Associations of Serum Osteoprotegerin Levels with Diabetes, Stroke, Bone Density, Fractures, and Mortality in Elderly Women*

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ABSTRACT

Osteoprotegerin (OPG) and its ligand are cytokines that regulate osteoclastogenesis and that may be involved in the regulation of vascular calcification. We examined whether serum OPG levels were associated with stroke, mortality, and cardiovascular risk factors, including diabetes, as well as with bone mineral density and fractures in a sample of 490 participants in a prospective cohort of white women, at least 65 yr of age. We found that OPG levels, assayed blinded from serum obtained at baseline, were about 30% greater in women with diabetes (mean ± sd, 0.30 ± 0.17 ng/mL) than in those without diabetes (0.23 ± 0.10 ng/mL; P = 0.0001). OPG levels were associated with all-cause mortality [age-adjusted odds ratio, 1.4/SD (0.11 ng/mL) increase in serum OPG level; 95% confidence interval, 1.2–1.8] and cardiovascular mortality (odds ratio, 1.4; 95% confidence interval, 1.1–1.8); these effects were not confounded by diabetes. OPG levels were not associated with baseline bone mineral density or with subsequent strokes or fractures. The association of serum OPG levels with diabetes and with cardiovascular mortality raises the possibility that OPG may be a cause of or a marker for vascular calcification.

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Subjects and Methods

Subjects

Ambulatory women, 65 yr of age or older, who had not previously had bilateral hip replacements were recruited from September 1986 to October 1988 at four clinical centers: The Kaiser-Permanente Center for Health Research (Portland, OR), University of Minnesota in Minneapolis, University of Maryland in Baltimore, and University of Pittsburgh (12). Men and black women were excluded because of their relatively low incidence of osteoporotic fractures. Written informed consent was obtained from all participants after the appropriate institutional review boards had approved the study protocol.

Measurements

Participants completed a questionnaire that was reviewed by an interviewer during the 3-h baseline examination. Unless otherwise noted, variables were dichotomized (yes/no). The questionnaire asked about use of cigarettes (in pack-years), college education, current use of estrogen replacement therapy, and physician-diagnosed diabetes mellitus. At a baseline examination, we measured knee height (to avoid the effects of vertebral osteoporosis on total height), weight, and blood
pressure; we calculated a modified body mass index. Hypertension was defined as taking a diuretic medication or having a measured blood pressure greater than 160/90 mm Hg. Confounders were defined as potential risk factors for mortality, cardiovascular disease, or stroke (i.e., age, history of hypertension, diabetes, pack-years of smoking, use of estrogen replacement therapy, modified body mass index, and serum levels of HDL and LDL cholesterol and C-reactive protein) or for fractures (i.e., age, pack-years of smoking, use of estrogen replacement therapy, and modified body mass index) that were associated (at \( P < 0.05 \)) with serum OPG levels. We also examined multivariate models that included all of these predictor variables. Odds ratios with 95% confidence intervals are reported. We also used models with quadratic terms as well as dividing participants into quintiles of OPG levels to look for J- and U-shaped associations. Mean levels of continuous variables were compared with Student’s t test or ANOVA, as appropriate. Categorical variables were compared using the \( \chi^2 \) test. Statistical significance was set at \( P < 0.05 \).

Because of the unusual design of this study, there was an excess number of participants who suffered strokes during follow-up. Thus, we performed analyses of the associations between OPG levels and clinical outcomes separately in the originally defined cases and controls. Power was reduced in these stratified analyses, so although the results were similar to those presented, some results that had been significant in the overall analyses were no longer significant in the stratified analyses. Measurements of bone mineral density using single photon absorptiometry at baseline were available in 483 (distal radius) to 488 (os calcis) of the 490 women; follow-up measurements of bone mineral density using dual energy x-ray absorptiometry were available in 439 (spine) to 445 (hip) women. Serum measurements were missing in at most 9 of the 490 women.

**Results**

Not surprisingly, subjects who suffered strokes or died during follow-up were older and more likely to have a history of hypertension or diabetes (Table 1). Of the 117 participants who died during follow-up, 81 were included in the 243 cases of stroke. There were 154 women who suffered fractures during follow-up, including 34 with wrist fractures and 28 with hip fractures.

Serum OPG levels were roughly normally distributed among these elderly women (Fig. 1). The mean (± sd) OPG level was 0.24 ± 0.12 ng/mL; the median value was 0.22 ng/mL, with an interquartile range (25th to 75th percentile) of 0.16 to 0.29 ng/mL. Only one woman had an OPG level that was not measurable. OPG levels increased with age (\( r = 0.18, P < 0.0001 \)), from a mean of 0.23 ± 0.12 ng/mL in women 65 to 74 yr old, to 0.26 ± 0.10 ng/mL in women 75 to 84 yr old, to 0.28 ± 0.12 ng/mL in women 85 yr of age or older (\( P = 0.01 \)).

**OPG levels and cardiovascular risk factors**

We found no difference in serum OPG levels by current smoking (Table 2). There were no correlations between serum OPG levels and body mass index (\( r = 0.04; P = 0.39 \)), serum LDL (\( r = -0.07; P = 0.11 \)) or HDL cholesterol levels (\( r = 0.02; P = 0.61 \)), or serum C-reactive protein levels (\( r = 0.05; P = 0.25 \)). OPG levels were slightly greater in women with hypertension (Table 2; \( P = 0.03 \)).

Serum OPG levels were correlated with serum fructosamine levels (\( r = 0.24; P < 0.0001 \)). This correlation was apparent only in women with diabetes (Fig. 2). OPG levels were about 30% greater in women with diabetes, either based on self-report of a physician diagnosis (0.29 ± 0.15 ng/mL; \( n = 56 \)) or a serum fructosamine level greater than 285 \( \mu \text{mol/L} \) (0.32 ± 0.25 ng/mL; \( n = 13 \)) than in the 413 women without diabetes (0.23 ± 0.10 ng/mL; \( P < 0.0001 \)). OPG levels
were also greater in the 70 women who were current users of hormone replacement therapy than in the 408 nonusers (Table 2; *P* < 0.01). Adjustment for age and body mass index had little effect on the associations between OPG levels and diabetes or hormone replacement therapy, but did account for the apparent associations with stroke and education.

Associations between OPG levels and subsequent mortality or stroke

Greater serum OPG levels were associated with increased all-cause and cardiovascular mortality (Table 3). The association between OPG and mortality was slightly diminished

![FIG. 1. Distribution of serum OPG levels in the 247 controls (women aged 65 yr and older, randomly selected from the cohort).](image)
TABLE 2. OPG levels by selected characteristics of participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Osteoprotegerin level (ng/mL)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Among participants</td>
<td></td>
</tr>
<tr>
<td></td>
<td>with characteristic</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Among participants</td>
<td></td>
</tr>
<tr>
<td></td>
<td>without characteristic</td>
<td></td>
</tr>
<tr>
<td>At baseline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.25 ± 0.11</td>
<td>0.23 ± 0.12</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.30 ± 0.17</td>
<td>0.23 ± 0.10</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>0.27 ± 0.14</td>
<td>0.24 ± 0.11</td>
</tr>
<tr>
<td>Hormone replacement</td>
<td>0.27 ± 0.18</td>
<td>0.23 ± 0.10</td>
</tr>
<tr>
<td>College education</td>
<td>0.23 ± 0.09</td>
<td>0.25 ± 0.13</td>
</tr>
<tr>
<td>Body mass index (&gt;305 kg/m²)</td>
<td>0.25 ± 0.11</td>
<td>0.24 ± 0.12</td>
</tr>
<tr>
<td>During follow-up</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroke</td>
<td>0.25 ± 0.13</td>
<td>0.23 ± 0.10</td>
</tr>
<tr>
<td>Death</td>
<td>0.28 ± 0.13</td>
<td>0.23 ± 0.11</td>
</tr>
</tbody>
</table>

Values are the mean ± SD.

*Represents the upper quartile of modified body mass index.

Association between OPG levels and fractures, bone mineral density, and measures of calcium metabolism

OPG levels were not associated with subsequent fractures of all types (Table 3). In post-hoc analyses, there was a significant association between OPG levels and subsequent hip fractures (age-adjusted odds ratio, 1.4; 95% confidence interval, 1.0–1.8; P = 0.03). OPG levels were also greater in women who died during follow-up regardless of whether they had diabetes (Fig. 3). The association between serum OPG levels and mortality was largely confined to women with OPG levels of 0.32 ng/mL or greater (the highest quintile). These women had a 3.0-fold (95% confidence interval, 1.5–6.2; P = 0.02) greater odds of mortality, including a 4.4-fold (95% confidence interval, 1.5–6.2; P = 0.007) greater odds of cardiovascular mortality, than those with levels of 0.15 ng/mL or less (the lowest quintile).

Discussion

We found that serum levels of OPG were greater in women with diabetes and in those who subsequently died of cardiovascular disease during follow-up than in control women. These associations were not affected by adjustment for age, body mass index, or other cardiovascular risk factors, including hypertension, smoking, and serum lipid levels. OPG levels were not associated with levels of C-reactive protein, suggesting that OPG is not only a nonspecific marker of inflammation.

Why should OPG levels be greater in women with diabetes than in control subjects? One possibility is that levels of serum glucose or glycosylated proteins affect the assay for OPG, but we found no correlation between levels of OPG and fructosamine in women without diabetes, suggesting that this is an unlikely explanation. Another hypothesis is that serum OPG levels reflect ongoing vascular disease, which is more common in patients with diabetes and in those who subsequently die. OPG levels, however, were not associated with the risk of nonfatal stroke. It is also possible that OPG levels are affected by an underlying condition that is common to both diabetes and vascular disease (15–17).

If opg-deficient mice, which have no measurable OPG in their blood, develop premature arterial calcification (mainly in the media of large vessels) (3) that is preventable by restoration of the gene (18), why are greater OPG levels in humans associated with diabetes and with an increased, rather than a decreased, risk of cardiovascular disease? One hypothesis is that increased serum OPG levels in humans are a response to rather than a cause of atherosclerosis or vascular calcification, perhaps in an attempt to regulate those processes. Another explanation is that the greater OPG levels are a result of decreased clearance of OPG, perhaps because of increased binding of OPG ligand. The results of this epidemiological study cannot be used to distinguish between these or other potential explanations.

OPG levels were also greater in women who were using hormone replacement therapy. This was not a randomized trial, however, and it is possible that OPG levels are a marker for health conditions that affected the likelihood that a woman used hormone replacement therapy rather than a consequence of the biological effects of estrogen.

Previous studies have suggested that patients with diabetes and peripheral vascular disease are more likely to have medial artery (macrovascular) calcification, which may be associated with an increased risk of vascular events (19–21).
It is important to emphasize, however, that we did not measure vascular calcification directly, at either the macrovascular or intimal level, and that the apparent similarity between the effects of diabetes in humans and those of opg deficiency in mice may well be coincidental.

We were unable to confirm the results of a recent report from Japan that found an association between OPG levels and bone mineral density (11). Those investigators indicated that OPG circulates as both a monomer and a homodimer; it remains to be determined whether the assay that we used measures the same form(s) of OPG as in that study (11). We did not find that OPG levels were associated with the risk of subsequent fractures, except perhaps that greater OPG levels were associated with an increased risk of hip fractures in a...
TABLE 3. Associations between serum OPG levels, mortality, and incident stroke and fracture during follow-up

<table>
<thead>
<tr>
<th>Outcome</th>
<th>No. of events</th>
<th>Odds ratio (^b) (95% confidence interval)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjusted for age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All-cause mortality</td>
<td>116</td>
<td>1.4 (1.2–1.8)</td>
<td>0.001</td>
</tr>
<tr>
<td>Cardiovascular mortality</td>
<td>55</td>
<td>1.4 (1.1–1.8)</td>
<td>0.009</td>
</tr>
<tr>
<td>Incident stroke</td>
<td>241</td>
<td>1.1 (0.9–1.4)</td>
<td>0.2</td>
</tr>
<tr>
<td>Fracture</td>
<td>154</td>
<td>1.1 (0.9–1.4)</td>
<td>0.3</td>
</tr>
<tr>
<td>Adjusted for age and current use of hormone replacement therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All-cause mortality</td>
<td>112</td>
<td>1.4 (1.2–1.8)</td>
<td>0.001</td>
</tr>
<tr>
<td>Cardiovascular mortality</td>
<td>54</td>
<td>1.4 (1.1–1.8)</td>
<td>0.01</td>
</tr>
<tr>
<td>Adjusted for age, diabetes, history of hypertension, college education, and current use of hormone replacement therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All-cause mortality</td>
<td>110</td>
<td>1.3 (1.0–1.6)</td>
<td>0.04</td>
</tr>
<tr>
<td>Cardiovascular mortality</td>
<td>53</td>
<td>1.3 (1.0–1.7)</td>
<td>0.06</td>
</tr>
<tr>
<td>Multivariate-adjusted(^c)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All-cause mortality</td>
<td>106</td>
<td>1.3 (1.0–1.6)</td>
<td>0.06</td>
</tr>
<tr>
<td>Cardiovascular mortality</td>
<td>51</td>
<td>1.4 (1.0–1.8)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

\(^a\) Numbers of events differ slightly among models because of missing data.

\(^b\) Per 30 (0.11 ng/mL) increase in serum osteoprotegerin level.

\(^c\) These variables were associated with OPG levels at \(P < 0.05\).

\(^d\) Adjusted for age, diabetes, history of hypertension, college education, current use of hormone replacement therapy, pack-years of smoking, modified body mass index, and serum levels of HDL and LDL cholesterol and C-reactive protein.

FIG. 3. Mean OPG levels in elderly women, stratified by diabetes at baseline and mortality during follow-up. The differences in OPG levels between those who survived and those who died were significant among women without diabetes \(52 \times 0.23; P = 0.0001\) and those with diabetes \(52 \times 0.23; P = 0.01\). The post-hoc analysis that involved only 28 women with hip fractures; this finding should be examined in other studies.

We found an inverse correlation between serum levels of osteocalcin and OPG. Osteocalcin is a small protein (molecular weight, 5800) that is synthesized by osteoblasts, and serum osteocalcin levels are a marker of bone formation (22). Osteocalcin and its messenger ribonucleic acid have also been identified in platelets (23). We cannot determine, however, whether OPG and osteocalcin have a true biological (e.g., counterregulatory) relation or are both affected by an unmeasured third factor. The inverse correlation that we observed between serum osteocalcin and fructosamine levels \(r = -0.23; P = 0.0001\) is consistent with a previous finding that osteocalcin levels increased with better glucose control in 16 middle-aged men with diabetes (24). Adjustment for serum fructosamine levels, however, did not affect the association between osteocalcin and OPG levels.

Our study has several other important limitations. We enrolled only elderly white women who were ambulatory at the time of the baseline examination. This study was primarily designed to look at risk factors for stroke, as reflecting in our sampling scheme. It is plausible, albeit unlikely, that oversampling stroke cases, compared with other women in the cohort, may have affected the estimated magnitude of the association between OPG level and mortality, as stroke deaths were overrepresented. There was no association, however, between OPG level and the risk of stroke, and our analyses had similar results, albeit with less power due to smaller sample sizes, when they were restricted to only control subjects. In addition, our results should be interpreted with caution; some of the statistically significant findings may have been due to chance.

Serum samples had been stored for several years before the assays were performed, and we cannot verify the long-term stability of OPG levels in frozen sera. However, we were able to assay OPG levels in all but one specimen. Moreover, degradation of OPG in serum would have made it more difficult to find an association among OPG levels, mortality, and diabetes. Because an assay was not available, we did not measure levels of OPG ligand in our samples. It seems reasonable to assume that these levels are important, and that their measurement would enhance our understanding of the effects of OPG.

Our results raise the possibility that the OPG system may be involved in vascular calcification in humans, as has been seen in genetically altered laboratory animals (3) and with other regulators of bone formation and resorption (25–32). Additional research is needed to confirm these findings in another sample, to clarify the importance of OPG ligand, and to determine whether serum OPG levels are a cause or an effect of vascular disease. OPG levels, at least as we measured them, were not associated with bone mineral density or overall fracture risk.

Acknowledgments

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References


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