Alkaline Phosphatase, Serum Phosphate, and Incident Cardiovascular Disease and Total Mortality in Older Men

Sasiwarang Goya Wannamethee, Naveed Sattar, Olia Papcosta, Lucy Lennon, Peter H. Whincup

Objective—We have examined the association between serum phosphate and alkaline phosphatase (ALP) with incident cardiovascular disease (CVD) outcomes and total mortality in older men.

Approach and Results—A prospective study of 3381 men, aged 60 to 79 years, without a history of myocardial infarction or stroke followed up for an average 11 years during which there were 605 major CVD events (fatal coronary heart disease and nonfatal myocardial infarction, stroke, and CVD death) and 984 total deaths. ALP but not serum phosphate was associated with increased risk of coronary heart disease and overall CVD events which persisted after adjustment for CVD risk factors and markers of inflammation and after exclusion of men with chronic kidney disease (adjusted hazard ratio per SD, 1.19 [1.05, 1.34]; \( P = 0.007 \) and 1.10 [1.01, 1.21]; \( P = 0.04 \)). In contrast, serum phosphate was only associated with increased CVD mortality owing to noncoronary heart disease or stroke causes (adjusted hazard ratio per SD, 1.35 [1.01, 1.83]; \( P = 0.04 \)). Both raised phosphate and ALP were associated with significantly increased total mortality after full adjustment and exclusion of men with chronic kidney disease.

Conclusions—ALP but not serum phosphate is associated with coronary heart disease risk in elderly men. High levels of ALP and serum phosphate are both associated with increased total mortality. (Arterioscler Thromb Vasc Biol. 2013;33:1070-1076.)

Key Words: alkaline phosphatase ■ cardiovascular disease ■ coronary heart disease ■ phosphate ■ total mortality

Altered patterns of mineral metabolism denoted by changes in alkaline phosphatase (ALP) and circulating phosphate concentrations are often observed in end-stage renal disease.1–3 Several clinical and epidemiological studies have linked higher circulating phosphorus or phosphate levels and serum ALP levels to increased coronary calcification and increased risk of cardiovascular disease (CVD) events and total mortality in persons with chronic kidney disease (CKD).1–4 There has been considerable interest in the relationship between markers of bone and mineral metabolism and CVD in the general population. Although several studies have shown serum phosphate to be associated with subclinical atherosclerosis,5,6 arterial calcification,7–9 and increased risk of CVD (variously defined) in individuals with preexisting CVD10,11 as well as in individuals in the general population,12–16 other studies have reported no association between phosphate and coronary heart disease (CHD) events in the general population.13,14,16 ALP commonly includes isoenzymes mainly derived from the liver, bones, and in lesser amounts from intestines, placenta, kidneys, and leukocytes.17 Although ALP is used to diagnose obstructive biliary disease, it is also considered a biochemical marker of bone turnover and is used to monitor metabolic bone disease associated with renal insufficiency.3 In the few population studies which have investigated the association between ALP and CVD, elevated ALP is associated with increased CVD mortality and hospitalization.17–19 ALP has been shown to be strongly associated with C-reactive protein (CRP), the definitive marker of inflammation20; however, whether the ALP–CVD relationship is independent of inflammation has not been well studied. Moreover, there is a paucity of data on mineral metabolism and CVD risk in older people. We have examined the association between serum phosphate and ALP with risk of CHD, stroke, CVD mortality, and total mortality in a population of older men aged 60 to 79 years with no history of myocardial infarction (MI) or stroke.

Materials and Methods
Materials and Methods are available in the online-only Supplement.21–27

Results
Baseline Characteristics by ALP and Serum Phosphate
Serum phosphate showed no correlation with ALP \(( r = -0.007 \)). The mean (SD) serum phosphate in the study population

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From the Department of Primary Care and Population Health, University College London, London, United Kingdom (S.G.W., O.P., L.L.); Institute of Cardiovascular and Medical Sciences, BHF Glasgow Cardiovascular Research Centre, University of Glasgow, Glasgow, United Kingdom (N.S.); and Department of Population Health Sciences and Education, St George’s, University of London, London, United Kingdom (P.H.W.).
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Correspondence to S. Goya Wannamethee, PhD, Department of Primary Care and Population Health, University College London Medical School, Royal Free Campus, Rowland Hill St, London NW3 2PF, United Kingdom. E-mail g.wannamethee@ucl.ac.uk
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1070
was 1.15 (0.16) mmol/L and the mean (interquartile range) ALP was 80.6 (67–95) U/L. Tables 1 and 2 show the baseline characteristics according to quarters of ALP and serum phosphate in men without CVD. ALP was strongly associated with many adverse CV risk factors, including age, smoking, physical inactivity, systolic blood pressure, low high-density lipoprotein-cholesterol, lung function, CRP, von Willebrand factor (vWF), and low estimated glomerular filtration rate (eGFR). However, phosphate was inversely (ie, favourably) associated with some CV risk factors, including blood pressure and high-density lipoprotein-cholesterol. Raised ALP and raised phosphate were both associated with increased levels of N-terminal prohormone of brain natriuretic peptide, a marker of cardiac function.

**Follow-Up CVD Events in Men Without Baseline MI or Stroke**

During the mean follow-up time of 11 years, there were 317 CHD events, 230 stroke events, 345 CVD deaths, and a total of 605 major incident CVD events (including stroke, CHD, and CVD deaths) in the 3381 men with no preexisting CVD.

**ALP and Associations With CVD Risk**

High ALP (highest quarter) was associated with significantly increased risk of major CHD events and CVD mortality and CVD events after adjustment for CV risk factors; age, smoking status, physical activity, alcohol intake, body mass index, systolic blood pressure, high-density lipoprotein-cholesterol, FEV1, eGFR, and diabetes mellitus (Table 3). The associations with CHD and CVD events were attenuated but remained significant after further adjustment for CRP and vWF. Further adjustment for D-dimer and N-terminal prohormone of brain natriuretic peptide did not alter the findings. In contrast to ALP, other liver enzymes, ALT, and gamma-glutamyl transferase were not independently associated with risk of CHD, and further adjustment for ALT and gamma-glutamyl transferase made little difference to the ALP–CHD findings. The positive association with CHD remained and was strengthened after exclusion of men (n=639) with eGFR <60 mL/min per 1.73 m².

**Serum Phosphate and Associations With CVD Risk**

In contrast, phosphate showed no associations with CHD or stroke risks but showed significant associations with CVD mortality, which was attenuated after adjustment for inflammation and exclusion of men with eGFR <60 mL/min per 1.73 m² (Table 4). However, phosphate remained significantly associated with increased CVD mortality owing to deaths from CVD causes other than CHD or stroke (eg, from aortic aneurysm [n=26], diseases of veins and lymphatics [n=9], peripheral vascular disease [n=9], and other form of heart disease [n=29]) after adjustment and exclusion of men with eGFR <60 mL/min per 1.73 m² (hazard ratio [HR] per SD increase, 1.35 [1.01, 1.83]; P=0.04). In contrast, ALP showed no association with CVD mortality owing to deaths from other CVD causes (HR per SD increase, 1.05 [0.79, 1.40]; P=0.71).

### Table 1. Baseline Characteristics According to Quartiles of Alkaline Phosphatase in Men With No Diagnosed Myocardial Infarction or Stroke

<table>
<thead>
<tr>
<th>Alkaline Phosphatase, U/L</th>
<th>1 (&lt;4.21)</th>
<th>2 (4.21–4.38)</th>
<th>3 (4.39–4.55)</th>
<th>4 (&gt;4.55)</th>
<th>P Value for Overall Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>848</td>
<td>866</td>
<td>803</td>
<td>864</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>68.1 (5.21)</td>
<td>68.4 (5.53)</td>
<td>68.4 (5.44)</td>
<td>68.8 (5.57)</td>
<td>0.03</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>7.9</td>
<td>10.7</td>
<td>15.8</td>
<td>17.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Active, %</td>
<td>30.3</td>
<td>29.6</td>
<td>32.4</td>
<td>37.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Manual, %</td>
<td>47.1</td>
<td>50.9</td>
<td>53.7</td>
<td>61.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mod/heavy drinkers, %</td>
<td>11.0</td>
<td>7.7</td>
<td>8.1</td>
<td>7.8</td>
<td>0.05</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>11.5</td>
<td>10.1</td>
<td>9.5</td>
<td>11.2</td>
<td>0.49</td>
</tr>
<tr>
<td>Use of antihypertensive drugs, %</td>
<td>28.4</td>
<td>25.9</td>
<td>25.0</td>
<td>29.6</td>
<td>0.12</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.9 (3.85)</td>
<td>26.8 (3.54)</td>
<td>26.7 (3.49)</td>
<td>26.6 (3.49)</td>
<td>0.39</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>147.9 (23.3)</td>
<td>149.7 (23.6)</td>
<td>150.0 (23.5)</td>
<td>151.9 (24.7)</td>
<td>0.008</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>6.06 (1.02)</td>
<td>6.08 (1.00)</td>
<td>6.03 (1.07)</td>
<td>6.03 (1.15)</td>
<td>0.63</td>
</tr>
<tr>
<td>High-density lipoprotein-cholesterol, mmol/L</td>
<td>1.39 (0.36)</td>
<td>1.33 (0.33)</td>
<td>1.34 (0.35)</td>
<td>1.28 (0.32)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Glucose,* mmol/L</td>
<td>5.75 (6.01–6.07)</td>
<td>5.81 (5.26–6.08)</td>
<td>5.75 (5.23–6.04)</td>
<td>5.92</td>
<td>0.02</td>
</tr>
<tr>
<td>FEV1, L</td>
<td>2.71 (0.66)</td>
<td>2.67 (0.67)</td>
<td>2.61 (0.67)</td>
<td>2.51 (0.62)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C-reactive protein,* mg/L</td>
<td>1.13 (0.58–2.10)</td>
<td>1.44 (0.73–2.77)</td>
<td>1.86 (0.89–3.70)</td>
<td>2.56 (1.14–5.55)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>von Willebrand factor, IU/dL</td>
<td>133.0 (44.2)</td>
<td>135.8 (42.1)</td>
<td>136.6 (45.4)</td>
<td>144.6 (48.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>D-dimer,* ng/mL</td>
<td>69.4 (43–101)</td>
<td>73.7 (45–108)</td>
<td>82.3 (48–127)</td>
<td>96.5 (55–138)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Estimated glomerular filtration rate, mL/min per 1.73 m²</td>
<td>73.6 (12.3)</td>
<td>72.7 (11.5)</td>
<td>73.2 (12.5)</td>
<td>71.2 (13.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>N-terminal prohormone of brain natriuretic peptide,* pg/mL</td>
<td>80.6 (41–148)</td>
<td>83.9 (42–159)</td>
<td>86.5 (39–160)</td>
<td>102.5 (48–200)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Values represent mean and SD.

*Geometric mean and interquartile range.
Total Mortality

ALP and serum phosphate were both associated with increased total mortality even after adjustment for inflammation and after excluding men with eGFR <60 mL/min per 1.73 m² (Table 5). Adjustment for liver function (ALT and gamma-glutamyl transferase) did not alter the findings. ALP and phosphate were also significantly associated with non-CVD mortality after adjustment for risk factors, including inflammation (data not shown). The HRs per SD increase were 1.11 (1.03, 1.20); \( P = 0.01 \) and 1.11 (1.03, 1.21); \( P = 0.007 \) for ALP and phosphate, respectively. Phosphate was significantly associated with cancer mortality (adjusted HR 1.15 [1.02, 1.30]; \( P = 0.02 \)). However, ALP showed no significant association with cancer mortality (adjusted HR 1.08 [0.96, 1.20]; \( P = 0.20 \)). Further exclusion of men with levels above the normal ranges for these metabolites (phosphate > 1.78 mmol/L; ALP > 140 U/L) made little difference to the findings.

Discussion

In this study of older British men without history of MI or stroke, ALP but not serum phosphate was associated with increased risk of CHD and overall CVD events which was to a large extent associated with cardiovascular risk factors and inflammation. Serum phosphate was only associated with other CVD mortality (deaths where CHD and stroke were not the underlying cause). Both ALP and phosphate were associated with significant increased risk of total mortality even after full adjustment and exclusion of men with CKD. Our findings confirm some previous findings on the association between ALP and phosphate with CHD/CVD and total mortality and extend the findings to older adults without prevalent CVD. They also take into account the role of inflammation on CV morbidity, not previously assessed in the older population.

Serum Phosphate and CVD

Although several studies have shown phosphate to be associated with increased risk of CVD events or mortality in the general population, most studies have shown no relationship between phosphate and incident CHD (fatal and nonfatal MI). We observed no association between serum phosphate and CHD and CVD event, which is consistent with findings from 2 other older population studies (the Atherosclerosis Risk in Communities Study and the Multiple Outcomes for Raloxifene Evaluation Trial of postmenopausal women in the United States). Strong evidence that phosphate is independently associated with incident CVD in the general population without established CVD comes from the Framingham offspring study which showed a positive association with CVD incidence. The differences in findings may be owing to the differences in definition of CVD used as peripheral vascular disease and heart failure were included in the outcome measure in the Framingham offspring. The association with CHD specifically was not examined separately in the Framingham study. Alternatively, it may relate to the difference in the age group studied because Framingham offspring participants were on average 25 years younger than British Regional Heart Study men. Although no association was seen with CHD, stroke, or overall CVD events, we observed an association.
between serum phosphate and total CVD mortality, which is consistent with findings in the National and Nutritional Survey of older people, aged ≥65 years, and the Uppsala Study in Sweden. The increased risk of CVD mortality was largely owing to deaths from CVD causes other than CHD or stroke (which particularly included aortic aneurysm, peripheral vascular diseases, diseases of veins and lymphatics, and other forms of heart disease such as pulmonary heart disease and cardiomyopathy). Because overall CVD events predominantly consisted of stroke and CHD events (88%), this would account for the lack of association between phosphate and overall CVD events.

The association between serum phosphate and other CVD mortality was not explained by traditional or novel CVD risk factors. Indeed, high phosphate was associated with a more favorable CVD risk profile, including lower blood pressure and high-density lipoprotein-cholesterol, which has been noted in other studies, and no association was seen with markers of coagulation (D-dimer) or endothelial dysfunction (vWF). However, a weak positive association was seen with inflammation and phosphate related positively to N-terminal prohormone of brain natriuretic peptide, a marker of cardiac function. Much work on the association between phosphate and CVD has focused on the link between phosphate and vascular calcification. Our finding that serum phosphate relates to CVD death of non-CHD causes is consistent with the recent finding that phosphate is more related to calcified plaques, which have been shown to be only weakly associated with the presence of coronary artery disease and less strongly associated with ischemic symptoms than noncalcified plaque. Thus, the association between phosphate and calcified rather than noncalcified plaques may explain the weak association with CHD outcome (nonfatal MI and CHD death), which involves rupture of atherosclerotic plaque. We did not have the power to examine the specific causes of CVD deaths from where CHD and stroke were not the underlying cause. Aortic aneurysm accounted for about one third of all other CVD deaths. The findings from this and other studies suggest that phosphate may be more related to calcified aortic valve disease than to MI.

### Table 3. Incidence Rates Per 1000 Person-Years and Adjusted Hazards Ratios (95% Confidence Interval) for Major Cardiovascular Events by Quartiles of Alkaline Phosphatase in Men Without Diagnosed Myocardial Infarction or Stroke

<table>
<thead>
<tr>
<th>Alkaline Phosphatase</th>
<th>1 (n=848)</th>
<th>2 (n=866)</th>
<th>3 (n=803)</th>
<th>4 (n=864)</th>
<th>1 SD increase in log Alkaline Phosphatase</th>
<th>Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronary heart disease events</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>65</td>
<td>69</td>
<td>62</td>
<td>121</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate per 1000 person-years</td>
<td>7.6</td>
<td>8.1</td>
<td>8.0</td>
<td>15.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age-adjusted</td>
<td>1.00</td>
<td>1.03 (0.73, 1.44)</td>
<td>1.03 (0.73, 1.46)</td>
<td>1.90 (1.40, 2.56)</td>
<td>1.28 (1.17, 1.39)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.00</td>
<td>0.97 (0.69, 1.37)</td>
<td>0.89 (0.63, 1.27)</td>
<td>1.45 (1.06, 1.98)</td>
<td>1.19 (1.07, 1.31)</td>
<td>0.0009</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.00</td>
<td>0.92 (0.65, 1.30)</td>
<td>0.84 (0.59, 1.20)</td>
<td>1.30 (0.94, 1.80)</td>
<td>1.15 (1.03, 1.28)</td>
<td>0.01</td>
</tr>
<tr>
<td>Exclude CKD</td>
<td>1.00</td>
<td>0.89 (0.60, 1.33)</td>
<td>0.93 (0.63, 1.39)</td>
<td>1.44 (1.00, 2.08)</td>
<td>1.19 (1.05, 1.34)</td>
<td>0.007</td>
</tr>
<tr>
<td>Stroke events</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>47</td>
<td>58</td>
<td>51</td>
<td>74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate per 1000 person-years</td>
<td>5.5</td>
<td>6.9</td>
<td>6.5</td>
<td>9.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age-adjusted</td>
<td>1.00</td>
<td>1.20 (0.82, 1.76)</td>
<td>1.16 (0.78, 1.73)</td>
<td>1.61 (1.12, 2.32)</td>
<td>1.15 (1.03, 1.29)</td>
<td>0.02</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.00</td>
<td>1.12 (0.76, 1.66)</td>
<td>1.08 (0.72, 1.62)</td>
<td>1.45 (0.99, 2.11)</td>
<td>1.12 (0.99, 1.27)</td>
<td>0.06</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.00</td>
<td>1.10 (0.74, 1.64)</td>
<td>1.04 (0.69, 1.57)</td>
<td>1.36 (0.92, 2.00)</td>
<td>1.09 (0.96, 1.25)</td>
<td>0.17</td>
</tr>
<tr>
<td>CVD deaths</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>66</td>
<td>80</td>
<td>80</td>
<td>119</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate per 1000 person-years</td>
<td>7.6</td>
<td>9.2</td>
<td>10.1</td>
<td>14.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age-adjusted</td>
<td>1.00</td>
<td>1.13 (0.82, 1.57)</td>
<td>1.29 (0.93, 1.79)</td>
<td>1.72 (1.27, 2.32)</td>
<td>1.21 (1.11, 1.32)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.00</td>
<td>1.09 (0.78, 1.51)</td>
<td>1.18 (0.85, 1.64)</td>
<td>1.34 (0.98, 1.83)</td>
<td>1.14 (1.03, 1.25)</td>
<td>0.01</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.00</td>
<td>1.00 (0.72, 1.40)</td>
<td>1.05 (0.75, 1.48)</td>
<td>1.12 (0.81, 1.55)</td>
<td>1.07 (0.96, 1.18)</td>
<td>0.22</td>
</tr>
<tr>
<td>CVD events</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>124</td>
<td>145</td>
<td>129</td>
<td>207</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate per 1000 person-years</td>
<td>14.8</td>
<td>16.8</td>
<td>27.3</td>
<td>20.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age-adjusted</td>
<td>1.00</td>
<td>1.15 (0.90, 1.46)</td>
<td>1.12 (0.87, 1.43)</td>
<td>1.73 (1.39, 2.16)</td>
<td>1.20 (1.12, 1.29)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.00</td>
<td>1.08 (0.85, 1.37)</td>
<td>1.02 (0.79, 1.31)</td>
<td>1.42 (1.13, 1.79)</td>
<td>1.14 (1.06, 1.22)</td>
<td>0.0008</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.00</td>
<td>1.04 (0.81, 1.32)</td>
<td>0.95 (0.74, 1.23)</td>
<td>1.27 (1.00, 1.62)</td>
<td>1.09 (1.01, 1.18)</td>
<td>0.02</td>
</tr>
<tr>
<td>Exclude CKD</td>
<td>1.00</td>
<td>1.01 (0.77, 1.33)</td>
<td>0.95 (0.72, 1.26)</td>
<td>1.30 (0.99, 1.70)</td>
<td>1.10 (1.01, 1.21)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

CKD indicates chronic kidney disease; and CVD, cardiovascular disease.
Model 1: adjusted for age, cigarette smoking, alcohol intake, physical activity, social class, body mass index, use of antihypertensive drugs, diabetes mellitus, lung function, systolic blood pressure, and estimated glomerular filtration rate.
Model 2: Adjusted for variables in model 1 and in addition for C-reactive protein and von Willebrand factor.
ALP and CVD

In contrast to serum phosphate, ALP was positively associated with CHD events as observed in previous studies, and ALP was strongly associated with many adverse risk factors, in particular low lung function, inflammation, endothelial dysfunction, and coagulation. It has been hypothesized that the association between ALP and CVD may be acting through inflammation. ALP has shown to be associated with CRP in this and other studies. ALP levels may, therefore, partially reflect inflammation of hepatic origin because CRP is hepatically derived. In vascular disease, atherosclerosis is associated with inflammatory processes (reflected in raised CRP levels), and in advanced atherosclerotic plaque there is calcification and increased expression of ALP. Thus, inflammation may be a common link between ALP and CHD. Because proinflammatory cytokine is known to stimulate release of vWF from vascular endothelium, this may explain the association between ALP and endothelial dysfunction, although other pathways may be clearly involved. The National Health and Nutrition Examination Survey study showed ALP to be associated with CVD mortality independent of CRP, but CRP was not measured with a high sensitivity assay. Although the association with CHD and CVD events in this study was to a large extent explained by adverse CV risk factors and inflammation, there remained a significant association between ALP and CHD events after adjustment for CV risk factors, inflammation (CRP), and vWF (a marker of endothelial dysfunction). The association between ALP and stroke events and CVD mortality in this study, however, was considerably weakened after adjustment for inflammation and vWF and after excluding men with evidence of CKD. Thus, inflammation seems to be one of the main pathways by which ALP is associated with increased CVD, with established risk factors also being highly relevant. In contrast to phosphate, ALP showed no association with CVD mortality of non-CHD causes. This suggests that the associations between phosphate and ALP with CVD mortality are operating through different mechanisms.

Total Mortality

Almost all population studies show phosphate and ALP to be associated with increased total mortality as was observed in the present study. This increased mortality, which was also evident for non-CVD causes, was seen even among men within the normal range of ALP (ALP<140 U/L) and phosphate (phosphate<1.78 mmol/L) and in men without evidence of CKD and was not explained by established cardiometabolic risk factors, markers of inflammation, or other markers of liver function (gamma-glutamyl transferase and ALT). Hypovitaminosis D is known to be associated with

Table 4. Incidence Rates Per 1000 Person-Years (Number of Cases) and Adjusted Hazards Ratios (95% Confidence Interval) for Major Cardiovascular Events by Quartiles of Serum Phosphate in Men Without Diagnosed Myocardial Infarction or Stroke

<table>
<thead>
<tr>
<th>Serum Phosphate</th>
<th>1 (n=861)</th>
<th>2 (n=825)</th>
<th>3 (n=844)</th>
<th>4 (n=832)</th>
<th>1 SD Increase in Phosphate</th>
<th>Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronary heart disease events</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>82</td>
<td>75</td>
<td>73</td>
<td>83</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate per 1000 person-years</td>
<td>9.6</td>
<td>9.4</td>
<td>8.9</td>
<td>10.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age-adjusted</td>
<td>1.00</td>
<td>0.93 (0.69, 1.26)</td>
<td>0.89 (0.66, 1.21)</td>
<td>1.09 (0.82, 1.46)</td>
<td>1.08 (0.96, 1.21)</td>
<td>0.19</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.00</td>
<td>1.00 (0.73, 1.38)</td>
<td>0.94 (0.69, 1.30)</td>
<td>1.06 (0.78, 1.45)</td>
<td>1.07 (0.95, 1.20)</td>
<td>0.26</td>
</tr>
<tr>
<td>Stroke events</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>67</td>
<td>57</td>
<td>50</td>
<td>54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate per 1000 person-years</td>
<td>7.9</td>
<td>7.2</td>
<td>6.1</td>
<td>7.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age-adjusted</td>
<td>1.00</td>
<td>0.88 (0.63, 1.23)</td>
<td>0.76 (0.54, 1.08)</td>
<td>0.89 (0.63, 1.26)</td>
<td>0.99 (0.86, 1.14)</td>
<td>0.89</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.00</td>
<td>0.87 (0.61, 1.24)</td>
<td>0.77 (0.53, 1.11)</td>
<td>0.88 (0.61, 1.26)</td>
<td>0.98 (0.85, 1.13)</td>
<td>0.81</td>
</tr>
<tr>
<td>CVD deaths</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>75</td>
<td>89</td>
<td>88</td>
<td>91</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate per 1000 person-years</td>
<td>8.5</td>
<td>11.0</td>
<td>10.5</td>
<td>11.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age-adjusted</td>
<td>1.00</td>
<td>1.15 (0.86, 1.53)</td>
<td>1.12 (0.84, 1.50)</td>
<td>1.27 (0.95, 1.69)</td>
<td>1.16 (1.03, 1.30)</td>
<td>0.01</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.00</td>
<td>1.23 (0.89, 1.68)</td>
<td>1.25 (0.92, 1.71)</td>
<td>1.25 (0.92, 1.70)</td>
<td>1.25 (1.01, 1.26)</td>
<td>0.04</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.00</td>
<td>1.25 (0.91, 1.71)</td>
<td>1.25 (0.91, 1.71)</td>
<td>1.21 (0.88, 1.65)</td>
<td>1.11 (0.99, 1.24)</td>
<td>0.07</td>
</tr>
<tr>
<td>Exclude chronic kidney disease</td>
<td>1.00</td>
<td>1.26 (0.87, 1.82)</td>
<td>1.26 (0.87, 1.82)</td>
<td>1.26 (0.87, 1.83)</td>
<td>1.10 (0.96, 1.26)</td>
<td>0.15</td>
</tr>
<tr>
<td>Major CVD events</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>155</td>
<td>149</td>
<td>140</td>
<td>156</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rate per 1000 person-years</td>
<td>18.7</td>
<td>19.0</td>
<td>17.3</td>
<td>20.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age-adjusted</td>
<td>1.00</td>
<td>0.98 (0.79, 1.21)</td>
<td>0.91 (0.73, 1.13)</td>
<td>1.10 (0.89, 1.36)</td>
<td>1.07 (0.98, 1.17)</td>
<td>0.11</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.00</td>
<td>1.01 (0.80, 1.26)</td>
<td>0.94 (0.75, 1.19)</td>
<td>1.07 (0.85, 1.34)</td>
<td>1.06 (0.97, 1.15)</td>
<td>0.19</td>
</tr>
</tbody>
</table>

CVD indicates cardiovascular disease;
Model 1: Adjusted for age, cigarette smoking, alcohol intake, physical activity, social class, body mass index, use of antihypertensive drugs, diabetes mellitus, lung function, systolic blood pressure, and estimated glomerular filtration rate.
Model 2: Adjusted for variables in model 1 and in addition for C-reactive protein and von Willebrand factor.
elevated serum ALP levels and phosphate levels, and meta-analysis and studies in older subjects have shown vitamin D to be associated with increased mortality. Thus, the increased total mortality associated with ALP and serum phosphate fits with epidemiological data on vitamin D levels, and therefore no comments on causal pathways should be inferred.

**Strengths and Limitations**

This study is based on a cohort of older (60–79 years) men. Although this is a group of considerable clinical interest as they constitute a high risk group in whom traditional risk factors become less predictive, our results need further confirmation both in similar study population and also in middle-aged populations and in women. The study population is socially representative of the United Kingdom, and follow-up rates in the British Regional Heart Study are exceptionally high. Ascertainment of CHD death and MI is based on standard methods, and both CHD mortality and MI incidence rates correspond closely with national data. We were able to take into account a wide range of CV risk factors, including several markers of inflammation and cardiac markers. However, blood measurements were based on a single measurement and we cannot preclude the possibility of residual confounding. An additional limitation is that data on isoforms of ALP were not available and we were not able to distinguish bone-specific ALP from the different isoforms of ALP. Nevertheless, the ALP–CHD association was independent of other liver tests, and thus other mechanisms potentially linked to systemic inflammation or calcified plaques may, in part, explain our observations. Further studies are needed to better understand the nature of the link between ALP and CHD. Moreover, we did not have measurements of vitamin D, an important determinant of phosphate levels.

**Conclusion**

In this study of older men without prevalent MI or stroke, ALP was associated with increased risk of CHD and CVD events, which was partially explained by its association with established risk factors and inflammation. By contrast, serum phosphate has weak or differential associations with established CVD risk factors and showed no association with CHD events and overall CVD events but a positive association with CVD mortality owing to non-CHD and stroke causes. Both ALP and serum phosphate were associated with increased total mortality independent of CKD and inflammation. Further studies are required to elucidate the differing association between ALP and phosphate with CHD and CVD outcomes.

**Sources of Funding**

The British Regional Heart Study is a British Heart Foundation (BHF) research group and receives support from BHF Program grant RG/08/013/25942. The examination of study men aged 60 to 79 years was supported by BHF project grant 97012.

**Disclosures**

None.

**References**

Further studies are required to elucidate the differing association between alkaline phosphatase and phosphate with CVD outcomes.

Significance

Altered patterns of mineral metabolism denoted by changes in alkaline phosphatase and circulating phosphate concentrations have been associated with increased risk of cardiovascular disease (CVD) events and total mortality in persons with chronic kidney disease. However, less is known about the association between mineral metabolism and CVD risk among the general older population in whom disturbances in mineral metabolism are highly prevalent. In this study of older men aged 60 to 79 years with no history of myocardial infarction or stroke, alkaline phosphatase but not serum phosphate was associated with increased risk of coronary heart disease and CVD events, which was not explained by known CVD risk factors. By contrast, serum phosphate was only associated with CVD mortality owing to non-coronary heart disease and stroke causes. Both raised phosphate and alkaline phosphatase were associated with increased total mortality. Further studies are required to elucidate the differing association between alkaline phosphatase and phosphate with CVD outcomes.
Alkaline Phosphatase, Serum Phosphate, and Incident Cardiovascular Disease and Total Mortality in Older Men
Sasiwarang Goya Wannamethee, Naveed Sattar, Olia Papcosta, Lucy Lennon and Peter H. Whincup

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MATERIAL AND METHODS

The British Regional Heart Study is a prospective study of cardiovascular disease involving 7735 men aged 40-59 years drawn from one general practice in each of 24 British towns, who were screened between 1978 and 1980 [1]. The population studied was socio-economically representative of British men but consisted almost entirely of white Europeans (>99%). In 1998-2000, all surviving men, now aged 60-79 years, were invited for a 20th year follow-up examination, on which the analyses presented here are based. All men completed a mailed questionnaire providing information on their lifestyle and medical history, had a physical examination and provided a fasting blood sample collected using the Sarstedt Monovette system. The samples were frozen and stored at -20°C on the day of collection and transferred in batches for storage at -70°C until analysis, carried out after no more than one freeze-thaw cycle. The men were asked whether a doctor had ever told them that they had angina or MI (heart attack, coronary thrombosis), heart failure or stroke and to bring their medication to the examination session. 4252 men (77% of survivors) attended for the 1998-2000 examination and blood serum samples were available from 4088 men; 4034 men had measurements of ALP or serum phosphate. Of these men, 653 men with pre-existing doctor diagnosed MI or stroke were excluded leaving 3381 men for analyses.

Cardiovascular risk factor measurements at 1998-2000

Anthropometric measurements including body weight, height and waist circumference (WC) were carried out. Details of measurement and classification methods for smoking status, physical activity, body mass index, WC, social class, alcohol intake, blood pressure, blood lipids and forced expiratory volume in 1 second (FEV1) in this cohort have been described [2-4]. Prevalent diabetes included men with a diagnosis of diabetes or men with fasting
blood glucose > 7 mmol/l. C-reactive protein (CRP) was assayed by ultra sensitive nephelometry (Dade Behring, Milton Keynes, UK). D-dimer were measured with enzyme-linked immunosorbent assays (Biopool AB, Umeå, Sweden) as was von Willebrand factor (VWF) antigen (DAKO, High Wycombe, UK). N-terminal pro-brain natriuretic peptide (NT-proBNP) was determined using the Elecsys 2010 electrochemiluminescence method (Roche Diagnostics, Burgess Hill, UK). Predicted glomerular filtration rate (eGFR), estimated from serum creatinine using the Modification of Diet in Renal Disease equation developed by Levy et al [5] was used as a measure of renal function. Serum phosphate and ALP were both analysed on a Hitachi autoanalyser using colorimetric assays (Roche). The serum phosphate assay was based on the detection of ammonium phosphomolybdate; the ALP assay was based on the detection of p-nitrophenol released by ALP activity.

Follow-up

All men have been followed up from initial examination (1978-1980) for cardiovascular morbidity [6] and follow-up has been achieved for 99% of the cohort. In the present analyses, total mortality and morbidity events are based on follow-up from re-screening in 1998-2000 at mean age 60-79 years to June 2010, a mean follow-up period of 11 years (range 10-12 years). Information on death was collected through the established "tagging" procedures provided by the National Health Service registers. Fatal stroke episodes were those coded on the death certificate to International Classification of Diseases (ICD) 430-438. Non-fatal stroke events were those which produced a neurological deficit that was present for more than 24 hours. Fatal CHD events were defined as death with CHD (ICD 9th revision, codes 410-414) as the underlying code. A non-fatal MI was diagnosed according to World Health Organisation criteria [7]. Cardiovascular deaths include all those with ICD-9 codes
Evidence regarding non-fatal MI and non-fatal stroke was obtained by on-going reports from general practitioners, by biennial reviews of the patients' practice records (including hospital and clinic correspondence) through to the end of the study period and from repeated personal questionnaires to surviving subjects after initial examination. Outcomes assessed in the current analyses were major CHD (defined as fatal or non-fatal MI) major stroke events (fatal or non-fatal), CVD death and all major CVD events (major CHD events, stroke events or CVD death).

Statistical methods

The distribution of ALP was skewed and log transformation was used. Cox's proportional hazards model was used to assess the multivariate-adjusted hazards ratio (relative risk) in a comparison of quarters of ALP and serum phosphate and for a 1 standard deviation increase in ALP and serum phosphate. In multivariate analyses, smoking (never, long term ex-smokers (>15 years), recent ex-smokers (<15 years) and current smokers), social class (manual vs non manual), physical activity (4 groups), alcohol intake (5 groups), diabetes (yes/no), BMI (<25, 25-27.5, 27.5-29.9 and 30+ kg/m2), and eGFR (<60, 60-69, >70 ml/min per 1.73m2) were fitted as categorical variables; FEV1, HDL-C, CRP, systolic blood pressure, vWF and NT-proBNP were fitted as continuous variables.
REFERENCES


