastolic blood pressure; left ventricular ejection fraction; hemoglobin; red blood cell distribution width; low-density lipoprotein cholesterol; high-density lipoprotein (HDL) cholesterol; fasting serum glucose; serum albumin; calcium; bilirubin; liver enzymes (serum glutamic oxaloacetic transaminase, γ-glutamyl transpeptidase, and serum glutamate-pyruvate transaminase); and randomization to pravastatin or placebo. We then used Cox proportional hazards models to examine the association between AlkP levels and clinical outcomes, with follow-up beginning on the date of randomization. We analyzed fully adjusted models that included all characteristics that were significantly (P<0.05) associated with serum AlkP in the highest tertile in logistic regression, together with age, sex, race, and serum phosphate. Tests for linear trend across tertiles were computed by including tertile as a continuous variable in the Cox models. In sensitivity analyses, we forced additional variables that might be associated with adverse outcomes but were not significantly associated with serum AlkP into the fully adjusted models. Finally, we examined the predictive power of combinations of AlkP and phosphate by evaluating the relative hazard of death in 4 mutually exclusive categories defined by the presence and absence of AlkP greater than median and serum phosphate ≥3.5 mg/dL (the cut point that separated low risk from high risk in our previous work).

Findings of the Cox regression analyses that were performed in the CARE data set were then validated with the mortality follow-up data from NHANES III. Where possible, Cox models for the NHANES III data were adjusted for the same covariates as in CARE, with the addition of 25-hydroxyvitamin D and C-reactive protein ≥3 mg/L, which were included in the multivariable-adjusted regression models for NHANES III but were not available in the CARE data set. By design, fasting plasma glucose was available for a subset of NHANES III participants (n=6529 of those who met the eligibility criteria for the present analysis), and data on γ-glutamyl transpeptidase were missing for 3117 participants. Therefore, these parameters were not included in the multivariable-adjusted regression models for NHANES III. Because of the increased statistical power available in the larger NHANES data set, we performed an exploratory analysis to investigate the possibility of a nonlinear relation between all-cause death and AlkP. In that analysis, we used restricted cubic...
spline regression with death as the dependent variable and AlkP as the independent variable, with adjustment for the same covariates as in the fully adjusted Cox model. Knots were placed at the 10th, 50th, and 90th percentiles (53, 77, and 112 IU/L, respectively).

For analyses in both data sets, we determined that the proportional hazard assumption was satisfied by examining plots of the log-negative-log of the within-group survivorship functions versus log-time and by comparing Kaplan-Meier (observed) with Cox (expected) survival curves. Values are reported with 95% confidence intervals (CIs) where appropriate, and all \( P \) values are 2-sided.

Analyses for CARE were performed with Stata 10 SE software (StataCorp, College Station, Tex) and with SUDAAN 9.1 for NHANES III (Research Triangle Institute, Research Triangle Park, NC) to account for the complex sampling design of the present study, which included unequal probabilities of selection, oversampling, and nonresponse. Sampling weights were applied for all NHANES III analyses to produce estimates that are representative of the national US population.

### Results

#### Baseline Characteristics: CARE

Of 4159 CARE participants, 4115 had AlkP measured at baseline and were eligible for the present analysis. The demographic and clinical characteristics of CARE participants included in the present analyses are shown in Table 1. AlkP ranged from 21 to 332 IU/L (median 89 IU/L, interquartile range 75 to 106 IU/L), and 371 participants (9.0%) had AlkP levels outside the normal range, with AlkP ≥38 IU/L for 7 participants (0.2%) and ≥126 IU/L for 364 participants (8.9%). Values corresponding to tertiles of AlkP were 80 IU/L (tertile 1), 80 to 99 IU/L (tertile 2), and ≥99 IU/L (tertile 3).

### Association Between AlkP Level and Adverse Outcomes in CARE

The median duration of follow-up was 58.9 months. After adjustment for age, sex, and race, higher levels of baseline serum AlkP were associated with increased risk of all-cause death (\( P_{\text{trend}}<0.001 \)). For example, the age-, sex-, and race–adjusted risk of death in the highest tertile of AlkP was 1.62 (95% CI 1.25 to 2.11) compared with the lowest tertile (Table 2). After full adjustment, a graded relation remained between serum AlkP and the risk of death (\( P_{\text{trend}}=0.02 \), and the risk of death in the highest tertile was significantly higher than in the...
There was a borderline significant trend between higher levels of baseline serum AlkP and the risk of new heart failure (\(P_{\text{trend}}/H11005\) 0.051). Participants with AlkP in the highest tertile had a fully adjusted HR for experiencing new heart failure of 1.38 (95% CI 1.01 to 1.88) compared with those with AlkP in the lowest tertile. After full adjustment, baseline AlkP was not independently associated with death due to coronary heart disease (\(P_{\text{trend}}/H11005\) 0.57) or myocardial infarction (\(P_{\text{trend}}/H11005\) 0.14), although there was a borderline association with the composite outcome of death due to coronary disease, nonfatal myocardial infarction, new congestive heart failure, or stroke (\(P_{\text{trend}}/H11005\) 0.06; Table 2).

A statistical test for interaction between CKD status and the risk of death associated with higher levels of AlkP was negative (\(P=0.89\)), which suggests that the association between AlkP and mortality was not limited to those with CKD. In fact, the hazard of death associated with the highest compared with the lowest tertile of serum AlkP was similar in participants with (1.35, 95% CI 0.82 to 2.20) and without (1.44, 95% CI 1.02 to 2.02) CKD. Similarly, there was no evidence that the risk of death at higher levels of AlkP varied among participants with or without proteinuria (\(P_{\text{for interaction}}/H11005\) 0.22), those who were or were not current smokers (\(P_{\text{for interaction}}/H11005\) 0.29), those with body mass index \(>30\) kg/m\(^2\) (\(P_{\text{for interaction}}/H11005\) 0.47), or those with or without diabetes mellitus (\(P=0.89\)).

### Sensitivity Analyses in CARE

We performed analyses that adjusted for additional baseline factors, including diabetes, baseline LDL cholesterol, waist-to-hip circumference ratio, use of pravastatin, left ventricular ejection fraction, and diastolic blood pressure. The inclusion of these characteristics in the fully adjusted model did not appreciably affect the association between higher levels of AlkP and the increased risk of death or new heart failure (\(P_{\text{for trend}}\) remained <0.05 in all models).

### Table 2. Association Between Tertile of Serum AlkP and Clinical Outcomes in CARE

<table>
<thead>
<tr>
<th>Event</th>
<th>Unadjusted Events, n (%)</th>
<th>Age, Sex, and Race Adjusted</th>
<th>Fully Adjusted†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR 95% CI (P)</td>
<td>HR 95% CI (P)</td>
<td></td>
</tr>
<tr>
<td><strong>All-cause death</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tertile 1</td>
<td>89 (7)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Tertile 2</td>
<td>131 (9)</td>
<td>1.38 (1.05–1.80) 0.02</td>
<td>1.39 (1.06–1.83) 0.02</td>
</tr>
<tr>
<td>Tertile 3</td>
<td>155 (11)</td>
<td>1.62 (1.25–2.11) &lt;0.001</td>
<td>1.43 (1.08–1.89) 0.01</td>
</tr>
<tr>
<td>(P_{\text{for trend}}^*)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Coronary heart disease death</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tertile 1</td>
<td>56 (4)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Tertile 2</td>
<td>79 (6)</td>
<td>1.32 (0.94–1.86) 0.11</td>
<td>1.30 (0.92–1.84) 0.14</td>
</tr>
<tr>
<td>Tertile 3</td>
<td>79 (6)</td>
<td>1.32 (0.94–1.87) 0.11</td>
<td>1.13 (0.78–1.63) 0.52</td>
</tr>
<tr>
<td>(P_{\text{for trend}}^*)</td>
<td>0.08</td>
<td>0.12</td>
<td>0.57</td>
</tr>
<tr>
<td><strong>Symptomatic heart failure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tertile 1</td>
<td>69 (5)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Tertile 2</td>
<td>102 (7)</td>
<td>1.39 (1.02–1.88) 0.04</td>
<td>1.32 (0.97–1.80) 0.08</td>
</tr>
<tr>
<td>Tertile 3</td>
<td>131 (10)</td>
<td>1.69 (1.26–2.27) &lt;0.001</td>
<td>1.38 (1.01–1.88) 0.04</td>
</tr>
<tr>
<td>(P_{\text{for trend}}^*)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.051</td>
</tr>
<tr>
<td><strong>Fatal or nonfatal myocardial infarction</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tertile 1</td>
<td>102 (8)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Tertile 2</td>
<td>121 (9)</td>
<td>1.15 (0.88–1.49) 0.31</td>
<td>1.17 (0.89–1.53) 0.27</td>
</tr>
<tr>
<td>Tertile 3</td>
<td>140 (10)</td>
<td>1.36 (1.05–1.76) 0.02</td>
<td>1.23 (0.94–1.62) 0.13</td>
</tr>
<tr>
<td>(P_{\text{for trend}}^*)</td>
<td>0.02</td>
<td>0.02</td>
<td>0.14</td>
</tr>
<tr>
<td><strong>Fatal coronary heart disease, nonfatal myocardial infarction, symptomatic heart failure, or stroke</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tertile 1</td>
<td>208 (15)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Tertile 2</td>
<td>260 (19)</td>
<td>1.20 (1.003–1.44) 0.05</td>
<td>1.20 (0.98–1.45) 0.05</td>
</tr>
<tr>
<td>Tertile 3</td>
<td>299 (22)</td>
<td>1.38 (1.16–1.65) &lt;0.001</td>
<td>1.21 (0.98–1.46) 0.05</td>
</tr>
<tr>
<td>(P_{\text{for trend}}^*)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.06</td>
</tr>
</tbody>
</table>

\*\(P_{\text{for trend}}\) refers to linear trend across tertiles 1 through 3.

†HRs have been adjusted for age, sex, race, serum phosphate, alcohol use, fasting serum glucose, use of \(\beta\)-adrenergic blockers, proteinuria, GFR, systolic blood pressure, angiotensin-converting enzyme inhibitors, serum albumin, red blood cell distribution width, hemoglobin, HDL cholesterol, serum calcium, serum bilirubin, serum glutamic oxaloacetic transaminase, \(\gamma\)-glutamyl transpeptidase, and serum glutamate-pyruvate transaminase.

**Table 2.
Figure 1. Risk of all-cause death by baseline level of phosphate (Phos) and/or AlkP. The Figure shows the HR of all-cause death in CARE (top) and NHANES III (bottom) associated with 4 mutually exclusive categories defined by the presence or absence of serum phosphate >3.5 mg/dL and AlkP >89 IU/L. HRs from CARE have been adjusted for age, sex, race, serum phosphate, alcohol use, fasting serum glucose, use of β-adrenergic blockers, proteinuria, glomerular filtration rate, systolic blood pressure, angiotensin-converting enzyme inhibitors, serum albumin, red blood cell distribution width, hemoglobin, HDL cholesterol, serum calcium, serum bilirubin, serum glutamic oxaloacetic transaminase, γ-glutamyl transpeptidase, and serum glutamate-pyruvate transaminase. HRs from NHANES III have been adjusted for age, sex, race, smoking status, systolic blood pressure, antihypertensive medication use, glomerular filtration rate <60 mL · min⁻¹ · 1.73 m², albuminuria, hemoglobin, red blood cell distribution width, serum albumin, HDL cholesterol, serum calcium, serum phosphorus, serum 25-hydroxyvitamin D, diabetes mellitus, serum bilirubin, C-reactive protein ≥3 mg/L, alcohol use, serum glutamic oxaloacetic transaminase, and serum glutamate-pyruvate transaminase. 

Association Between Adverse Outcomes and Combinations of Serum Phosphate and AlkP in CARE

A graded, independent relation between the risk of death and the presence of higher serum phosphate and/or serum AlkP was present. Participants with neither characteristic were at lowest risk (referent), and those with higher levels of serum phosphate alone (HR 1.25, 95% CI 0.83 to 1.90), higher levels of serum AlkP alone (HR 1.40, 95% CI 1.07 to 1.84), and both characteristics (HR 1.62, 95% CI 1.09 to 2.41) were at progressively higher risk (P_trend=0.003; Figure 1).

Validation of Findings From CARE in the NHANES III Data Set

Factors associated with AlkP among NHANES III participants were similar to those associated with AlkP in CARE (Table 3), and 1711 participants (weighted percentage 9.1%) had values outside the normal range. Among NHANES III participants, graded associations between AlkP and all-cause and cardiovascular mortality were present (Table 4). Results were similar after adjustment for age, sex, and race, as well as after multivariable adjustment. As in the CARE data set, there was no significant interaction between the presence of CKD and the excess risk of death associated with higher levels of AlkP (P=0.88). Specifically, the HR of all-cause mortality associated with the highest tertile of AlkP (compared with the lowest) among NHANES III participants with CKD was 1.25 (95% CI 0.84 to 1.87), similar to that among participants without CKD (HR 1.24, 95% CI 1.02 to 1.51). In addition, there was no evidence that the risk of death at higher levels of AlkP varied among NHANES III participants with or without proteinuria (P for interaction=0.23), those who were or were not current smokers (P=0.37), those with body mass index > or ≤30 kg/m² (P=0.62), those with or without diabetes (P=0.60), or those with C-reactive protein >3 or ≤3 mg/L (P=0.22).

Findings remained consistent when analyses were restricted to participants without a baseline history of coronary disease (data not shown) and when NHANES III participants were classified by baseline quintile of AlkP (rather than by tertile). For example, the adjusted HRs of all-cause death in quintiles 2 through 5 were 1.05 (95% CI 0.82 to 1.32), 1.12 (95% CI 0.92 to 1.35), 1.22 (95% CI 0.92 to 1.62), and 1.23 (95% CI 0.99 to 1.53) compared with the lowest quintile (P_trend=0.03). Results were similar for cardiovascular death (data not shown). When cubic spline regression was used to explore the adjusted association between higher levels of AlkP and all-cause mortality, we observed an approximately linear relation, with HRs significantly greater than unity at approximately 110 IU/L (Figure 2).

As was observed in the CARE data set, higher levels of AlkP and serum phosphate were associated with an increased risk for both all-cause and cardiovascular death (P_trend<0.001 and 0.008, respectively). For example, the HRs for all-cause and cardiovascular death among participants with higher levels of both AlkP and phosphate (ie, ≥77.4 IU/L and 3.5 mg/dL, respectively) were 1.41 (95% CI 1.16 to 1.71) and 1.58 (95% CI 1.13 to 2.21), respectively, compared with those with neither characteristic.

Discussion

In clinical practice, elevated levels of serum AlkP usually reflect bony or hepatic disease, such as vitamin D deficiency, renal osteodystrophy, or cholestasis. Recent publications have shown an association between higher serum AlkP and mortality in dialysis patients, which has been interpreted as reflecting a link between the abnormal mineral metabolism of kidney failure and excess cardiovascular mortality.14,15 In the present study, we found a graded, independent association...
between baseline AlkP level and adverse clinical outcomes in people free of kidney failure, both in those with established vascular disease and in a representative sample of the general population. Most of these people (≈91%) had AlkP levels within the range that is considered normal, and results were consistent among people without evidence of non–dialysis-dependent kidney disease (estimated GFR ≥60 mL · min$^{-1}$ · 1.73 m$^{-2}$).

### Table 3. Baseline Characteristics of NHANES III Participants, by Tertile of Serum AlkP

| AlkP Tertile | Age, y | Male | Black | Current smoker | Alcohol consumption* | Body mass index, kg/m$^2$ | Diabetes mellitus | Antihypertensive medication, % | eGFR, mL · min$^{-1}$ · 1.73 m$^{-2}$ | Albuminuria† | SBP, mm Hg | Hemoglobin, mg/dL | Red cell distribution width, % | HDL, mg/dl | Phosphate, mg/dL | Albumin, mg/dL | Calcium, mg/dL | Bilirubin, mg/dL | SGOT, IU/L | SGPT, IU/L | 25(OH) vitamin D, ng/mL | C-reactive protein | eGFR indicates estimated GFR; SBP, systolic blood pressure; SGOT, serum glutamic oxaloacetic transaminase; and SGPT, serum glutamate-pyruvate transaminase. Values are mean (SE), percentage, or median (interquartile range). AlkP tertile cut points were based on weighted NHANES III data; therefore, the sample size is not equally distributed across tertiles. *More than 12 drinks per year. †Albumin to creatinine ratio ≥30 mg/g.
| Tertile 1: <68.2 IU/L (n=3890) | 40.2 (0.4) | 38.5 | 10.7 | 24.2 | 61.1 | 25.1 (0.1) | 2.4 | 55.4 | 94.6 (0.7) | 5.1 | 117.1 (0.4) | 4.22 (0.02) | 54.8 (0.5) | 3.45 (0.02) | 0.63 (0.01) | 17 (15–21) | 17.2 | 31.2 (0.5) | 5.1 | 0.001 |
| Tertile 2: 68.2–87 IU/L (n=4763) | 44.0 (0.6) | 52.4 | 10.8 | 30.4 | 56.6 | 26.7 (0.1) | 5.3 | 48.0 | 93.2 (0.7) | 7.7 | 122.1 (0.5) | 4.21 (0.02) | 49.3 (0.3) | 3.42 (0.01) | 0.62 (0.01) | 19 (16–23) | 26.5 | 29.3 (0.4) | 11.4 | 0.001 |
| Tertile 3: >87 IU/L (n=6063) | 48.1 (0.5) | 53.4 | 12.6 | 31.0 | 45.9 | 27.8 (0.2) | 9.7 | 51.8 | 91.6 (0.5) | 12.0 | 127.0 (0.5) | 4.13 (0.02) | 48.1 (0.4) | 3.46 (0.01) | 0.60 (0.01) | 20 (16–25) | 41.2 | 28.2 (0.4) | 1.27 | 0.006 |

### Table 4. Association Between Tertile of Serum AlkP and Clinical Outcomes in NHANES III

Table 4. Association Between Tertile of Serum AlkP and Clinical Outcomes in NHANES III

<table>
<thead>
<tr>
<th>All-cause death</th>
<th>Unadjusted Events, n (%)</th>
<th>Age, Sex, and Race Adjusted</th>
<th>Fully Adjusted†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tertile 1</td>
<td>347 (4.7)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Tertile 2</td>
<td>623 (8.2)</td>
<td>1.21</td>
<td>1.02–1.45</td>
</tr>
<tr>
<td>Tertile 3</td>
<td>1094 (13.0)</td>
<td>1.58</td>
<td>1.29–1.93</td>
</tr>
<tr>
<td>P for trend*</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.006</td>
</tr>
</tbody>
</table>

**Cardiovascular death**

| Tertile 1 | 156 (1.9) | 1 | 1 |
| Tertile 2 | 284 (3.5) | 1.20 | 0.91–1.59 | 0.19 | 1.11 | 0.84–1.47 | 0.44 |
| Tertile 3 | 499 (5.9) | 1.61 | 1.24–2.09 | <0.001 | 1.27 | 0.98–1.65 | 0.07 |
| P for trend* | <0.001 | <0.001 | 0.038 |

*P for trend refers to linear trend across tertiles 1 through 3. †HRs have been adjusted for age, sex, race, smoking status, systolic blood pressure, antihypertensive medication use, glomerular filtration rate <60 mL · min$^{-1}$ · 1.73 m$^{-2}$; albuminuria, hemoglobin, red blood cell distribution width, serum albumin, HDL cholesterol, serum calcium, serum phosphorus, serum 25-hydroxyvitamin D, diabetes mellitus, serum bilirubin, C-reactive protein ≥3 mg/L, alcohol use, serum glutamic oxaloacetic transaminase, and serum glutamate-pyruvate transaminase.
serum phosphate, estimated GFR, proteinuria, body mass index, smoking status, diabetes mellitus, hepatic enzymes, and (in the NHANES data set) C-reactive protein and 25-hydroxyvitamin D attenuated but did not eliminate the association between higher AlkP levels and adverse clinical outcomes. In fact, people with higher levels of both AlkP and serum phosphate had a risk of death that was \( \approx 60\% \) higher than those with neither characteristic. These findings suggest that in addition to providing possible pathophysiological insight, AlkP may provide useful prognostic information, especially when used together with serum phosphate. If the results of the present study are confirmed by future studies, this would provide additional support for the conduction of trials of therapies that might lower AlkP levels without unduly increasing serum phosphate (such as vitamin D\(^27\)).

To reduce the risk of confounding, we adjusted for multiple characteristics known to influence the risk of cardiovascular events, as well as for those that were found to be associated with higher levels of AlkP at baseline in the CARE data set. We then validated the results in the CARE study (in which all participants had a history of myocardial infarction) by repeating the analyses in an independent, nationally representative sample of adults from the general US population. Findings were consistent in both populations, which suggests the findings are not due to chance and that higher AlkP is independently associated with mortality and adverse cardiovascular outcomes in a broad range of individuals, including those with and without clinically evident coronary disease at baseline.

It currently is speculated that the association between hyperphosphatemia, higher AlkP, and increased mortality among dialysis patients is driven by cardiovascular calcification.\(^{14,15}\) This hypothesis is plausible given the frequency and severity of hyperphosphatemia and vascular calcification among dialysis patients, data linking disordered bone metabolism in this population with excess mortality, and the fact that AlkP inactivates inorganic pyrophosphate,\(^{28}\) which in turn has local and systemic inhibitory effects on vascular calcification.\(^{13}\) The mechanisms by which cardiovascular calcification might lead to excess mortality are controversial but may include deleterious effects on plaque stability, vascular stiffness, valvular heart disease, and calciphylaxis.\(^{11}\)

Recent attention has focused on the independent association between serum phosphate and the risk of death and other adverse clinical outcomes among people with apparently normal kidney function or with only moderate CKD.\(^6,8\) A recent study showed that higher levels of serum phosphate within the normal range were associated with increased calcification of coronary arteries, mitral and aortic valves, and the thoracic aorta among people with GFR <60 mL \( \cdot \) min\(^{-1} \cdot 1.73 \) m\(^{-2}\) but not kidney failure.\(^{50}\) However, none of these previous analyses adjusted for AlkP.

Why were higher levels of AlkP associated with excess mortality even among participants with normal kidney function? One possibility is that the putative link between AlkP and vascular calcification is not restricted to kidney failure. Vascular calcification was once considered a passive process driven by passive deposition of calcium and phosphate ions, but it is now thought to be an active, cell-mediated process to which multiple circulating promoters and inhibitors also contribute.\(^{12}\) The present finding that the excess risks associated with higher levels of AlkP and higher serum phosphate were additive is consistent with the hypothesis that the balance of inorganic pyrophosphate to serum phosphate regulates vascular calcification. However, because we did not have information on vascular calcification or bone-specific AlkP, this suggestion remains speculative.

A second possibility is that higher levels of AlkP and phosphate themselves do not cause adverse outcomes but instead identify the presence of another characteristic that increases mortality directly. One such entity might be fibroblast growth factor-23,\(^{31}\) which is thought to be associated with phosphate balance and is associated with higher mortality in dialysis patients. Other potential candidates might include vitamin D or parathyroid hormone status. Although findings in NHANES III participants were unaffected by adjustment for 25-hydroxyvitamin D status, we did not have data on parathyroid hormone levels in either NHANES III or CARE. Previous work suggests that the association between AlkP and mortality is independent of parathyroid hormone among dialysis patients,\(^{14,15}\) but this may not be true in the absence of kidney failure, and future studies in the general population should evaluate this potential mechanism.

A third possibility is that the association between AlkP and mortality is unrelated to mineral metabolism, but instead represents confounding by another characteristic, such as subclinical liver dysfunction or inflammation. Because the present analyses adjusted for other liver enzymes, we believe that the former is unlikely. Although results from NHANES III were independent of C-reactive protein levels, data on C-reactive protein were not obtained with a high-sensitivity...
assay, and thus, we cannot exclude the possibility that higher AlkP is a marker for patients with an underlying inflammatory state. Other mechanisms unrelated to vascular calcification, liver function, and inflammation are also possible, and future studies in this area should measure specific AlkP isoenzymes to allow better characterization of the pathophysiology that links higher AlkP with excess mortality.

Strengths of the present analysis include the large populations studied, the use of central laboratories for each data set, and the validation of the findings from CARE in an independent, nationally representative sample. However, the present study also has some limitations that should be considered. First, we cannot rule out the possibility of residual confounding by unmeasured characteristics. However, we adjusted for multiple potential confounders, including factors that were associated with AlkP levels and those known to be associated with adverse clinical outcomes. Second, although it is clear that AlkP is associated with adverse cardiovascular outcomes in a much broader population than was previously recognized, the mechanism for this association remains to be confirmed. Third, we did not demonstrate a significant relation between AlkP and the risk of stroke or myocardial infarction in CARE, perhaps because of inadequate statistical power. Alternatively, if the association between AlkP and adverse outcomes is mediated by vascular calcification, this link might be stronger for cardiovascular outcomes that do not involve rupture of atherosclerotic plaque (ie, heart failure) than for those that do (ie, myocardial infarction). Fourth, the prevalence of proteinuria and reduced kidney function was increased at higher levels of AlkP. Although we adjusted for proteinuria and estimated GFR in our multivariable models, it is possible that residual confounding by impaired kidney disease was partially responsible for the present findings. Fifth, although the outcomes studied were clinically relevant and objectively assessed, the method for outcome ascertainment differed between the 2 data sets in the present study. Finally, as for all observational studies, the present findings do not conclusively demonstrate that the association between AlkP and mortality is causal.

In conclusion, we found a graded, independent relation between higher levels of AlkP and adverse clinical outcomes among survivors of myocardial infarction and the general US population. The excess risk of death was present in people without evidence of kidney disease and was particularly high among people with higher levels of both AlkP and serum phosphate. Future studies should identify factors that are associated with higher levels of these characteristics and evaluate the possibility of a causal link to vascular calcification.

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References
CLINICAL PERSPECTIVE

Recent reports have shown that vascular calcification and markers of mineral metabolism such as higher levels of serum phosphate are associated with adverse outcomes among people with normal kidney function. These findings have prompted examination of the link between vascular calcification, mineral metabolism, and symptomatic vascular disease in the general population. Alkaline phosphatase (AlkP) catalyzes the hydrolysis of organic pyrophosphate, an inhibitor of vascular calcification. Two recent articles reported a strong independent association between AlkP and the risk of adverse outcomes in patients with kidney failure. The authors speculated that this association was due to abnormal bone metabolism, perhaps mediated by vascular calcification, which is common among dialysis patients. We first studied people with a prior history of myocardial infarction who were free of kidney failure at baseline. We then validated findings from this first data set in an independent, nationally representative sample of the general US population. We found a graded, independent relation between higher levels of AlkP and all-cause mortality that was consistent in both the derivation and validation samples. The excess risk of death was present in people without evidence of kidney disease and was particularly high among people with higher levels of both AlkP and serum phosphate. Future studies should identify factors that are associated with higher levels of AlkP and evaluate the possibility of a causal link to vascular calcification.
Relation Between Alkaline Phosphatase, Serum Phosphate, and All-Cause or Cardiovascular Mortality
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