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Concentration of Adipogenic and Proinflammatory Cytokines in the Bone Marrow Supernatant Fluid of Osteoporotic Women

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ABSTRACT

Osteoporosis is characterized by low bone mass, microarchitectural deterioration of bone tissue leading to increased bone fragility, and a resulting susceptibility to fractures. Distinctive environmental bone marrow conditions appear to support the development and maintenance of the unbalance between bone resorption and bone formation; these complex bone marrow circumstances would be reflected in the fluid surrounding bone marrow cells. The content of regulatory molecules in the extracellular fluid from the human bone marrow is practically unknown. Since the content of cytokines such as adiponectin, leptin, osteoprogeterin (OPG), soluble receptor activator of nuclear factor κB ligand (s-RANKL), tumor necrosis factor α, and interleukin 6 (IL-6) may elicit conditions promoting or sustaining osteoporosis, in this work we compared the concentrations of the above-mentioned cytokines and also the level of the soluble receptors for both IL-6 and leptin in the extracellular fluid from the bone marrow of nonosteoporotic and osteoporotic human donors. A supernatant fluid (bone marrow supernatant fluid [BMSF]) was obtained after spinning the aspirated bone marrow samples; donors were classified as nonosteoporotic or osteoporotic after dual-energy X-ray absorptiometry (DXA) measuring. Specific commercially available kits were used for all measurements. The cytokines' concentration in BMSF showed differently among nonosteoporotic and osteoporotic women; this last group was characterized by higher content of proinflammatory and adipogenic cytokines. Also, osteoporotic BMSF differentiated by decreased leptin bioavailability, suggesting that insufficient leptin action may distinguish the osteoporotic bone marrow. © 2010 American Society for Bone and Mineral Research.

KEY WORDS: BONE MARROW FLUID; OSTEOPOROSIS; ADIPOKINES; PROINFLAMMATORY CYTOKINES; LEPTIN BIOAVAILABILITY

Introduction

The functional relationship among the several phenotypes of cells found in bone marrow generates a distinctive microenvironment via locally produced soluble factors, the extracellular matrix components, and systemic factors.[1,2] Taking into consideration only the primary cellular components of the marrow stroma, it is anticipated that a broad range of signals result from the activity of adipocytes, macrophages, fibroblasts, osteoprogenitors, hematopoietic stem cells, and their progeny, as well as from endothelial and reticular cells. At present, the content of signaling molecules in the extracellular fluid from human bone marrow is practically unknown, making it difficult to infer their physiologic relevance to bone marrow processes.

Osteoporosis is characterized by low bone mass, microarchitectural deterioration of bone tissue leading to increased bone fragility, and a resultingt susceptibility to fractures.[3] Distinctive environmental bone marrow conditions appear to provide support for the development and maintenance of the unbalance between bone resorption and bone formation. These complex bone marrow circumstances would be noticeable in the fluid surrounding bone marrow cells, collecting some characteristic assortment of signaling molecules such as adipokines and proosteoclastic and proinflammatory cytokines, among others.

Measurement of soluble molecules found in the human bone marrow has been particularly difficult not only because of tissue seclusion but also because of the complicated anatomy and blood perfusion of bone. Knowledge on the intramedullar concentration of compounds with recognized regulatory effects on bone formation or resorption is scarce, limited to some pathologic condition or estimated from measurements in plasma.[4–7]

The progress of osteoporosis and other bone loss–related diseases appearsto be strongly associated with bone marrow

adipogenesis because both osteoblasts and adipocytes originate from common precursor cells, the mesenchymal stem cells (MSCs) present in bone marrow.[8–11] In osteoporosis, there is very early alteration of the differentiation potential of MSCs, leading to unbalanced osteogenic/adipogenic processes favoring adipose tissue accretion in the bone marrow, which is counterbalanced by reduced production of osteogenic cells.[9,10] Iliac crest biopsies from elderly women showed that bone marrow from osteoporotic patients had a considerable accumulation of adipocytes in relation to that of healthy elderly women.[12] Subsequent studies confirmed a negative association between bone marrow fat and the rate of bone formation.[13–15]

Until recently, fatty infiltration of the bone marrow was considered an inconsequential product of normal aging, whereas now the potential responsibility of bone marrow adipocytes in the promotion of bone loss environmental conditions, through the secretion of several compounds having paracrine action on bone cells, is acknowledged.[9,16]

The aim of this work was to evaluate in part the potential regulatory activity of the extracellular fluid from bone marrow of nonosteoporotic and osteoporotic human donors because we conjectured that the content of some cytokines in the bone marrow may elicit conditions promoting or sustaining osteoporosis. It could be expected that concentrations measured in the bone marrow supernatant fluid (BMSF) may more reliably reflect the physiologically relevant concentrations in the interstitial compartment surrounding the bone cells than values found in blood. Considering the complex organization in such a regulatory milieu, we opted for evaluating some molecules recognized as markers of either adipocyte[17–23] proinflammatory[24–27] or osteoclastic/osteoblastic[28–30] activity affecting consequently the balance between bone resorption and bone formation.

Therefore, in this work, after obtaining a supernatant fluid from the bone marrow samples from characterized donors, the concentrations of the following cytokines or receptors were measured: adiponectin, interleukine 6 (IL-6), soluble IL-6 receptor (sIL-6R), tumor necrosis factor-α (TNF-α), leptin, soluble leptin receptor (sOB-R), osteoprotegerin (OPG), and soluble receptor activator of nuclear factor κB ligand (s-RANKL). In addition, the concentration of the C-terminal telopeptide cross-link of type I collagen (CTX) was measured.

Materials and Methods

Subjects

Postmenopausal women 60 to 75 years of age who required bone surgery because of bone fracture or arthroplasty at the Trauma Section of Hospital Sótero del Río, Santiago, Chile, were asked to volunteer as bone marrow donors. Written informed consent was obtained from all subjects, and ethical approval was obtained from the ethical committees of both the Hospital Sótero del Río and the Instituto de Nutrición y Tecnología de los Alimentos (INTA). Bone marrow was obtained by iliac crest aspiration during surgical procedures.[31] Bone mineral density (BMD) at the lumbar spine (L2–4) was measured for each subject within 4 weeks of surgery using dual-energy X-ray absorptiometry (DXA) (LUNAR, Prodigy, General Electric Medical Systems,

Madison, WI, USA). Donors were classified as nonosteoporotic or osteoporotic according to their BMD value; nonosteoporotic donors had BMD higher than −2.5 SD of the mean BMD for young adults, and osteoporotic donors had BMD lower than −2.5 SD.[32] All donors considered themselves healthy, except for the surgery, and were not under glucocorticoid or estrogen replacement therapy. Sixty percent of osteoporotic donors presented hip fracture, whereas this condition was seen in 13% of the osteopenic nonosteoporotic donors.

Body mass index (BMI) and other anthropometric measurements were determined using values obtained during the DXA procedure.

Bone marrow supernatant fluid (BMSF)

Bone marrow supernatant fluid was obtained after spinning the bone marrow–aspirated sample ($\sim$2 mL) for 5 minutes at $600 \times g$.[4] Approximately 500 to 800 μL of bone marrow supernatant fluid was collected and kept at −20°C until cytokine measurement.

Measurement of cytokines and soluble cytokines receptors

Commercially available ELISA kits were used for the following measurements: Leptin was assessed using the Linco Research (St. Charles, MO, USA) kit, with a sensitivity of 0.125 ng/mL. For measurement of IL-6 and the sIL-6R and leptin, the kits used were from R&D Systems, Inc. (Minneapolis, MN, USA), with sensitivities of 0.039 pg/mL, 6.5 pg/mL, and 0.02 ng/mL, respectively. The kits used for the determination of both adiponectin and TNF-α were from US Biological (Swampscott, MA, USA), which had sensitivities of 0.1 μg/mL and 16 pg/mL, respectively. OPG, s-RANKL, and CTX were measured using kits from Immunodiagnostic Systems (Fountain Hills, AZ, USA), with detection limits of 0.14 pmol/L, 0.02 pmol/L, and 0.02 ng/ml, respectively.

Statistical analysis

All results are expressed as mean $\pm$ SD. Comparison between nonosteoporotic and osteoporotic groups was done using a two-sample Student's t test; Pearson's correlation coefficients were used to examine bivariate correlation. A $p < .05$ was considered to represent a statistically significant difference. Adjustement of data for weight, age, or both was done through the general linear method procedure (SAS Institute, Cary, NC, USA).

Results

Characteristics of subjects

Anthropometric characteristics of donors are shown in Table 1. The mean BMI was significantly different between the two groups, with the mean BMI of nonosteoporotics falling in the overweight range and the mean BMI of osteoporotics being normal. Accordingly, a significant difference in the percent of fat mass also was observed between groups.

Table 1. Characteristics of Nonosteoporotic and Osteoporotic Bone Marrow Donors

	Nonosteoporotics (n = 32)	Osteoporotics (n = 15)	P Value
Age (years)	71.4 ± 4.7	72.5 ± 5.7	.270
T-score	−1.1 ± 1.1	−3.8 ± 0.8	.001
Weight (kg)	62.9 ± 7.7	57.3 ± 9.0	.014
BMI (kg/m^2)	26.4 ± 2.5	23.9 ± 2.8	.010
Fat (%)	48.6 ± 5.9	41.9 ± 7.3	.003

Values are mean ± SD.

Bone marrow supernatant fluid

All bone marrow samples were obtained by iliac crest suction performed by the same surgeon (GS), attaining standardized volume of sampling; donor bone health was defined by L2–4 DXA after surgery. These conditions guaranteed representative sampling of bone marrow, which on careful centrifugation originated the portion denominated as *bone marrow supernatant fluid* (BMSF). This fraction appears to comprise the bone marrow milieu, although it also might contain components derived from the blood perfusion of tissue, as well as compounds from broken marrow cells.[4]

Bone resorption marker in BMSF

The bone resorption marker serum C-telopeptide cross-link of type 1 collagen (sCTX) was measured in BMSF samples because it is a highly sensitive indicator of increased bone resorption in menopausal women. As expected, sCTX significantly differed between nonosteoporotics and osteoporotics, being 0.55 ± 0.16 and 0.87 ± 0.38 ng/mL, respectively ($p < .027$; $n = 8$).

Cytokine levels in BMSF

Selected cytokines, sIL-6R, and leptin concentrations were measured in the BMSF obtained from nonosteoporotic and osteoporotic donors (Table 2). All concentration values showed statistical difference between both groups, except for soluble leptin receptor. The levels of IL-6, TNF-α, and OPG were higher in osteoporotic than in nonosteoporotic donors, whereas leptin, adiponectin, and s-RANKL concentrations were higher in nonosteoporotic than in osteoporotic BMSF. The concentration
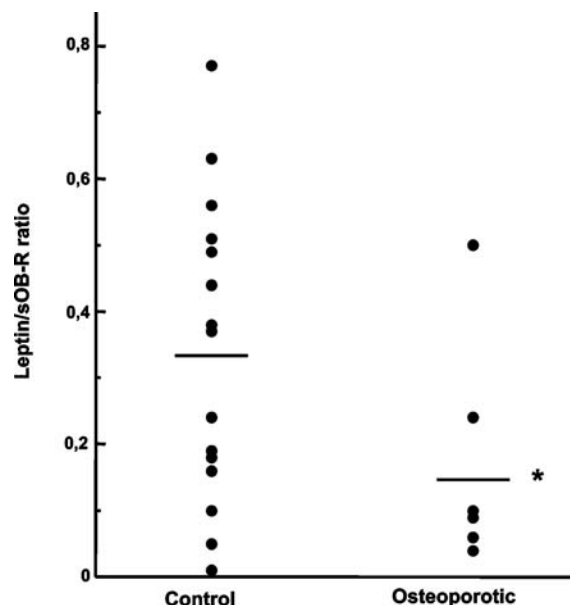
Fig. 1. Leptin bioavailability in bone marrow supernatant fluid (BMSF) of nonosteoporotic and osteoporotic donors. Results show the ratio between leptin and the soluble leptin receptor (sOB-R) concentrations in samples of BMSF. The bar indicates the mean value for each group. $^*p < .05$.

of sIL-6R was higher in osteoporotic samples, whereas in both groups the sOB-R concentrations were similar.

Since the groups studied differed in body weight (and its derived anthropometric values), in addition to values of T-score, the leptin concentrations shown in Table 2 were adjusted for weight, age, or both, having in mind the influence of body weight and/or fat on circulating leptin values and of age on T-scores. We substantiated that mean leptin concentration values and the significance range among groups were essentially maintained after these statistical adjustments (values not shown).

In plasma, the bioactivity of some cytokines appears to depend on the concentration of their soluble receptors. Thus the available leptin concentration for cells depends on the ratio between the concentration of this cytokine and its specific soluble receptor. As shown in Fig. 1, leptin bioavailability, based on this ratio, was significantly diminished in the BMSF of

Table 2. Concentration of Cytokines and of the Soluble Receptors for Interleukin-6 and Leptin in the Bone Marrow Supernatant Fluid

	Nonosteoporotics	Osteoporotics	P Value
Interleukin-6 (pg/mL)	4.8 ± 2.5 (n = 23)	6.2 ± 2.5 (n = 15)	.044
Soluble interleukin-6 receptor (ng/mL)	33.7 ± 13.1 (n = 12)	47.0 ± 13.7 (n = 10)	.02
TNF-α (pg/mL)	72.3 ± 55.0 (n = 15)	148.9 ± 82.0 (n = 12)	.036
Adiponectin (μg/mL)	9.5 ± 2.4 (n = 15)	5.7 ± 2.7 (n = 14)	.04
Soluble RANKL (pmol/L)	0.27 ± 0.16 (n = 8)	0.14 ± 0.05 (n = 8)	.021
Osteoprotegerin (pmol/L)	2.9 ± 0.9 (n = 8)	4.4 ± 1.8 (n = 8)	.035
Leptin (ng/mL)	14.5 ± 11.3 (n = 24)	7.0 ± 4.4 (n = 12)	.004
Soluble leptin receptor (ng/mL)	44.6 ± 14.7 (n = 16)	48.9 ± 17.8 (n = 8)	.282

Values are mean ± SD. Number of samples indicated in parenthesis.

Table 3. Statistically Significant Bivariate Correlation Coefficients Between Measured Variables

	BMI	Fat	sIL-6R	Leptin	Leptin/sOB-R	OPG
T-score	0.40293 (.01098)	0.3334 (.03807)	n.s.	0.46493 (.00428)	0.5739 (.00336)	n.s.
BMI	1	0.69759 (<.0001)	n.s	0.35813 (.03465)	0.62382 (.00112)	n.s.
Fat	n.s	1	n.s.	0.64375 (<.0001)	0.61532 (.00137)	n.s.
sIL-6R	n.s	n.s	1	n.s.	−0.57457 (.03999)	n.s.
Leptin	n.s	n.s	n.s.	1	0.92349 (<.0001)	n.s.
sOB-R	n.s	n.s	n.s.	n.s.	−0.59293 (.00226)	0.86835 (.02486)
IL-6	n.s.	n.s	0.43852 (.0412)	n.s.	n.s.	n.s.

BMI = body mass index; IL-6 = interleukine 6; sIL-6R = soluble interleukine 6 receptor; TNF-α = tumor necrosis factor α; sOB-R = soluble leptin receptor. p values are indicated in parentheses. n.s. = nonsignificant.

osteoporotic donors compared with that of nonosteoporotic donors (0.15 ± 0.16 and 0.33 ± 0.22, respectively). In contrast, the functional relationship for the IL-6 system appears to depend on a simultaneous elevation in both IL-6 and sIL-6R, which was observed in the osteoporotic group (see Table 2).

On the other hand, osteoclast activity is likely to depend, at least in part, on the relative balance of RANKL and OPG. We found that the RANKL/OPG ratio appears significantly increased ($p < .010; n = 8$) in BMSF of nonosteoporotic versus osteoporotic donors (0.095 ± 0.034 versus 0.042 ± 0.018, respectively).

No influence of the fracture condition of donors was noticed in the parameter values measured in the BMSF samples (not shown).

All factors measured in samples of nonosteoporotic and osteoporotic subjects were analyzed in a matrix of linear correlation; statistically significant results are shown in Table 3. T-scores showed a significant positive linear correlation with BMI, the percent of fat, leptin concentration, and the leptin/sOB-R ratio. Furthermore, BMI correlated positively with the percent of fat, leptin concentration, and leptin/sOB-R ratio. In addition, the percent of fat showed a significant positive correlation with both leptin level and the leptin/sOB-R ratio. While the concentration of IL-6 correlated directly with the concentration of its soluble receptor, the level of sOB-R correlated significantly with the leptin/sOB-R ratio and OPG (see Table 3). No significant linear correlation was appreciated between TNF-α, adiponectin, or RANKL levels and the other parameters measured (not shown).

Discussion

Results showed that in the bone marrow microenvironment, the concentrations of functional cell signals such as OPG, RANKL, TNF-α, IL-6, and leptin are different in nonosteoporotic and osteoporotic subjects. Thus, although the concentration range for all parameters measured in the BMSF was equivalent to the respective range in normal blood plasma,[6,7,18,30,33,34] the concentration of cytokines in the BMSF of nonosteoporotic and osteoporotic subjects proved to be different enough to discriminate bewteen these groups. Several in vitro studies show direct action of these cytokines on bone cells,[28,35–37] and one in vivo study has shown that estrogen withdrawal at menopause promotes a display of proinflammatory cytokines.[26] Nevertheless, no consistent difference in the levels of these cytokines has been shown in plasma between nonosteoporotic and

osteoporotic subjects,[7,18,28,38–40] except for OPG.[41,42] Our results suggest that in the bone marrow environment these cytokines attain levels that show less variability than those found in blood. Hence the cytokine levels measured in BMSF may give a better account of their influence on bone condition.

Our results substantiate that in the BMSF of osteoporotic subjects, proinflammatory and proadipogenic conditions prevail, given that the levels of IL-6, sIL-6R, and TNF-α were increased, whereas those of adiponectin and leptin were diminished compared with nonosteoporotic subjects. The relative increase in the proinflammatory cytokines in the BMSF of osteoporotic subjects is in agreement with the recognized role of IL-6 and TNF-α as mediators of bone loss not only in postmenopausal osteoporosis but also in other diseases associated with bone loss.[26] Moreover, the associated increase in sIL-6R in the BMSF of osteoporotic subjects compared with nonosteoporotic subjects corroborates its enhanced proinflammatory potential as it could be expected from the recognized relevance of the IL-6/sIL-6R system in several bone loss conditions.[43,44]

Considering the adipokine levels found in the BMSF of osteoporotic and nonosteoporotic subjects, it could be inferred that the adipose tissue of both groups presents distinctive activities. Hence, in the BMSF of nonosteoporotic subjects, the levels of both leptin and adiponectin appear appropriate, implying a balanced activity of bone marrow adipose tissue. Adequate leptin production by nonosteoporotic subjects could result in part from their high BMI and percent of fat mass; hence, in these individuals, leptin could exert an efficient protective effect on bone.[20] In contrast, the levels of these cytokines in the BMSF of osteoporotic subjects suggest an increased and/or disturbed bone marrow adipogenesis, given that adiponectin deficiency is a typical feature of extramedullar adipose tissue of patients with obesity, diabetes, or insulin resistance.[45,46] The observed reduction in the production of leptin by osteoporotic bone marrow appears paradoxical because it is known that osteoporosis and other bone loss diseases are characterized by an increase in the buildup of adipose tissue in bone marrow together with a decrease in bone volume.[9,12,47,48] All osteoporotic women in this study were either normal or overweight, as assessed by BMI, thus having a fat mass that essentially should be sufficient for normal leptin production. The decrease in leptin concentration could result in part from the increased IL-6 and TNF-α levels in osteoporotic bone marrow; these cytokines have been shown to inhibit leptin production

and secretion by bone marrow adipocytes.[21] Hence, it could be argued that in osteoporosis, an imbalance in bone marrow adipogenesis leads to a disruption in the production and/or action of signaling molecules, consequently preserving the adipogenic condition. Former in vitro studies support this conclusion in relation to a direct action of leptin on MSCs.[37,49]

On the other hand, decreased RANKL and increased OPG levels were detected in the BMSF of osteoporotic subjects compared with nonosteoporotic subjects, resulting in a low RANKL/OPG ratio. This observation could result in part from the methodology employed because reagents for OPG detection recognize all OPG forms, but measurement for RANKL is restricted to the free soluble RANKL form, underestimating therefore other functional RANKL forms. The level of OPG has been found increased also in plasma of osteoporotic women compared with nonosteoporotic women, hypothetically resulting from some sort of compensatory activity.[41,42] Further, it could be suggested that the above-mentioned increased and/or altered adipogenic conditions in the bone marrow of osteoporotic patients also could affect OPG levels because adipocytes and other bone marrow cells synthesize this cytokine, whereas its expression is under leptin regulation.[50–52] Moreover, it could be inferred that in osteoporotic subjects, the OPG protective effect on bone is by some means hindered despite its high content.

We observed that the bioavailability of leptin in BMSF is higher in nonosteoporotic subjects than in osteoporotic subjects, suggesting insufficient leptin action in the latter group, at least at the bone marrow level. It is of notice that a failure of leptin signaling has been recognized as underlying several other clinical states.[53] A decrease in leptin availability in osteoporotic bone marrow could represent to some extent the aforementioned decrease in the biologic activity of leptin detected on MSCs.[37,49] Reduced leptin bioavailability in the osteoporotic BMSF appears to result from both diminished leptin production and the preservation of sOB-R levels. Our present data suggest that this receptor, by its binding to the scarce leptin being produced, may disrupt effective leptin signaling in osteoporotic bone marrow. The mechanisms regulating sOB-R concentration are not clear, but several in vitro and experimental studies have suggested that leptin suppresses the expression of its own receptor.[54–57] Moreover, the administration of leptin has been shown to repress sOB-R levels in humans.[58] Also, sOB-R is low in subjects who are insulin resistant, and it has been concluded that abdominal fat may exert a suppressive effect on sOB-R levels.[57]

Concerning the anthropometric characteristics of the studied subjects, nonosteoporotic subjects had higher weight, BMI, fat mass, and percent of fat than osteoporotic subjects, although the latter group was far from thin; these observations match with several reports pointing out these types of differences.[7,59]

The preceding observations were corroborated by linear correlation analyses showing the expected relationships among T-score and both BMI and percent of fat mass, which has been interpreted as indicative of a protective effect of weight against osteoporosis.[20] In addition, T-score correlated with both leptin levels and the leptin/sOB-R ratio, both parameters directly reflecting leptin availability in the bone marrow environment, emphasizing the positive consequences of effective leptin action on bone. Leptin levels in the BMSF correlated positively with both BMI and percent of fat mass, probably reflecting the preponderance of adipose tissue as a source of the hormone. Finally, the significant correlation found between the levels of IL-6 and sIL-6R and between the levels of sOB-R and OPG, as well as the negative relationship between sIL-6R and the leptin/sOB-R ratio, suggest a potential regulatory relationship among these signals in the bone marrow.

In summary, the measurement of cytokine concentrations in BMSF showed a distinctive supply of these signaling molecules among nonosteoporotic and osteoporotic elderly women; the latter group was characterized by a higher content of proinflammatory and adipogenic cytokines. Also, decreased leptin bioavailability appears characteristic of osteoporotic BMSF, suggesting that insufficient leptin action may distinguish the osteoporotic bone marrow.

Disclosures

The authors state that they have no conflicts of interest.

Acknowledgments

References

1. Zipori D. The stem state: mesenchymal plasticity as a paradigm. Curr Stem Cell Res Ther. 2006;1:95–102.

2. Minguell JJ, Erices A, Conget P. Mesenchymal stem cells. Exp Biol Med. 2001;226:507–520.

3. Gass M, Dawson-Hughes B. Preventing osteoporosis-related fractures: an overview. Am J Med. 2006;119:3S–11S.

4. Wiig H, Berggreen E, Borge BA, Iversen PO. Demonstration of altered signaling responses in bone marrow extracellular fluid during increased hematopoiesis in rats using a centrifugation method. Am J Physiol Heart Circ Physiol. 2004;286:H2028–H2034.

5. Iversen PO, Wiig H. tumor necrosis factor α and adiponectin in bone marrow interstitial fluid from patients with acute myeloid leukemia inhibit normal hematopoiesis. Clin Cancer Res. 2005;11:6793–6799.

6. Lee WY, Kang MI, Oh ES, et al. The role of cytokines in the changes in bone turnover following bone marrow transplantation. Osteoporos Int. 2002;13:62–68.

7. Khosla S, Peterson JM, Egan K, Jones JD, Riggs BL. Circulating cytokine levels in osteoporotic and normal women. J Clin Endocrinol Metab. 1994;79:707–611.

8. Nuttall ME, Gimble JM. Controlling the balance between osteoblastogenesis and adipogenesis and the consequent therapeutic implications. Curr Opin Pharmacol. 2004;4:290–294.

9. Rosen CJ, Bouxsein ML. Mechanisms of disease: is osteoporosis the obesity of bone? Nat Clin Prac Rheumatol. 2006;2:35–43.

10. Rodríguez JP, Astudillo P, Ríos S, Pino AM. Involvement of adipogenic potential of human bone marrow mesenchymal stem cells (MSCs) in osteoporosis. Stem Cell Res Ther. 2008;3:208–218.

11. Pittenger MF, Mackay AM, Beck SC, et al. Multineage potential of adult human mesenchymal stem cells. Science. 1999;284:143–147.

12. Meunier P, Aaron J, Edouard C, Vignon G. Osteoporosis and the replacement of cell populations of the marrow by adipose tissue: a quantitative study of 84 iliac bone biopsies. Clin Orthop Relat Res. 1971;80:147–154.

13. Ogawa S, Urano T, Hosoi T, et al. Association of bone mineral density with a polymorphism of the peroxisome proliferator-activated receptor gamma gene: *PPARγ* expression in osteoblasts. Biochem Biophys Res Commun. 1999;260:122–126.

14. Bodine PV, Zhao W, Kharode YP, et al. The Wnt antagonist secreted frizzled related protein 1 is a negative regulator of trabecular bone formation in adult mice. Mol Endocrinol. 2004;18:1222–1237.

15. Bennett CN, Longo KA, Wright WS, et al. Regulation of osteoblastogenesis and bone mass by Wnt10b. Proc Natl Acad Sci USA. 2005; 102:3324–3329.

16. Schäffler A, Müller-Ladner U, Schölmerich J, Büchler C. Role of adipose tissue as an inflammatory organ in human diseases. Endocr Rev. 2006;27:449–467.

17. Yokota T, Meka CS, Medina KL, et al. Paracrine regulation of fat cell formation in bone marrow cultures via adiponectin and prostaglandins. J Clin Invest. 2002;109:1303–1310.

18. Richards JB, Valdes AM, Burling K, Perks UC, Spector TD. Serum adiponectin and bone mineral density in women. J Clin Endocrinol Metab. 2007;92:1517–1523.

19. Nishida M, Funahashi T, Shimomura I. Pathophysiological significance of adiponectin. Med Mol Morphol. 2007;40:55–67.

20. Thomas T, Burguera B. Is leptin the link between fat and bone mass? J Bone Miner Res. 2002;17:1563–1569.

21. Laharrague P, Truel N, Fontanilles AM, Corberand JX, Penicaud L, Casteilla L. Regulation by cytokines of leptin expression in human bone marrow adipocytes. Horm Metab Res. 2000;32:381–385.

22. Lammert A, Kiess W, Bottner A, Glasow A, Kratzsch J. Soluble leptin receptor represents the main leptin binding activity in human blood. Biochem Biophys Res Commun. 2001;283:982–988.

23. Sinha MK, Opentanova I, Ohannesian JP, et al. Evidence of free and bound leptin in human circulation: studies in lean and obese subjects and during short-term fasting. J Clin Invest. 1996;98: 1277–1282.

24. Bertolini DR, Nedwin GE, Bringman TS, Smith DD, Mundy GR. Stimulation of bone resorption and inhibition of bone formation in vitro by human tumour necrosis factors. Nature. 1986;319:516–518.

25. Ishimi Y, Miyaura C, Jin CH, et al. IL-6 is produced by osteoblasts and induces bone resorption. J Immunol. 1990;145:3297–3303.

26. Mundy GR. Osteoporosis and inflammation. Nutr Rev. 2007;65:S147–151.

27. Giuliani N, Sansoni P, Girasole G, et al. Serum interleukin-6, soluble interleukin-6 receptor and soluble gp130 exhibit different patterns of age- and menopause-related changes. Exp Gerontol. 2001;36:547–57.

28. Kearns AE, Khosla S, Kostenuik PJ. Receptor activator of nuclear factor κB ligand and osteoprotegerin regulation of bone remodeling in health and disease. Endocr Rev. 2008;29:155–192.

29. Mezquita-Raya P, de la Higuera M, Fernández García D, et al. The contribution of serum osteoprotegerin to bone mass and vertebral fractures in postmenopausal women. Osteoporos Int. 2005;16:1368–1374.

30. Schett G, Kiechl S, Redlich K, et al. Soluble RANKL and risk of nontraumatic fracture. JAMA. 2004;291:1108–1113.

31. Rodríguez JP, Garat S, Gajardo H, Pino AM, Seitz G. Abnormal osteogenesis in osteoporotic patients is reflected by altered mesenchymal stem cell dynamics. J Cell Biochem. 1999;75:414–423.

32. Raisz LG. The osteoporosis revolution. Ann Intern Med. 1997;126: 458–462.

33. Indridason OS, Franzson L, Sigurdsson G. Serum osteoprotegerin and its relationship with bone mineral density and markers of bone turnover. Osteoporos Int. 2005;16:417–423.

34. Glover SJ, Garnero P, Naylor K, Rogers A, Eastell R. Establishing a reference range for bone turnover markers in young, healthy women. Bone. 2008;42:623–630.

35. Tsuda E, Goto M, Mochizuki SI, et al. Isolation of a novel cytokine from human fibroblasts that specifically inhibits osteoclastogenesis. Biochem Biophys Res Commun. 1997;234:137–142.

36. Yasuda H, Shima N, Nakagawa N, 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;95:3597–3602.

37. Hess R, Pino AM, Ríos S, Fernández M, Rodríguez JP. High affinity leptin receptors are present in human mesenchymal stem cells (MSCs) derived from control and osteoporotic donors. J Cell Biochem. 2005;94:50–57.

38. Odabaşi E, Ozata M, Turan M, et al. Plasma leptin concentrations in postmenopausal women with osteoporosis. Eur J Endocrinol. 2000; 142:170–173.

39. Shaarawy M, Abassi AF, Hassan H, Salem ME. Relationship between serum leptin concentrations and bone mineral density as well as biochemical markers of bone turnover in women with postmenopausal osteoporosis. Fertil Steril. 2003;79:919–924.

40. Weiss LA, Barrett-Connor E, von Mühlen D, Clark P. Leptin predicts BMD and bone resorption in older women but not older men: the Rancho Bernardo Study. J Bone Miner Res. 2006;21:758–764.

41. Yano K, Tsuda N, Kobayashi F, et al. Immunological characterization of circulating osteoprotegerin/osteoclastogenesis inhibitory factor: increased serum concentrations in postmenopausal women with osteoporosis. J Bone Miner Res. 1999;14:518–527.

42. Riggs BL, Khosla S, Atkinson EJ, Dunstan CR, Melton LJ III. Evidence that type I osteoporosis results from enhanced responsiveness of bone to estrogen deficiency. Osteoporos Int. 2003; 14:728–733.

43. Abrahamsen B, Bopnnevie-Nielsen V, Nils Ebbesen E, Gram J, Beck-Nielsen H. Cytokines and bone loss in a 5-year longitudinal study-hormone replacement therapy suppresses serum soluble interleukin-6 receptor and increases interleukin-1-receptor antagonist: the Danish Osteoporosis Prevention Study. J Bone Miner Res. 2000;15:1545–1554.

44. Nakchbandi I, Mitnick MA, Lang R, Gundberg C, Kinder B, Insogna K. Circulating levels of interleukin-6 soluble receptor predict rates of bone loss in patients with primary hyperparathyroidism. J Clin Endocrinol Metab. 2002;87:4946–4951.

45. Arita Y, Kihara S, Ouchi N, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun. 1999;257:79–83.

46. Hotta K, Funahashi T, Arita Y, et al. Plasma concentration of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol. 2000;20:1595–1599.

47. Moerman EJ, Teng K, Lipschitz DA, Lecka-Czernik B. Aging activates adipogenic and suppresses osteogenic programs in mesenchymal marrow stroma/stem cells: the role of PPAR-γ2 transcription factor and TGF-β/BMP signaling pathways. Aging Cell. 2004;3:379–389.

48. Zayzafon M, Gathings WE, McDonald JM. Modeled microgravity inhibits osteogenic differentiation of human mesenchymal stem cells and increases adipogenesis. Endocrinology. 2004;145:2421–2432.

49. Astudillo P, Ríos S, Pastenes L, Pino AM, Rodríguez JP. Increased adipogenesis of osteoporotic human-msenchymal stem cells (MSCs) characterizes by impaired leptin action. J Cell Biochem. 2008;103: 1054–1065.

50. Hozumi A, Osaki M, Goto H, Sakamoto K, Inokuchi S, Shindo H. Bone marrow adipocytes support dexamethasone-induced osteoclast differentiation. Biochem Biophys Res Commun. 2009;382:780–784.

51. Holloway WR, Collier FM, Aitken CJ, et al. Leptin inhibits osteoclast generation. J Bone Miner Res. 2002;17:200–209.

52. Martin A, de Vittoris R, David V, et al. Leptin modulates both resorption and formation while preventing disuse-induced bone loss in tail-suspended female rats. Endocrinology. 2005;146:3652–3659.

53. Unger RH. Lipid overload and overflow: metabolic trauma and the metabolic syndrome. Trends Endocrinol Metab. 2003;14:398–403.

54. Uotani S, Bjorbaek C, Tornoe J, Flier JS. Functional properties of leptin receptor isoforms: internalization and degradation of leptin and ligand-induced receptor down regulation. Diabetes. 1999;48:279–286.

55. Wang ZW, Zhou YT, Kakuma T, et al. Comparing the hypothalamic and extrahypothalamic actions of endogenous hyperleptinemia. Proc Natl Acad Sci USA. 1999;96:10373–10378.

56. Martin RL, Perez E, He YJ, Dawson R Jr, Millard WJ. Leptin resistance is associated with hypothalamic leptin receptor mRNA and protein downregulation. Metabolism. 2000;49:1479–1484.

57. Sandhofer A, Laimer M, Ebenbichler CF, Kaser S, Paulweber B, Patsch JR. Soluble leptin receptor and soluble receptor-bound fraction of leptin in the metabolic syndrome. Obes Res. 2003;11:760–768.

58. Chan JL, Bluher S, Yiannakouris N, Suchard MA, Kratzsch J, Mantzoros CS. Regulation of circulating soluble leptin receptor levels by gender, adiposity, sex steroids, and leptin: observational and interventional studies in humans. Diabetes. 2002;51:2105–2112.

59. Jiang L-S, Zhang Z-M, Jiang S-D, Chen W-H, Dai L-Y. Differential bone metabolism between postmenopausal women with osteoarthritis and osteoporosis. J Bone Miner Res. 2008;23:475–483.