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Age-Related Changes in Cortical Bone Content of Insulin-Like Growth Factor Binding Protein (IGFBP)-3, IGFBP-5, Osteoprotegerin, and Calcium in Postmenopausal Osteoporosis: A Cross-Sectional Study

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Serum GH and IGF-I levels decline with increasing age, whereas osteoprotegerin (OPG) increases. IGFs as well as OPG are present in bone matrix and mediate the effects of many upstream hormones (*e.g.* estrogen). To evaluate whether changes in these proteins may to some extent explain the decrease in bone mass in postmenopausal or senile osteoporosis, we measured bone contents of IGF-I, IGF-II, IGF binding protein (IGFBP)-3, IGFBP-5, and OPG in combined extracts obtained after EDTA and guanidine hydrochloride extraction in 60 postmenopausal women aged 47–74 (mean, 63) yr with a previous distal forearm fracture and a hip or spine Z-score less than 0. We found age-related increases in IGFBP-3 ($r = 0.35$; $P < 0.01$), IGFBP-5 ($r = 0.59$; $P < 0.001$), and OPG ($r = 0.36$; $P < 0.01$) in cortical bone, significantly inversely correlated with femoral neck and lumbar spine BMD. A correlation between age and OPG was also detected in trabecular bone ($r = 0.27$; $P < 0.05$). A pronounced age-related decrease in cortical calcium contents ($r = -0.60$; $P < 0.001$), positively correlated with femoral neck and lumbar spine BMD, was also found. No age-related changes were detected for IGF-I or IGF-II. The present study demonstrates age-related changes in cortical bone contents of IGFBPs, calcium, and OPG, possibly related to the pathophysiology of postmenopausal osteoporosis. As for OPG, our findings probably represent compensatory responses to increased osteoclastic resorption. (*J Clin Endocrinol Metab* 88: 1014–1018, 2003)

THE IGF SYSTEM has an important role in the regulation of bone cell metabolism. IGFs affect proliferation and differentiation of bone cells and stimulate cell activity and collagen synthesis (1–3). Moreover, IGFs and their binding proteins (IGFBPs) may be released by bone resorption to act as local determinants of site-specific coupled bone formation (4). Several lines of evidence suggest the IGF system as a mediator in postmenopausal osteoporosis. Serum levels of GH and IGF-I decline with increasing age and have been implicated in the age-related decrease in bone mass (2). However, circulating levels may not necessarily reflect levels in the bone tissue, and although some studies have found correlations between serum levels of IGF-I and bone mass or occurrence of osteoporotic fractures, other studies fail to demonstrate this (5–9). Although several studies have investigated the age-related changes in bone matrix IGFs, only one has examined trabecular biopsies from postmenopausal women (10).

Recently, osteoprotegerin (OPG) and its cognate ligand, receptor activator of nuclear factor-κB ligand (RANKL), have been identified as important factors in the paracrine signaling between osteoblasts and osteoclasts (11). OPG acts as a soluble receptor that inhibits osteoclast differentiation by binding RANKL and competitively inhibiting interaction between RANKL and its receptor, RANK, on the osteoclast surface (11). Thus, increased OPG in serum from postmenopausal women was recently reported, suggesting that OPG may be regulated by age-related factor(s) (12, 13). Moreover, OPG is measurable in bone and, to our knowledge, no one has quantitated bone matrix levels in postmenopausal women.

The aim of the present study was to evaluate whether bone content of IGF-I, IGF-II, IGFBP-3, IGFBP-5, OPG, and calcium is related to age and bone mass in postmenopausal women.

Abbreviations: BMD, Bone mineral density; ICTP, carboxy-terminal telopeptide of type I collagen; IGFBP, IGF binding protein; OPG, osteoprotegerin; RANKL, receptor activator of nuclear factor-κB ligand; s, serum; sAP, serum alkaline phosphatase.

Patients and Methods

Patients

Sixty postmenopausal women aged 47–74 yr (mean, 63 yr), who had previously suffered a distal forearm fracture and had low BMD in the spine or hip (Z-score < 0), were included in the study. From each patient, an iliac crest bone biopsy was obtained under local anesthesia, using a modified Bordier trephine (inner diameter, 9 mm) from the standard site 2 cm below the iliac crest and 2 cm behind the anterior superior iliac spine. The samples were frozen at −80 C immediately after removal. Still frozen, the biopsies were later sawed carefully to divide cortical and trabecular bone. None of the patients received treatment for osteoporosis when iliac crest bone biopsy was performed. Written informed consent for blood and bone biopsy collection was obtained from all patients. The study was conducted according to the *Declaration of Helsinki II* and the *Guidelines of Good Clinical Practice* and approved by the local ethical committee.

Biochemistry

Blood samples were drawn after an overnight fast and either frozen at −80 C until analysis or analyzed immediately. Serum alkaline phosphatase (sAP) was determined by spectrophotometry using *p*-nitrophenylphosphate as substrate (14). PTH (1–84) was determined by RIA (Nichols Institute Diagnostics, Nijmegen, The Netherlands). Serum crosslinked carboxy-terminal telopeptide of type I collagen (sICTP) was measured by an equilibrium RIA (Orion, Diagnostica, Finland). Serum osteocalcin was measured by an in-house RIA using rabbit antiserum against bovine gla-protein (15). Intra- and interassay coefficients of variation were less than 10% for all assays.

Densitometry

Bone mineral density (BMD) was determined by dual energy x-ray absorptiometry in the lumbar spine (L1–L4) and the left femoral neck using a QDR-1000/W scanner (Hologic, Inc., Waltham, MA).

Extraction procedures and assays for IGF-I/II, IGFBP-3/5, osteocalcin, and OPG

Levels of IGF-I, IGF-II, IGFBP-3, IGFBP-5, osteocalcin, OPG, and total protein were determined in extracts obtained after EDTA and guanidine hydrochloride extraction as previously described (16, 17), with the exception that total protein was measured spectrophotometrically directly after desalting at 280 nm using BSA as a standard as described by others (17, 18). Calcium content was determined in extracts after HCl hydrolysis (16, 17). IGF-I and IGFBP-3 were analyzed using RIAs (Nichols Institute Diagnostics). IGF-II was analyzed using an IRMA from Diagnostic Systems Laboratories, Inc. (Webster, TX). Before assay of IGF-I and IGF-II, IGFBPs were separated by acid gel filtration (19). Osteocalcin was measured by RIA using a kit from DiaSorin, Inc. (Stillwater, MN). This assay requires full carboxylation of osteocalcin. Bone matrix levels of OPG were quantified by enzyme immunoassay using matched commercially available antibodies according to the manufacturer's descriptions (R&D Systems, Minneapolis, MN). We have recently validated this assay for bone samples (17). Cortical and trabecular bone extracts (5 μg protein on each lane) were subjected to Western ligand blot to attain a semiquantitative estimate of IGFBP-5 levels as previously described (16, 17). Cortical and trabecular bone was analyzed on separate occasions in the present study. We therefore refrain from comparing protein or calcium levels in the two compartments. Normalizing for total protein makes sense when performing cross-sectional or longitudinal studies. However, when analyzing relationships between variables, correcting for protein content may lead to covariance and perhaps overestimate relationships between variables, as well as increase the analytical variation.

Statistical analysis

To examine the relationship between BMD and cortical and trabecular bone contents of calcium, IGF-I, IGF-II, IGFBP-3, IGFBP-5, osteocalcin, and OPG, we used simple linear (bivariate) regression analysis. A subsequent multiple linear regression analysis was performed with stepwise addition of the variables that had *P* values less than 0.2 in our *a priori* analysis and upon bivariate regression. Normality was evaluated by comparing the distribution of standardized residuals to a normal distribution. When necessary, values were logarithmically transformed before performing regression analysis. Data are shown nontransformed to simplify interpretation (Fig. 1).

Results

The demographic and baseline features of the study population are presented in Table 1.

Age-related changes

The age-related distribution of the bone matrix content of measured variables is presented in Fig. 1. Age-related

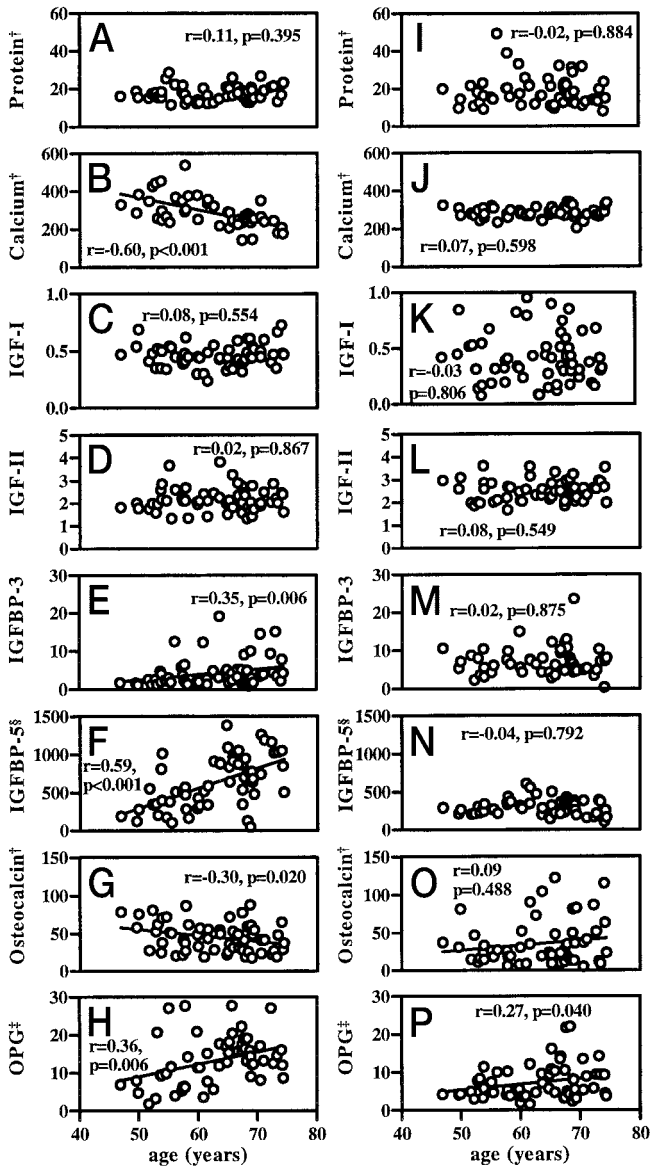

FIG. 1. Correlation analysis (Pearson) of cortical (A–H) and trabecular (I–P) bone contents of protein (A, I), calcium (B, J), IGF-I (C, K), IGF-II (D, L), IGFBP-3 (E, M), IGFBP-5 (F, N), osteocalcin (G, O), and OPG (H, P) *vs.* age in 60 postmenopausal women. Data are expressed as nanograms per milligram dry bone, unless otherwise specified. †, Micrograms per milligram dry bone; ‡, picograms per milligram dry bone; §, arbitrary units per square millimeter.

decreases were found for cortical calcium (r = −0.60; $P < 0.001$) and osteocalcin (r = −0.30; $P = 0.020$), whereas age-related increases were observed for IGFBP-3 (r = 0.35; $P = 0.006$), IGFBP-5 (r = 0.59; $P < 0.001$), and OPG (r = 0.36; $P = 0.006$). In contrast, only trabecular bone content of OPG was positively associated with age (r = 0.27; $P = 0.040$). No age-related changes were found for other bone matrix parameters. BMD of both the spine and femoral neck were correlated with age (r = −0.66, $P < 0.001$; and r = −0.45, $P = 0.001$, respectively). Of the biochemical bone markers, only ICTP was correlated with age (r = 0.23; $P = 0.049$).

Associations with BMD

Table 2 shows correlations between bone matrix proteins and BMD. Lumbar spine and femoral neck BMD was positively correlated with cortical calcium contents and negatively correlated with cortical IGFBP-5 and OPG. In addition, cortical IGFBP-3 was negatively correlated with lumbar spine BMD. In contrast, trabecular calcium and osteocalcin content were negatively correlated with femoral neck BMD, but not lumbar spine BMD. Of the biochemical bone markers, only ICTP ($r = -0.27$; $P = 0.045$) and sAP ($r = -0.32$; $P = 0.042$) were correlated with lumbar spine BMD.

Determinants of bone mass

To identify determinants of BMD, stepwise linear regression analysis was performed. Variables that had P values less than 0.2 in bivariate analysis (Table 2) were included in the model. Linear regression revealed that age, trabecular osteocalcin, and cortical bone contents of OPG explained 36% ($\beta = -0.45$; $t = -3.37$; $P = 0.002$) of the variation in femoral neck BMD, with osteocalcin and OPG contributing 8% ($\beta = -0.28$; $t = -2.25$; $P = 0.03$) and 7% ($\beta = -0.28$; $t = -2.08$; $P = 0.043$), respectively. Age was the only variable, explaining 43% ($\beta = -0.66$; $t = -6.29$; $P < 0.001$) of the variation in lumbar spine BMD.

TABLE 1. Baseline characteristics of patients with postmenopausal osteoporosis

	Postmenopausal osteoporosis	Normal postmenopausal range
Age in yr (mean, range)	63 (47–74)	
BMD (g/cm^2)		
Lumbar spine	0.71 ± 0.10	0.89 ± 0.11[a]
Femoral neck	0.61 ± 0.07	0.73 ± 0.10[a]
Years since menopause	15 ± 10	
Prevalent vertebral compression fractures		
0	52	
1	5	
>1	3	
sPTH (ng/liter)	36 ± 14	30 ± 11
sICTP (μg/liter)	3.2 ± 1.1	3.2 ± 8.4
sAP (U/liter)	157 ± 45	137 ± 71
sOsteocalcin (μg/liter)	20 ± 5	11 ± 5

[a] Standard Hologic QDR1000 reference curves, age 60 yr.

TABLE 2. Correlation analysis (Pearson) of cortical and trabecular bone contents of calcium, IGF-I, IGF-II, IGFBP-3, IGFBP-5, osteocalcin, and OPG *vs.* BMD in 60 postmenopausal women

	Cortical		Trabecular	
	BMD FN	BMD LS	BMD FN	BMD LS
Calcium	0.359[b]	0.521[d]	−0.288[b]	−0.146
IGF-I	−0.073	−0.033	−0.027	0.004
IGF-II	−0.180[a]	−0.121	−0.261[a]	−0.185[a]
IGFBP-3	−0.131	−0.282[b]	0.006	0.048
IGFBP-5	−0.277[b]	−0.313[b]	0.065	−0.020
Osteocalcin	0.078	0.091	−0.341[b]	−0.177[a]
OPG	−0.424[c]	−0.424[c]	−0.019	−0.026

FN, Femoral neck; LS, lumbar spine.
[a] $P < 0.2$ (included in stepwise linear regression); [b] $P < 0.05$; [c] $P < 0.01$; [d] $P < 0.001$.

Discussion

Both *in vitro* and *in vivo* models suggest that bone loss during estrogen deficiency is partly mediated by an up-regulation of RANKL and down-regulation of OPG (11). Estrogen suppresses RANKL-induced osteoclast differentiation by different mechanisms (20) and increases OPG expression and protein levels in human osteoblasts (21). We found an age-related increase in the bone matrix accumulation of OPG in postmenopausal women, significantly negatively correlated with bone mass in cortical bone. This is, to our knowledge, the first assessment of bone matrix and reflects previous findings by others in serum (12, 13). Furthermore, multiple regression analysis identified OPG as a significant negative predictor of BMD, although these effects were modest compared with age, suggesting that other age-related factors not accounted for in the present study may be more prominent predictors of BMD. Yano *et al.* (12) suggested that the age-related increase in serum OPG was a compensatory response to increased osteoclastic resorption. Thus, although OPG is produced in many tissues, bone-derived OPG may be released into the circulation, and serum levels reflect the situation locally in bone. To fully evaluate the OPG system in humans, RANKL should be determined. We have attempted this previously, but our assay was not sensitive enough to detect RANKL in bone samples (17).

A major finding in the present study was the pronounced age-related decrease in cortical calcium, amounting to an annual loss of approximately 2.0%/yr (data not shown), which could signify a serious undermineralization of the bone matrix in the older patients, resulting in decreased biomechanical competence (22). We did not find any serological indications of osteomalacia because sAP was only marginally elevated. Also, cortical calcium was not correlated with sPTH, and there was no difference in occurrence of prevalent vertebral compression fractures across age. Finally, an annual decline in BMD of approximately 1.0% is within the normal range. Still, bone biopsies were not available for histomorphometry. The most rapid loss of calcium occurred in the oldest patients (data not shown). This could indicate a shift in remodeling from the trabecular to the cortical compartment due to increased endocortical and intracortical remodeling, in addition to a modest secondary hyperparathyroidism, further increasing remodeling at this envelope (23, 24). The age-related decrease in the cortical concentration of osteocalcin could be explained by high bone turnover, increasing release of osteocalcin from the extracellular matrix to serum. However, because our assay requires full carboxylation of osteocalcin, an age-related decrease in the relative carboxylation of the protein (25) could also explain these results. We take the unchanged calcium levels in trabecular bone in relation to age, weakly associated with increased BMD, to mean that decreases in areal bone density at cancellous sites could be due to trabecular thinning with loss of bone mass, without a proportional loss of bone mineral (24).

Several groups have previously investigated bone matrix IGFs in relation to age and bone mass, but the results are inconsistent (26). In autopsy specimens, a negative correlation between IGF-I levels and age was found in women (27),

whereas Seck *et al.* (10), using live donors, found a weak however significant age-related decline in trabecular IGF-I, but not IGF-II in postmenopausal women, with no correlation to bone mass. Our findings suggest that IGFBPs may be involved in the pathophysiology of postmenopausal osteoporosis, possibly by regulating the bioavailability of IGFs, and thereby their anabolic potential (28). IGF-independent effects on both osteoblasts and osteoclasts have also been reported (29). Further experimental studies (*e.g.* IGFBP-5 transgenic/knockout mice) are needed to elucidate the clinical importance of these findings. Finally, because circulating IGFBP-3 and IGFBP-5 decline with increasing age in postmenopausal women (8, 9, 30, 31), serum levels do not seem to reflect levels of growth factors accumulated in bone, and other proteins (*e.g.* cytokines and growth factors) in the local environment may partly determine IGFBP expression. In fact, IL-6, a major bone-resorbing cytokine implicated in postmenopausal bone loss (32, 33), increases *in vitro* osteoblastic production of IGFBP-5 (34).

Surprisingly, changes in bone matrix protein concentrations were restricted to cortical bone in the present study. Our study was not designed to elucidate different regulatory mechanisms between trabecular and cortical bone, and we can only speculate on the differences found in the present study. Except OPG, no age-related changes were detected for bone matrix proteins in trabecular bone, suggesting regional differences in pathophysiology. This could be attributed to difference in bone turnover, increased skeletal blood flow in trabecular bone (35) making it more metabolically active and responsive to treatment (36), systemic factors, and circulating immune cells that may impact bone metabolism (37, 38).

In conclusion, we found age-related increases in cortical bone matrix levels of OPG, IGFBP-3, and IGFBP-5 in postmenopausal women. Furthermore, these changes were inversely correlated with BMD, possibly providing a link to the pathophysiology of postmenopausal osteoporosis. We also measured a rapid age-related loss of cortical, but not trabecular, bone matrix calcium. Future studies are needed to elucidate the clinical importance of these results. As for OPG, our findings probably represent compensatory responses to increased osteoclastic resorption.

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References

1. **Canalis E, McCarthy T, Centrella M** 1988 Growth factors and the regulation of bone remodeling. J Clin Invest 81:277–281
2. **Rosen CJ, Donahue LR, Hunter SJ** 1994 Insulin-like growth factors and bone: the osteoporosis connection. Proc Soc Exp Biol Med 206:83–102
3. **Wergedal JE, Mohan S, Lundy M, Baylink DJ** 1990 Skeletal growth factor and other growth factors known to be present in bone matrix stimulate proliferation and protein synthesis in human bone cells. J Bone Miner Res 5:179–186
4. **Hayden JM, Mohan S, Baylink DJ** 1995 The insulin-like growth factor system and the coupling of formation to resorption. Bone 17:93S–98S
5. **Seck T, Scheidt-Nave C, Leidig-Bruckner G, Ziegler R, Pfeilschifter J** 2001 Low serum concentrations of insulin-like growth factor I are associated with femoral bone loss in a population-based sample of postmenopausal women. Clin Endocrinol (Oxf) 55:101–106
6. **Martini G, Valenti R, Giovani S, Franci B, Campagna S, Nuti R** 2001 Influence of insulin-like growth factor-1 and leptin on bone mass in healthy postmenopausal women. Bone 28:113–117
7. **Collins D, Woods A, Herd R, Blake G, Fogelman I, Wheeler M, Swaminathan R** 1998 Insulin-like growth factor-I and bone mineral density. Bone 23:13–16
8. **Sugimoto T, Nishiyama K, Kuribayashi F, Chihara K** 1997 Serum levels of insulin-like growth factor (IGF) I, IGF-binding protein (IGFBP)-2, and IGFBP-3 in osteoporotic patients with and without spinal fractures. J Bone Miner Res 12:1272–1279
9. **Boonen S, Mohan S, Dequeker J, Aerssens J, Vanderschueren D, Verbeke G, Broos P, Bouillon R, Baylink DJ** 1999 Down-regulation of the serum stimulatory components of the insulin-like growth factor (IGF) system (IGF-I, IGF-II, IGF binding protein [BP]-3, and IGFBP-5) in age-related (type II) femoral neck osteoporosis. J Bone Miner Res 14:2150–2158
10. **Seck T, Scheppach B, Scharla S, Diel I, Blum WF, Bismar H, Schmid G, Krempien B, Ziegler R, Pfeilschifter J** 1998 Concentration of insulin-like growth factor (IGF)-I and -II in iliac crest bone matrix from pre- and postmenopausal women: relationship to age, menopause, bone turnover, bone volume, and circulating IGFs. J Clin Endocrinol Metab 83:2331–2337
11. **Hofbauer LC, Khosla S, Dunstan CR, Lacey DL, Boyle WJ, Riggs BL** 2000 The roles of osteoprotegerin and osteoprotegerin ligand in the paracrine regulation of bone resorption. J Bone Miner Res 15:2–12
12. **Yano K, Tsuda E, Washida N, Kobayashi F, Goto M, Harada A, Ikeda K, Higashio K, Yamada Y** 1999 Immunological characterization of circulating osteoprotegerin/osteoclastogenesis inhibitory factor: increased serum concentrations in postmenopausal women with osteoporosis. J Bone Miner Res 14: 518–527
13. **Browner WS, Lui LY, Cummings SR** 2001 Associations of serum osteoprotegerin levels with diabetes, stroke, bone density, fractures, and mortality in elderly women. J Clin Endocrinol Metab 86:631–637
14. **Keiding NR, Hörder M, Gerhardt W, Pitkänen E, Tenhunen R, Strömme JH, Waldenström J, Tryding N, Westlund L** 1974 Recommended methods for the determination of four enzymes in blood. Scand J Clin Lab Invest 33:291–306
15. **Brixen K, Nielsen HK, Eriksen EF, Charles P, Mosekilde L** 1989 Efficacy of wheat germ lectin-precipitated alkaline phosphatase in serum as an estimator of bone mineralization rate: comparison to serum total alkaline phosphatase and serum bone Gla-protein. Calcif Tissue Int 44:93–98
16. **Ueland T, Bollerslev J, Hansen TB, Ebbesen EN, Mosekilde L, Brixen K, Flyvbjerg A, Djoseland O** 1999 Increased cortical bone content of insulin-like growth factors in acromegalic patients. J Clin Endocrinol Metab 84:123–127
17. **Ueland T, Bollerslev J, Flyvbjerg A, Hansen TB, Vahl N, Mosekilde L** 2002 Effects of 12 months of GH treatment on cortical and trabecular bone content of IGFs and OPG in adults with acquired GH deficiency: a double-blind, randomized, placebo-controlled study. J Clin Endocrinol Metab 87:2760–2763
18. **Boonen S, Aerssens J, Dequeker J, Nicholson P, Cheng X, Lowet G, Verbeke G, Bouillon R** 1997 Age-associated decline in human femoral neck cortical and trabecular content of insulin-like growth factor I: potential implications for age-related (type II) osteoporotic fracture occurrence. Calcif Tissue Int 61: 173–178
19. **Mohan S, Baylink DJ** 1995 Development of a simple valid method for the complete removal of insulin-like growth factor (IGF)-binding proteins from IGFs in human serum and other biological fluids: comparison with acid-ethanol treatment and C18 Sep-Pak separation. J Clin Endocrinol Metab 80: 637–647
20. **Shevde NK, Bendixen AC, Dienger KM, Pike JW** 2000 Estrogens suppress RANK ligand-induced osteoclast differentiation via a stromal cell independent mechanism involving c-Jun repression. Proc Natl Acad Sci USA 97:7829–7834
21. **Hofbauer LC, Khosla S, Dunstan CR, Lacey DL, Spelsberg TC, Riggs BL** 1999 Estrogen stimulates gene expression and protein production of osteoprotegerin in human osteoblastic cells. Endocrinology 140:4367–4370
22. **Currey JD** 1984 Effects of differences in mineralization on the mechanical properties of bone. Philos Trans R Soc Lond B Biol Sci 304:509–518
23. **Riggs BL, Khosla S, Melton III LJ** 2002 Sex steroids and the construction and conservation of the adult skeleton. Endocr Rev 23:279–302
24. **Seeman E** 2002 Pathogenesis of bone fragility in women and men. Lancet 359:1841–1850
25. **Plantalech L, Guillaumont M, Vergnaud P, Leclercq M, Delmas PD** 1991 Impairment of γ carboxylation of circulating osteocalcin (bone gla protein) in elderly women. J Bone Miner Res 6:1211–1216
26. **Aerssens J, Boonen S, Joly J, Dequeker J** 1997 Variations in trabecular bone composition with anatomical site and age: potential implications for bone quality assessment. J Endocrinol 155:411–421
27. **Seck T, Bretz A, Krempien R, Krempien B, Ziegler R, Pfeilschifter J** 1999 Age-related changes in insulin-like growth factor I and II in human femoral cortical bone: lack of correlation with bone mass. Bone 24:387–393
28. **Kiefer MC, Schmid C, Waldvogel M, Schlapfer I, Futo E, Masiarz FR, Green K, Barr PJ, Zapf J** 1992 Characterization of recombinant human insulin-like growth factor binding proteins 4, 5, and 6 produced in yeast. J Biol Chem 267:12692–12699
29. **Andress DL** 2001 IGF-binding protein-5 stimulates osteoblast activity and bone accretion in ovariectomized mice. Am J Physiol Endocrinol Metab **2**81: E283–E288

30. **Gamero P, Sornay-Rendu E, Delmas PD** 2000 Low serum IGF-1 and occurrence of osteoporotic fractures in postmenopausal women. Lancet 355:898–899
31. **Calo L, Castrignano R, Davis PA, Carraro G, Pagnin E, Giannini S, Semplicini A, D'Angelo A** 2000 Role of insulin-like growth factor-I in primary osteoporosis: a correlative study. J Endocrinol Invest 23:223–227
32. **Scheidt-Nave C, Bismar H, Leidig-Bruckner G, Woitge H, Seibel MJ, Ziegler R, Pfeilschifter J** 2001 Serum interleukin 6 is a major predictor of bone loss in women specific to the first decade past menopause. J Clin Endocrinol Metab 86:2032–2042
33. **Seck T, Diel I, Bismar H, Ziegler R, Pfeilschifter J** 2002 Expression of interleukin-6 (IL-6) and IL-6 receptor mRNA in human bone samples from pre- and postmenopausal women. Bone 30:217–222
34. **Franchimont N, Durant D, Canalis E** 1997 Interleukin-6 and its soluble receptor regulate the expression of insulin-like growth factor binding protein-5 in osteoblast cultures. Endocrinology 138:3380–3386
35. **Cook GJ, Lodge MA, Blake GM, Marsden PK, Fogelman I** 2000 Differences in skeletal kinetics between vertebral and humeral bone measured by 18F-fluoride positron emission tomography in postmenopausal women. J Bone Miner Res 15:763–769
36. **Duan Y, Tabensky A, DeLuca V, Seeman E** 1997 The benefit of hormone replacement therapy on bone mass is greater at the vertebral body than posterior processes or proximal femur. Bone 21:447–451
37. **Abrahamsen B, Bendtzen K, Beck-Nielsen H** 1997 Cytokines and T-lymphocyte subsets in healthy post-menopausal women: estrogen retards bone loss without affecting the release of IL-1 or IL-1ra. Bone 20:251–258
38. **Roggia C, Gao Y, Cenci S, Weitzmann MN, Toraldo G, Isaia G, Pacifici R** 2001 Up-regulation of TNF-producing T cells in the bone marrow: a key mechanism by which estrogen deficiency induces bone loss in vivo. Proc Natl Acad Sci USA 98:13960–13965