Circulating Estradiol and Osteoprotegerin as Determinants of Bone Turnover and Bone Density in Postmenopausal Women

A. ROGERS, G. SALEH, R. A. HANNON, D. GREENFIELD, AND R. EASTELL
Bone Metabolism Group, University of Sheffield (A.R., R.A.H., D.G., R.E.), Sheffield S5 7AU; and Division of Biomedical Sciences, Sheffield Hallam University (G.S.), Sheffield S1 1WB, United Kingdom

Osteoprotegerin (OPG) is a recently identified cytokine that acts as a decoy receptor for the receptor activator of NFκB ligand. OPG has been shown to be an important inhibitor of osteoclast differentiation and activation in rodent models. Estrogen is known to suppress bone resorption, and the action of estrogen on bone may be mediated by OPG. The relationship between endogenous estrogen and circulating OPG levels and bone status in human populations is unclear. Thus, the aim of this study was to investigate the relationship between biochemical markers of bone turnover and bone density and circulating OPG and endogenous estradiol levels in a population-based cohort of postmenopausal women.

Subjects were 180 women ages 55–91 yr (mean age, 67 yr). Serum estradiol was measured using an auto-analyzer. Serum concentrations of OPG were determined by ELISA. Markers of bone formation and resorption were measured by standard methods. Bone mineral density at total body, total hip, femoral neck, and lumbar spine was measured by dual energy x-ray absorptiometry.

There was a significant inverse relationship between estradiol and all bone turnover markers (r-values from −0.41 to −0.23; P < 0.05). Serum estradiol was positively related to absolute bone density at all sites and to change in bone density at the hip and femoral neck by univariate analysis (r-values from 0.15–0.29; P < 0.05). We observed a weak inverse association between OPG and serum-based bone turnover markers (r-values −0.18 and −0.16; P < 0.05). There was a significant positive relationship between OPG and bone mineral density at total body, total hip, and femoral neck (r-values from 0.17–0.2; P < 0.05) by univariate analysis, which was lost after adjustment for age and body mass index. There was a significant weak positive relationship between circulating OPG and serum estradiol (r = 0.18; P < 0.02). We observed no significant relationships between OPG and bone turnover markers measured in urine.

We conclude that the variation in circulating endogenous estradiol levels is an important factor contributing to levels of bone turnover and bone density at the menopause. Our observations also suggest that circulating levels of OPG may reflect OPG activity in bone and are related to circulating endogenous levels of estradiol. We have previously reported high levels of variability in urine markers of bone resorption, and we suggest that this could account for the absence of a significant association between these markers and circulating OPG. (J Clin Endocrinol Metab 87: 4470–4475, 2002)

The mechanism of action of estrogen on bone is still not fully understood. There is evidence to suggest that estrogen may suppress the production of locally produced proinflammatory cytokines (4–6). Recent studies in vitro have shown that estradiol increases the production of the novel peptide osteoprotegerin (OPG) in human osteoblast-like cells (7). This may be an important signaling pathway for the action of estrogen on bone.

The discovery of OPG in 1997 has enhanced our understanding of the way in which the processes of bone remodeling are regulated (8, 9). In vitro and animal studies have unambiguously revealed the role of OPG as a decoy receptor for receptor activator of NFκB ligand (RANKL), neutralizing the effect of RANKL on the differentiation and proliferation of osteoclasts.

The critical importance of this cytokine is observed in OPG knockout mice that develop severe osteoporosis (10) and also in transgenic mice designed to overexpress OPG, which develops osteopetrosis (8, 11).

Since this early work was performed, there have been several studies designed to assess the importance of OPG to the skeleton in human populations. The results of these epidemiology studies have been conflicting. In one study, women with osteoporosis were shown to have higher cir-

Abbreviations: BMD, Bone mineral density; BMI, body mass index; Bone ALP, bone-specific alkaline phosphatase; CV, coefficient(s) of variation; FNBD, femoral neck BMD; IFDPD, immunoreactive free deoxypyridinoline; LSBDM, lumbar spine BMD; OPG, osteoprotegerin; RANKL, receptor activator of NFκB ligand; S-CTX, serum cross-linked C-telopeptides of type I collagen; TBBMD, total body BMD; THBMD, total hip BMD; U-NTX, urinary cross-linked N-telopeptides of type I collagen.
Calculating levels of OPG than controls (13). Another study has shown no difference between serum OPG levels in osteoporotic vs. healthy postmenopausal women (14). OPG has also been administered as a therapeutic agent, resulting in a dramatic reduction in bone turnover state, but little is known of its long-term effect on bone density (15). The relationship between serum concentrations of endogenous OPG and bone turnover is uncertain, with different studies yielding differing results. It is unclear whether circulating concentrations of OPG reflect the activity of OPG in the bone microenvironment. A relationship between circulating OPG and estradiol in vivo in humans has yet to be established.

In this study, we examine the association between circulating levels of OPG and estradiol, bone turnover, bone density, and rate of bone loss in a population-based cohort of 180 postmenopausal women.

Subjects and Methods

Study cohort

A population-based sample of 375 postmenopausal women was recruited by age-stratified randomization from three general practitioners in Sheffield, United Kingdom, as part of the European Vertebral Osteoporosis Study group’s work (16). Strata were half decades of age. Subjects were recruited to take part in a longitudinal study of the epidemiology of osteoporosis. Women were excluded if they were too ill to take part (e.g. terminal illness) or if they were unable to give informed consent. A total of 242 women returned after 5 yr. Bone density measurements were made at baseline and at 5 yr. Serum and urine samples for measurements of biochemical markers of bone turnover were performed at the 5-yr visit. Serum levels of OPG and estradiol were measured in a subset of 180 women in which samples were available. The mean age of this group at the 5-yr visit was 67 yr (8). None of the women in this subset were taking or had ever taken estrogen replacement therapy at the time of sampling. All participants gave their written informed consent, and the studies were approved by the North Sheffield Local Research Ethics Committee (Sheffield, UK).

Sample collection

All samples were collected between 0900 and 0945 h after an overnight fast. Blood samples for serum were collected in serum separator tubes (Vacutainer, Cowley, Oxford, UK). Blood was allowed to clot for 30 min at room temperature and was then centrifuged at 2500 g for 10 min at 4°C and stored at −70°C until assay. Twenty-four-hour urine samples were collected and stored at −20°C until assay.

Laboratory analyses

Markers of bone turnover. Bone-specific alkaline phosphatase (Bone ALP), a marker of bone formation, was measured by ELISA in serum [Alkphase-B, Metra Biosystems Inc., Mountain View, CA; intra-assay coefficient of variation (CV), 2.5%; interassay CV, 4.5%].

Immunoassay for the deoxypyridinoline (IFDPD), a marker of bone resorption, was measured by ELISA in urine (Pyrilinks-D, Metra Biosystems Inc., Mountain View, CA; intra-assay CV, 5.5%; interassay CV, 8.8%). Urinary cross-linked N-telopeptides of type I collagen (U-NTX), a marker of bone resorption was measured by ELISA (Osteomark, OsteX International, Inc., Seattle, WA; intra-assay CV, 6.9%; interassay CV, 10.8%). Serum cross-linked C-telopeptides of type I collagen (S-CTX), a marker of bone resorption, was measured using an Elecsys Autoanalyzer (Roche Diagnostics, Basel, Switzerland; intra-assay CV, 3%; interassay CV, 6%).

Other biochemicals.

Analyses were performed using Statgraphics for Windows software (Manugistics, Inc., Rockville, MD).

Statistical analysis

Correlations between biochemical markers and serum levels of OPG and estradiol were made using Pearson’s correlation with and without an adjustment for age and body mass index (BMI). Values of biochemical markers and OPG and estradiol were not normally distributed and so were log-transformed before analysis. Correlations between bone density and serum OPG and estradiol were made with and without adjustment for age and BMI. ANOVA was used to determine differences in OPG and estradiol between tertiles of hip and spine bone density.

Results

Table 1 shows descriptive statistics for the subjects taking part in the study at the 5-yr visit. Associations between variables were made using Pearson’s correlations.

Serum estradiol concentration was inversely related to age (r = −0.40; P < 0.0001) and positively related to BMI (r = 0.18; P = 0.03). There was a weak significant positive association between serum estradiol and OPG (r = 0.18; P < 0.02). Serum estradiol was significantly inversely associated with all markers of bone turnover (r-values from −0.46 to −0.23; Fig. 1). This association remained significant after adjustment for age. Serum estradiol was also weakly posi-

![Table 1. Characteristics of 180 postmenopausal women from the European Vertebral Osteoporosis Study](https://academic.oup.com/jcem/article-abstract/87/10/4470/2846388/25/April/2019)
tively correlated to bone density at all sites measured (r-values from 0.14–0.20; Table 2) and to percentage change in bone density at the total hip and femoral neck (r-values of 0.28 and 0.15, respectively; Table 2) using a univariate analysis. This association was lost after adjustment for age and BMI, with the exception of change in THBMD (r = 0.21; P = 0.03).

A cut-point analysis, used to compare tertiles of LSBMD and FNBMD, showed that serum estradiol was higher in the upper tertile of LSBMD compared with the middle and lower tertiles (P = 0.04; Table 3). Serum estradiol was also higher in the upper tertile of THBMD although this was not significant (P = 0.09; Table 3).

Pearson correlations between markers of bone turnover measured in serum and OPG showed significant negative relationships with r-values of −0.16 (Bone ALP) and −0.18 (S-CTX; Fig. 2). Analysis of bone resorption markers mea-

TABLE 2. Pearson’s correlations between serum estradiol and BMD and annual percentage change in BMD over 5 yr

<table>
<thead>
<tr>
<th>Variable</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBBMD</td>
<td>0.24</td>
<td>0.002</td>
</tr>
<tr>
<td>Change in TBBMD</td>
<td>0.23</td>
<td>0.73</td>
</tr>
<tr>
<td>LSBMD</td>
<td>0.19</td>
<td>0.01</td>
</tr>
<tr>
<td>Change in LSBMD</td>
<td>0.08</td>
<td>0.26</td>
</tr>
<tr>
<td>FNBMD</td>
<td>0.20</td>
<td>0.01</td>
</tr>
<tr>
<td>Change in FNBMD</td>
<td>0.15</td>
<td>0.05</td>
</tr>
<tr>
<td>THBMD</td>
<td>0.13</td>
<td>0.03</td>
</tr>
<tr>
<td>Change in THBMD</td>
<td>0.29</td>
<td>0.009</td>
</tr>
</tbody>
</table>

TABLE 3. Serum estradiol and OPG (pmol/liter) in upper, middle, and lower tertiles of LSBMD and THBMD

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estradiol</th>
<th>OPG</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSBMD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper tertile</td>
<td>70.5 (6.8)</td>
<td>13.3 (0.9)</td>
</tr>
<tr>
<td>Middle tertile</td>
<td>60.6 (4.3)</td>
<td>12.1 (0.9)</td>
</tr>
<tr>
<td>Lower tertile</td>
<td>56.0 (3.7)</td>
<td>11.5 (0.7)</td>
</tr>
<tr>
<td>P</td>
<td>0.04</td>
<td>0.06</td>
</tr>
<tr>
<td>THBMD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper tertile</td>
<td>69.8 (6.0)</td>
<td>13.5 (0.9)</td>
</tr>
<tr>
<td>Middle tertile</td>
<td>61.3 (5.8)</td>
<td>12.2 (0.8)</td>
</tr>
<tr>
<td>Lower tertile</td>
<td>57.9 (4.6)</td>
<td>12.7 (1.1)</td>
</tr>
<tr>
<td>P</td>
<td>0.09</td>
<td>0.1</td>
</tr>
</tbody>
</table>

P values determined by ANOVA. Values are mean (SEM).
sured in urine (U-NTX, U-IFDPD) showed no significant relationships with serum OPG (Fig. 2). Multiple regression analysis revealed age as a possible confounder of bone marker measurements. When adjustment for age was made, all significant associations remained significant, with a $P$ value of less than 0.05.

TBBMD, FNBMD, and THBMD were positively associated with serum OPG levels by univariate analysis; $r$-values ranged from 0.17–0.20 ($P < 0.05$). However, the significance of this association was lost after adjustment for age and BMI. There was no association between serum OPG and LSBMD.

When assessed as a continuous variable, there was no association between change in BMD and OPG. However, those women with a reduction in BMD of more than 3% over 5 yr at the lumbar spine ($n = 50$) had lower levels of serum OPG compared with the rest ($P < 0.05$). There was no association between bone loss at the femoral neck or total hip in relation to serum OPG concentration. Serum OPG was not significantly related to age in this cohort ($r = 0.12; P = 0.10$).

Serum OPG was higher in the upper tertile of LSBMD and also in the upper tertile of THBMD compared with the middle and lower tertiles; however, this did not reach significance ($P = 0.06$ and 0.1, respectively; Table 3).

**Discussion**

In this study we examined the relationships between the concentration of circulating OPG and estradiol, and bone turnover and bone density in a cohort of postmenopausal women. We were able to detect estradiol in all women using an automated electrochemiluminescent assay. Estradiol levels were highly significantly correlated to markers of bone turnover and explained up to 16% of the variation in bone turnover. The relationship with bone density and change in bone density was weaker. Estradiol explained less than 3% of the variation in bone density in this cohort and was also related to change in bone density, especially in the region of the hip. Interestingly, the relationship between estradiol and bone turnover in this study was stronger than that observed in previous studies (1–3). The reasons for this are not clear, but the use of a sensitive reproducible assay and the relative ages of the populations may be influencing factors.

We have shown an inverse relationship between circulating levels of OPG and serum-based markers of bone turnover; the $r$-values, although significant, were small ($r = -0.16$ and $-0.18$), indicating a rather weak relationship between these variables. Although urine-based bone turnover mark-
findings of different studies. The methodology for the de-
vide more insight into the significance of circulating OPG
icant negative correlation between serum osteocalcin and
ments of bone resorption in urine (17). Previous studies have
in men above the age of 40, but not in younger men. Bone formation markers, however, were not
in this male study.
levels of OPG have been shown to increase with age in
estradiol and OPG in a cohort of 252 men. In this study,
differences may be due to the dif-
aleestradiol may inhibit
matured OPG to measure.
Serum levels of OPG have been shown to increase with age in
men above the age of 40, but not in younger men. Bone formation markers, however, were not associated with OPG in this male study.

It has been suggested that circulating OPG levels are higher in osteoporotic women, compared with controls, and
that this occurs as a protective mechanism to slow down the
increased bone resorption and subsequent bone loss seen in
osteoporosis (13, 20). Our results do not appear to endorse
this observation. In our study, higher levels of OPG were relat-
ed to higher bone density, whereas in the study by
Browner et al. (18), there was no relationship between OPG
and bone density. These differences may be due to the dif-
ferent study populations and design and also the assays used.
In the study by Yano et al. (13), those women with the
severest osteoporosis had substantially higher levels of OPG.

In our study, none of the women had osteoporosis as defined
by the World Health Organization criteria, i.e. a T score of less
than –2.5, which may be an explanation for our discrepant
findings. When we used a cut point analysis to compare
levels of OPG by tertiles of LSBMD and THBMD, we ob-
erved higher levels of OPG in the upper tertiles of bone
density.

There is evidence from studies in vitro that 17β-estradiol
stimulates the expression of OPG (7). In this study, there was
a positive relationship between OPG and serum estradiol
levels. This finding is in accordance with that of Szulc et al.
(19), who also showed a positive correlation between serum
estradiol and OPG in a cohort of 252 men. In this study,
multiple regression analysis revealed that estradiol levels
were more closely related to bone turnover and bone density
than to OPG. This suggests that 17β-estradiol may inhibit
osteoclastogenesis by other pathways independent of
OPG/RANKL.

Circulating OPG levels may not fully reflect the activity of
OPG within the bone microenvironment. OPG is synthetized
by both skeletal and nonskeletal cell types and is regulated
by a variety of hormones and cytokines (21). It is also likely
that the biological activity of OPG is dependent on the
relative levels of both OPG and its ligand (RANKL; Ref. 22).
Measurement of the ratio of OPG to RANKL may thus pro-
vide more insight into the significance of circulating OPG
concentrations in humans.

The limitations of these serum measurements may also par-

References
1. Garnero P, Sonnay-Rendu E, Claustrat B, Delmas PD 2000 Biochemical mark-
ers of bone turnover, endogenous hormones and the risk of fractures in post-
estradiol and sex hormone-binding globulin and the risk of hip fracture in
3. Chapurlat RD, Bauer DC, Cummings SR 2001 Association between endog-
enous hormones and sex hormone-binding globulin and bone turnover in
older women: study of osteoporotic fractures. Bone 29:381–387
4. Paciﬁc R 1998 Cytokines, estrogen, and postmenopausal osteoporosis—the
second decade. Endocrinology 139:2699–2661
5. Rogers A, Eastell R 1998 Effects of estrogen therapy of postmenopausal
women on cytokines measured in peripheral blood. J Bone Miner Res 13:
1577–1586
in cultures of peripheral blood. Bone 29:30–34
Estrogen stimulates gene expression and protein production of osteoprote-
gerin in human osteoblastic cells. Endocrinology 140:4367–4370
HQ, Wooden S, Bennett L, Boone T, Shimamoto G, DeRose M, Elliott R,
L, Hughes TM, Hill D, Pattison W, Campbell P, Boyle WJ 1997 Osteopro-
tegerin: a novel secreted protein involved in the regulation of bone density.
Cell 89:309–319
es showed a similar relationship, they did not reach signif-
icance. We believe that this may be due to the high level of
biological variability encountered when making measure-
ments of bone resorption in urine (17). Previous studies have
observed inverse associations between serum OPG and bone
turnover markers. In a study by Browner et al. (18), a sig-
nificant negative correlation between serum osteocalcin and
OPG was observed in a cohort of 490 elderly women. Szulc
et al. (19) observed a significant negative association between
U-IFPD and OPG in men over the age of 40, but not in
younger men. Bone formation markers, however, were not
associated with OPG in this male study.

In our study, none of the women had osteoporosis as defined
by the World Health Organization criteria, i.e. a T score of less
than –2.5, which may be an explanation for our discrepant
findings. When we used a cut point analysis to compare
levels of OPG by tertiles of LSBMD and THBMD, we ob-
erved higher levels of OPG in the upper tertiles of bone
density.

We acknowledge the help of the nurses and radiographers at the
Osteoporosis Centre, Northern General Hospital (Sheffield, UK) for their
help in the recruitment and scanning of the subjects for this study.

Received March 13, 2002. Accepted June 27, 2002.
Address all correspondence and requests for reprints to: Dr. Angela Rogers, Clinical Sciences Centre (North), Northern General Hospital, Herries Road, Sheffield S5 7AU, United Kingdom. E-mail: angela.rogers@sheffield.ac.uk.

This work was funded in part by Programme Grant E0510 from the Arthritis Research Council UK.

Acknowledgments
We acknowledge the help of the nurses and radiographers at the
Osteoporosis Centre, Northern General Hospital (Sheffield, UK) for their
help in the recruitment and scanning of the subjects for this study.

Received March 13, 2002. Accepted June 27, 2002.
Address all correspondence and requests for reprints to: Dr.
Angela Rogers, Clinical Sciences Centre (North), Northern General Hospital,
Herries Road, Sheffield S5 7AU, United Kingdom. E-mail: angela.rogers@sheffield.ac.uk.

This work was funded in part by Programme Grant E0510 from the Arthritis Research Council UK.

References
1. Garnero P, Sonnay-Rendu E, Claustrat B, Delmas PD 2000 Biochemical mark-
ers of bone turnover, endogenous hormones and the risk of fractures in post-
estradiol and sex hormone-binding globulin and the risk of hip fracture in
3. Chapurlat RD, Bauer DC, Cummings SR 2001 Association between endog-
enous hormones and sex hormone-binding globulin and bone turnover in
older women: study of osteoporotic fractures. Bone 29:381–387
4. Pacific R 1998 Cytokines, estrogen, and postmenopausal osteoporosis—the
second decade. Endocrinology 139:2699–2661
5. Rogers A, Eastell R 1998 Effects of estrogen therapy of postmenopausal
women on cytokines measured in peripheral blood. J Bone Miner Res 13:
1577–1586
in cultures of peripheral blood. Bone 29:30–34
Estrogen stimulates gene expression and protein production of osteoprote-
gerin in human osteoblastic cells. Endocrinology 140:4367–4370
HQ, Wooden S, Bennett L, Boone T, Shimamoto G, DeRose M, Elliott R,
L, Hughes TM, Hill D, Pattison W, Campbell P, Boyle WJ 1997 Osteopro-
tegerin: a novel secreted protein involved in the regulation of bone density.
Cell 89:309–319

4474 J Clin Endocrinol Metab, October 2002, 87(10):4470–4475 Rogers et al. • Circulating Estradiol and OPG

Downloaded from https://academic.oup.com/jcem/article-abstract/87/10/4470/2846388 by guest on 25 April 2019


12. Deleted in proof.


