Osteoporos Int (2002) 13:394–399

Osteoporosis International

Original Article

Correlates of Osteoprotegerin Levels in Women and Men

S. Khosla[1], H. M. Arrighi[3], L. J. Melton, III[2], E. J. Atkinson[2], W. M. O'Fallon[2], C. Dunstan[3] and B. L. Riggs[1]

[1]Endocrine Research Unit, Division of Endocrinology, Metabolism, and Nutrition, Department of Internal Medicine, [2]Department of Health Sciences Research, Mayo Clinic and Foundation, Rochester, MN; and [3]Amgen Inc., Thousand Oaks, CA, USA

Abstract. Osteoprotegerin (OPG) is a potent antiresorptive molecule that binds the final effector for osteoclastogenesis, receptor activator of NF-κB ligand (RANK-L). OPG production is regulated by a number of cytokines and hormones, including sex steroids, but there are few data on age and gender effects on circulating serum OPG levels, as well as possible relationships between OPG levels and bone turnover markers or bone mineral density (BMD). Thus, we measured serum OPG levels in an age-stratified, random sample of men (n = 346 age range, 23–90 years) and women (n = 304; age range 21–93 years) and related them to sex steroid levels, bone turnover markers and BMD. Serum OPG levels increased with age in both men (R = 0.39, $p < 0.001$) and women (R = 0.18, $p < 0.01$). Premenopausal women had higher OPG levels than men under age 50 years (171 ± 6 pg/ml vs 134 ± 6 pg/ml, respectively, $p < 0.001$), whereas serum OPG levels were no different in postmenopausal women compared with men = 50 years (195 ± 7 pg/ml vs 188 ± 7 pg/ml, respectively, p = 0.179). OPG levels correlated inversely with serum bioavailable testosterone levels in men = 50 years (R = –0.27, $p < 0.001$), but no associations were present with either estrogen or testosterone levels in the women. In the men, there was a trend for OPG levels to be associated positively with bone resorption markers and inversely with BMD. Collectively, the gender difference in OPG levels suggests that sex steroids may regulate OPG production in vivo, as has been found in vitro. Moreover, OPG production may also rise with increases in bone turnover, probably as a homeostatic mechanism to limit bone loss. Further studies directly testing these hypotheses should provide additional insights into the potential role of OPG in bone loss related to aging and sex steroid deficiency.

Keywords: BMD; Bone turnover; OPG; RANK-L; Sex steroids

Correspondence and offprint requests to: Sundeep Khosla, MD, Mayo Clinic, 200 First Street SW, 5-194 Joseph, Rochester, MN 55905, USA. Tel: +1 (507) 255 6788. Fax: +1 (507) 255 4828. e-mail: Khosla.Sundeep@Mayo.Edu

Introduction

Osteoprotegerin (OPG) is a soluble decoy receptor that binds what appears to be the final effector molecule for osteoclastogenesis, receptor activator of NF-κB ligand (RANK-L) [1,2]. It is one of the most potent antiresorptive agents known, since transgenic mice overexpressing OPG have profound osteosclerosis and a virtual absence of osteoclasts [3] and, conversely, OPG knockout mice have marked osteopenia and increased numbers of osteoclasts [4,5]. OPG production by osteoblastic cells is regulated by a number of cytokines and hormones, including sex steroids [6]. Thus, several studies have found that estrogen (E) increases OPG production [7,8], whereas testosterone (T) tends to inhibit OPG mRNA and protein levels in osteoblastic cells [9].

OPG is also present in the circulation in detectable if low amounts, although the skeletal versus nonskeletal contributions to circulating OPG levels are unclear at this point [10,11]. In addition, recombinant OPG has been found to be rapidly cleared from the blood in rats [12], perhaps due to the heparin binding nature of OPG [13], and thus clearance rate may be a significant determinant of circulating levels. Nonetheless, it is of interest to characterize possible gender and age effects

on circulating OPG levels and to relate OPG levels to bone mineral density (BMD) and bone turnover markers, since this may provide potential insights into the role of OPG in bone loss related to sex steroid deficiency and aging. Thus, in the present study, we measured serum OPG levels in a well-characterized cohort of men and women and examined potential relationships between circulating OPG and sex steroid levels as well as between OPG levels and bone turnover markers and BMD.

Subject and Methods

Study Subjects

Subjects were recruited from age-stratified random samples of Rochester, Minnesota, men and women selected using the medical records linkage system of the Rochester Epidemiology Project [14]. Over half of the Rochester population is identified annually in this system and the majority are seen in any 3-year period. Thus, the enumerated population approximates the underlying population of the community, including both free-living and institutionalized individuals. Altogether, 1138 men and 938 women aged 20 years and over were approached, but 239 men and 126 women were ineligible (amongst the men, 109 were demented and could not give informed consent, 13 were radiation workers, 91 died before contact, 25 were debilitated due to terminal cancer, and 1 was unable to participate due to pending legal action; amongst the women, 89 were demented, 11 were pregnant, 9 were radiation workers, 8 were participants in an ongoing clinical trial of osteoporosis prophylaxis, and 9 died before they could be contacted). Of the 899 eligible men, 348 participated and provided full study data, although 2 were excluded from this analysis because one was on testosterone therapy and one had inexplicably high (into the range of premenopausal women) estradiol and estrone levels. Of the 812 eligible women, 351 participated and provided full study data, although 47 of the 213 postmenopausal women were receiving estrogen replacement therapy and were also excluded from this analysis. Thus, the total number of subjects included in this analysis was 650 (346 men, 304 women). All but 13 men and 2 women were Caucasian, reflecting the ethnic composition of the population (96% white in 1990). The men ranged in age from 23 to 90 years, and the women ranged in age from 21 to 93 years.

Study Protocol

BMD (g/cm^2) was determined for the mid-distal radius, spine (L2–L4) and femoral neck using dual-energy X-ray absorptiometry (DXA) with the Hologic QDR2000 instrument (Hologic, Waltham, MA) using software version 5.40. Since we did not specifically exclude subjects with spinal osteoarthritis or aortic calcification, which can confound the BMD measurement [15], we assessed the mid-lateral instead of the anteroposterior spine, which largely excludes these confounders from the scanning field. The coefficients of variation (CVs) for the radius, lateral spine and femoral neck were 1.7%, 2.1%, and 1.8%, respectively.

Fasting-state serum samples were obtained between 0800 and 0900 hours and a 24-h urine collection was turned in. All samples were stored at –70 °C until analyzed.

Laboratory Methods

Fasting serum samples were assayed by radioimmunoassay (RIA) for total testosterone (T; Diagnostic Products, Los Angeles, CA; interassay CV = 11%), estradiol (E_2; Diagnostic Products, Los Angeles, CA; interassay CV $<16\%$) and estrone (E_1; Diagnostic Systems Laboratories, Webster, TX; interassay CV = 9%). In addition, the non-SHBG-bound (bioavailable) fraction of total T and E_2 was measured using a modification of the technique of O'Connor et al. [16] and Tremblay and Dube [17]. Briefly, tracer amounts of ^{3}H-labeled T, E_2 or E_1 were added to serum aliquots. An equal volume of saturated solution of ammonium sulfate (final concentration 50%) was added to precipitate SHBG with its bound steroid. Separation of the SHBG fraction was done by centrifugation at 1100 *g* for 30 min at 4 °C. The percentage of labeled steroid remaining in the supernatant (the free and albumin-bound fractions) was then calculated. The bioavailable steroid concentration was then obtained by multiplying the total steroid concentration, as determined by RIA, by the fraction that was non-SHBG-bound.

Bone formation was assessed by serum osteocalcin, measured by RIA (interassay CV $<6\%$) [18] as well as by serum bone-specific alkaline phosphatase (BSAP) measured by enzyme-linked immunosorbent assay [18] (ELISA; interassay CV $<11\%$). Bone resorption was evaluated by measurement of 24-h urinary levels of the N-telopeptide of type I collagen (NTx) and free deoxypyridinoline (f-Dpd), both assessed as nanomoles per liter of glomerular filtrate. NTx and f-Dpd were measured by ELISA kits (Osteomark, Ostex, Seattle, WA; interassay CV = 10%, and Pyrilinks-D, Metra Biosystems, Mountain View, CA; interassay CV = 14%). The glomerular filtration rate was assessed by creatinine clearance.

Serum OPG levels were measured with an ELISA using a mouse monoclonal antibody as capture antibody and a rabbit polyclonal antibody for detection (Amgen, Thousand Oaks, CA) [3]. This assay detects both monomeric and dimeric forms of OPG, as well as OPG bound to RANK-L. Interassay CV is less than 15%.

Statistical Analysis

Demographic characteristics and variables of interest were described using appropriate summary statistics. The Spearman rank correlation was used to relate serum OPG levels to sex steroid levels, bone turnover markers and BMD. The Wilcoxon rank sum test was used to compare characteristics between two groups. Regression models were used to compare differences in slope between men and women (since the distribution of OPG was non-Gaussian, the log transformation was applied for this modeling). A p value of less than 0.05 was considered statistically significant.

Results

Table 1 shows the characteristics of the study population. The men over the age of 50 years showed the expected decreases in sex steroid levels, particularly the bioavailable fractions. In addition, BMD at the various sites was lower in the older men compared with the young men, and bone turnover tended to be higher. The postmenopausal women showed, as expected, profound decrements in sex steroid levels, with significant reductions in BMD and increases in bone turnover markers compared with the premenopausal women.

Figure 1 shows the changes in serum OPG levels with age in the men and women, respectively. Serum OPG levels increased with age in both men and women, although the slope of this relationship was greater in the men compared with the women (p = 0.001 for the difference in slope in men versus women).

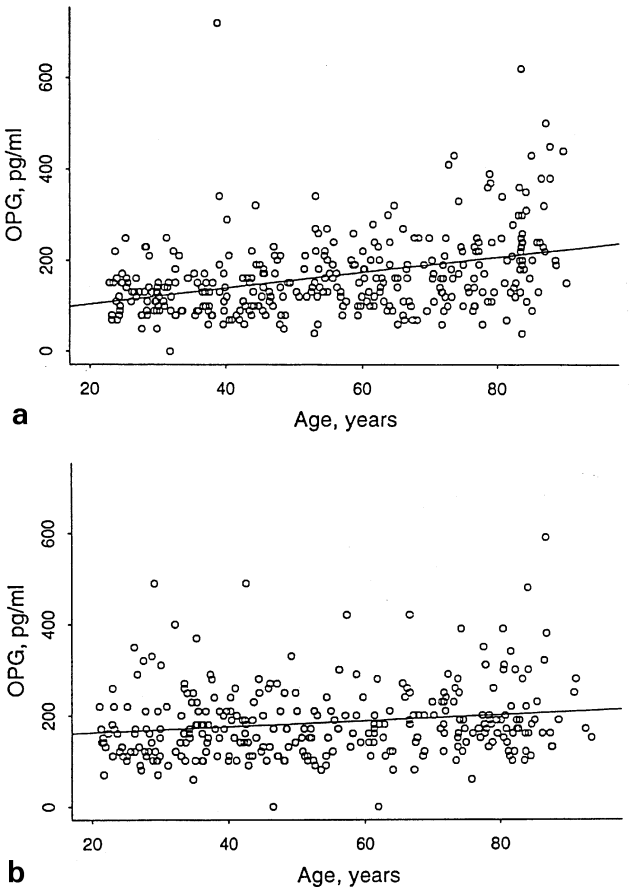

Fig. 1. Serum OPG levels among age-stratified samples of Rochester, Minnesota men (**a**) and women (**b**) as a function of age. R = 0.39, $p < 0.001$ and R = 0.18, $p < 0.01$ for correlation coefficients with age in the men and women, respectively.

Table 1. Characteristics of the study population

	Men < age 50 years	Men ⩾ age 50 years	Premenopausal women	Postmenopausal women
Age (years)	35.8 ± 0.7	69.8 ± 0.8	35.0 ± 0.7	69.8 ± 1.0
Sex steroids				
T (ng/dl)	537 ± 14	467 ± 14**	40 ± 1	33 ± 2***
Bio T (ng/dl)	167 ± 4	96 ± 4***	7.3 ± 0.4	5.9 ± 0.4**
E_2 (pg/ml)	32 ± 1	30 ± 1	120 ± 8	23 ± 3***
E_1 (pg/ml)	39 ± 1	35 ± 1*	71 ± 4	32 ± 2***
Bio E_2 (pg/ml)	17 ± 1	12 ± 1***	36 ± 2	7 ± 1***
Bone markers				
Serum				
OC (ng/ml)	6.6 ± 0.2	6.7 ± 0.2	4.9 ± 0.1	6.5 ± 0.2***
BSAP (U/l)	23.5 ± 0.7	22.2 ± 0.6*	17.4 ± 0.5	22.3 ± 0.6***
NTx (nmol/l)	12.4 ± 0.4	13.0 ± 0.5	10.6 ± 0.4	14.1 ± 0.5***
Urine				
NTx (nmol/l GF)	2.56 ± 0.11	2.98 ± 0.17	2.18 ± 0.08	3.14 ± 0.14***
Dpd (nmol/l GF)	0.38 ± 0.01	0.47 ± 0.02***	0.41 ± 0.01	0.51 ± 0.01***
Bone density				
Mid-radius (g/cm^2)	0.68 ± 0.004	0.62 ± 0.006***	0.60 ± 0.004	0.49 ± 0.007***
Mid-lateral spine (g/cm^2)	0.83 ± 0.012	0.68 ± 0.013***	0.81 ± 0.010	0.57 ± 0.015***
Femoral neck (g/cm^2)	0.94 ± 0.011	0.81 ± 0.010***	0.87 ± 0.010	0.68 ± 0.010***

Data are mean ± SEM. p value is for comparison of the men ⩾ age 50 years versus men < age 50 years (in the men), or postmenopausal women versus the premenopausal women (in the women). T, testosterone; Bio, bioavailable; E_2, estradiol; E_1, estrone; BMD, bone mineral density; OC, osteocalcin; BSAP, bone-specific alkaline phosphatase; NTx, N-telopeptide of type I collagen; Dpd, deoxypyridinoline.
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Table 2. Serum OPG levels (pg/ml) in the various groups

Men	Women	*p* value
All men	*All women*	
165 ± 5	184 ± 5	< 0.001
Men < 50 years	*Premenopausal women*	
134 ± 6	171 ± 6	< 0.001
Men ⩾ 50 years	*Postmenopausal women*	
188 ± 7	195 ± 7	0.179

Data are mean ± SEM.

Women had significantly higher serum OPG levels than men, and this was entirely due to higher levels in premenopausal women compared with young men (Table 2). Serum OPG levels were similar in postmenopausal women and men over the age of 50 years.

Table 3 shows the Spearman correlation coefficients between serum OPG and sex steroid levels. OPG levels were negatively correlated with serum total and bioavailable T levels in the men over age 50 years, and with bioavailable E_2 levels in both groups of men. None of the sex steroids was related to OPG levels in the women. OPG levels were positively associated with urinary NTx and Dpd in the men over the age of 50 years and with serum BSAP in the men under the age of 50 years (Table 3). By contrast, none of the bone turnover markers was related to serum OPG levels in the women. Mid-radius BMD was inversely associated with serum OPG levels in the men, but not in the women (Table 3). In addition, mid-lateral spine BMD was also inversely associated with BMD in the men under the age of 50 years. We also analyzed the data by combining all the men and women and then adjusting for age, body weight and gender (Table 3). In this analysis, bioavailable T was still inversely (albeit weakly) associated with serum OPG levels, with a persistent weak but positive association between urinary Dpd and serum OPG. Finally, since the distribution of serum OPG levels was non-Gaussian (Fig. 1), we also analyzed the data using quintiles of OPG, and found no associations between quintiles of OPG and BMD at the various sites (data not shown).

Discussion

We report the characterization of circulating OPG levels in a well-defined cohort of men and women. OPG levels were significantly higher in women compared with men, although this was largely due to higher levels in premenopausal women compared with men under the age of 50 years; men aged 50 years and over and postmenopausal women had relatively similar circulating OPG levels. In men aged 50 years and over, OPG levels were inversely associated with serum total and bioavailable T levels and with bioavailable E_2 levels, although no association with sex steroid levels were found in the women.

In vitro, E has been shown to increase OPG production [7,8], whereas T and the non-aromatizable androgen, 5α-dihydrotestosterone, appear to have the opposite effect [9]. This may, in part, explain the lower OPG levels in the young men compared with the premenopausal women, as well as the negative association with T levels in the older men. Consistent with this, in a direct interventional study in which men were made acutely hypogonadal and then replaced with either E or T, or both, we have recently found that E tended to increase and T (in the presence of an aromatase inhibitor) to decrease circulating OPG levels [19]. Surprisingly, in the present study, serum OPG levels were not associated with E levels in the women and, in fact, tended to be inversely associated with E levels in the men. The latter finding may be due to the association

Table 3. Spearman correlation coefficients between serum OPG levels and serum sex steroid levels, bone turnover markers and BMD

	Men < 50 years	Men ⩾ 50 years	Premenopausal women	Postmenopausal women	All groups, adjusted for age, weight and gender
Sex steroids					
T (ng/dl)	0.04	–0.16*	0.01	0.13	–0.02
Bio T (ng/dl)	–0.03	–0.27***	–0.06	0.07	–0.10*
E_2 (pg/ml)	–0.10	–0.02	0.06	0.07	0.03
E_1 (pg/ml)	–0.14	–0.02	0.03	0.12	0.04
Bio E_2 (pg/ml)	–0.21*	–0.18*	0.02	0.03	–0.03
Bone markers					
OC (ng/ml)	–0.03	0.05	–0.11	–0.001	–0.09*
BSAP (U/l)	0.17*	0.11	–0.11	0.03	0.04
NTx (nmol/l)	0.05	0.10	–0.02	0.05	0.01
NTx (nmol/l GF)	0.10	0.16*	–0.16	0.13	0.03
Dpd (nmol/l GF)	0.10	0.26***	–0.10	0.14	0.09*
Bone density					
Mid-radius (g/cm^2)	–0.19*	–0.17*	–0.01	–0.07	–0.07
Mid-lateral spine (g/cm^2)	–0.20*	–0.07	–0.01	0.04	0.02
Femoral neck (g/cm^2)	–0.13	–0.06	–0.09	–0.12	0.01

*$p < 0.05$; ***$p < 0.001$.

between bioavailable E_2 and T levels, since after adjusting for the effects of bioavailable T, bioavailable E_2 levels were no longer associated with OPG levels in men age 50 years and over (adjusted $R = –0.07$, $p = 0.351$). However, the inverse association between bioavailable E_2 and OPG persisted in the men under the age of 50 years (adjusted $R = –0.21$, $p = 0.012$). Since T is aromatized locally in bone to E [20], it is possible that the inverse association between bioavailable E_2 and OPG still reflects the association between bioavailable T and OPG, since higher T levels would provide more substrate for aromatization to E.

During the preparation of this manuscript, Szulc et al. [21] reported serum OPG levels in a cohort of men and, in contrast to our findings, found weak, positive associations between OPG and the free T and the free E_2 indices, but only after adjusting for age and body weight. Since in our study we related OPG levels to directly measured bioavailable T and E_2 levels, we repeated the analysis using the free T and E_2 indices. In this analysis, the free T index was also negatively associated with OPG levels in the men under the age of 50 years ($r = -0.17$, $p < 0.05$) as well as in the men age 50 years and over ($r = –0.27$, $p < 0.001$), although these associations were no longer significant after adjusting for age and body weight. The free E_2 index in the older men was not associated with OPG levels ($r = –0.10$), whereas in the younger men a negative association persisted ($r = -0.18$, $p < 0.05$), even after adjusting for age and body weight. The reason(s) for the different findings in the two studies are unclear, but may relate to the different OPG assays used, and the fact that the associations with sex steroids we and Szulc et al. [21] found were relatively weak, highlighting the need for direct interventional studies [19] in assessing sex steroid regulation of OPG in vivo in men.

In the men, OPG levels tended to be positively associated with bone turnover markers and negatively associated with BMD, although no such associations were present in the women. Consistent with our findings in the women, Browner et al. [11] did not find any association between serum OPG levels and either BMD or fractures in a cohort of postmenopausal women. By contrast, Yano et al. [10] did note a positive association between serum OPG levels and markers of bone turnover and a negative association between OPG and BMD in postmenopausal women, although they did not report comparable data in men. It is of note that the assay used by Yano et al. [10] gave significantly higher circulating OPG levels and only detected monomeric OPG, and the different results of these studies may reflect the measurement of different circulating OPG species. While not all the data are entirely consistent, due perhaps to the limitations of assessing OPG levels in the peripheral circulation, a plausible hypothesis is that OPG levels may increase with increases in bone turnover, possibly as a compensatory mechanism serving to limit bone resorption. Consistent with this, we have recently found that circulating OPG levels were significantly higher in a group of osteoporotic women with high bone turnover compared with age-matched controls [22]. Further studies directly assessing OPG levels in the bone microenvironment in various physiologic and pathologic states are needed to further test this hypothesis.

Consistent with previous reports [10,21], we also found that serum OPG levels clearly increase with age, although in our study this increase appeared to be larger in men than in women. The reason(s) for this age-related increase are unclear, and further studies assessing whether this is due to increased OPG production or decreased clearance with age are needed.

The OPG assay used in the present study detects both monomeric and dimeric forms of OPG, as well as OPG bound to RANK-L. In mammalian cell systems, dimeric OPG is secreted in greater amounts than monomeric OPG and is biologically more active than monomeric OPG [23]. Since it is at present unclear what proportion of circulating OPG is monomeric, dimeric or bound to RANK-L [10,24], it seems reasonable to use an assay that detects all forms of circulating OPG. Clearly, additional studies using assays specific for each of these forms of OPG, as well as unligated OPG, are needed to further address this issue.

In summary, our study describes age and gender effects on circulating OPG levels. These findings are consistent with the hypotheses that E and T may have opposite effects on OPG production, and that OPG production may increase with increases in bone turnover, perhaps as a compensatory mechanism serving to limit bone loss. Studies directly testing these hypotheses should provide additional insights into the possible role of OPG in bone loss related to sex steroid deficiency and aging.

Acknowledgements. We would like to thank Ms Vickie Gathje and Ms Joan Muhs for their help in recruiting and studying the subjects, Ms Roberta Soderberg for sample handling and Ms Cynthia Crowson for help with the statistical analyses. This work was supported by research grant AR27065 from the National Institute of Arthritis, Musculoskeletal and Skin Diseases, United States Public Health Service.

References

1. Lacey DL, Timms E, Tan H-L, Kelley MJ, Dunstan CR, Burgess T, et al. Osteoprotegerin (OPG) ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 1998;93:165–76.
2. Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S-I, et al. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Proc Natl Acad Sci USA 1998;95:3597–602.
3. Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang M-S, Lüthy R, et al. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 1997;89:309–19.
4. Bucay N, Sarosi I, Dunstan CR, Morony S, Tarpley J, Capparelli C, et al. Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. Genes Dev 1998;12:1260–8.
5. Mizuno A, Amizuka N, Irie K, Murakami A, Fujise N, Kanno T, et al. Severe osteoporosis in mice lacking osteoclastogenesis

inhibitory factor/osteoprotegerin. Biochem Biophys Res Commun 1998;247:610–5.
6. Hofbauer LC, Khosla S, Dunstan CR, Lacey DL, Riggs BL. The roles of osteoprotegerin and osteoprotegerin ligand in the paracrine regulation of bone resorption. J Bone Miner Res 2000;15:2–12.
7. Hofbauer LC, Khosla S, Dunstan CR, Lacey DL, Spelsberg TC, Riggs BL. Estrogen stimulates gene expression and protein production of osteoprotegerin in human osteoblastic cells. Endocrinology 1999;140:4367–70.
8. Saika M, Inoue D, Kido S, Matsumoto T. 17β-Estradiol stimulates expression of osteoprotegerin by a mouse stromal cell line, ST-2, via estrogen receptor-β. Endocrinology 2001;142:2205–12.
9. Hofbauer LC, Hicok KC, Chen D, Khosla S. Regulation of osteoprotegerin gene expression and protein production by androgens and anti-androgens in human osteoblastic cells [abstract]. In: 83rd Annual Meeting of the Endocrine Society, 2001:#P1–496.
10. Yano K, Tsuda E, Washida N, Kobayashi F, Goto M, Harada A, et al. Immunological characterization of circulating osteoprotegerin/osteoclastogenesis inhibitory factor: increased serum concentrations in postmenopausal women with osteoporosis. J Bone Miner Res 1999;14:518–27.
11. Browner WS, Lui LY, Cummings SR. Associations of serum osteoprotegerin levels with diabetes, stroke, bone density, fractures, and mortality in elderly women. J Clin Endocrinol Metab 2001;86:631–7.
12. Tomoyasu A, Goto M, Fujise N, Mochizuki S, Yasuda H, Morinaga T, et al. Characterization of monomeric and homodimeric forms of osteoclastogenesis inhibitory factor. Biochem Biophys Res Commun 1998;245:382–7.
13. Yamaguchi K, Kinosaki M, Goto M, Kobayashi F, Tsuda E, Morinaga T, et al. Characterization of structural domains of human osteoclastogenesis inhibitory factor. J Biol Chem 1998;273:5117–23.
14. Melton LJ III. History of the Rochester Epidemiology Project. Mayo Clin Proc 1996;71:266–74.
15. Reid IR, Evans MC, Ames R, Wattie DJ. The influence of osteophytes and aortic calcification on spinal mineral density in postmenopausal women. J Clin Endocrinol Metab 1991;72:1372–4.
16. O'Connor S, Baker HWG, Dulmanis A, Hudson B. The measurement of sex steroid binding globulin by differential ammonium sulphate precipitation. J Steroid Biochem 1973;4:331–9.
17. Tremblay RR, Dube JY. Plasma concentrations of free and non-TeBG bound testosterone in women on oral contraceptives. Contraception 1974;10:599–605.
18. Delmas PD, Stenner D, Wahner HW, Mann KG, Riggs BL. Increase in serum bone γ-carboxyglutamic acid protein with aging in women: implications for the mechanism of age-related bone loss. J Clin Invest 1983;71:1316–21.
19. Khosla S, Atkinson EJ, Dunstan CR, O'Fallon WM. Effect of estrogen versus testosterone on circulating osteoprotegerin and other cytokine levels in normal elderly men. J Clin Endocrinol Metab (In press).
20. Eyre LJ, Bland R, Bujalska IJ, Sheppard MC, Stewart PM, Hewison M. Characterization of aromatase and 17β-hydroxysteroid dehydrogenase expression in rat osteoblastic cells. J Bone Miner Res 1998;13:996–1004.
21. Szulc P, Hofbauer LC, Heufelder AE, Rothe S, Delmas PD. Osteoprotegerin serum levels in men: correlation with age, estrogen, and testosterone status. J Clin Endocrinol Metab 2001;86:3162–5.
22. Riggs BL, Melton LJ III, Atkinson EJ, O'Fallon WM, Dunstan C, Khosla S. Evidence that postmenopausal women with vertebral fractures due to type I osteoporosis have enhanced responsiveness of bone to estrogen deficiency. J Bone Miner Res 2001;16(Suppl 1):S281.
23. Tomoyasu A, Masaaki G, Fujise N, Mochizuki S, Yasuda H, Morinaga T, et al. Characterization of monomeric and homodimeric forms of osteoclastogenesis inhibitory factor. Biochem Biophys Res Commun 1998;245:382–7.
24. Chen D, Sarikaya NA, Gunn H, Martin SW, Young JD. ELISA methodology for detection of modified osteoprotegerin in clinical studies. Clin Chem 2001;47:747–9.

Received for publication 14 August 2001
Accepted in revised form 20 November 2001