Circulating Leptin and Bone Mineral Density in Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** To evaluate the association between circulating leptin and bone mineral density (BMD) in patients with rheumatoid arthritis (RA).

Methods. One-hundred thirty postmenopausal women with RA were assessed for body mass index (BMI), disease characteristics, history of drug use, rheumatoid factor, and erythrocyte sedimentation rate (ESR). BMD (g/cm^2) was determined in the hip and spine by DEXA. Serum leptin concentrations were measured by ELISA. Spearman's correlation coefficients (rho) were determined between BMD and leptin and other variables. A multiple regression analysis was used to adjust for confounders.

Results. Patients' serum leptin levels varied widely (range 2–128 ng/ml). Thirty-three patients (25%) had osteoporosis. Higher levels of leptin correlated significantly with BMD in the lumbar spine (rho = 0.17, p = 0.04) and total hip (rho = 0.21, p = 0.01). The variables that were negatively correlated with BMD were age, duration of menopause, and ESR. After adjustment for confounders, leptin was no longer associated with BMD. In the multivariate model, factors that remained associated with BMD in the total hip were age (p = 0.021) and BMI (p = 0.003); and the factors that remained associated with BMD in the lumbar spine were BMI (p = 0.03) and ESR (p = 0.01).

Conclusion. No relevant association was found between circulating leptin levels and BMD in patients with RA in this cross-sectional study. Followup studies are needed to evaluate whether abnormal leptin levels confer a risk for fractures due to osteoporosis. (First Release Jan 15 2009; J Rheumatol 2009;36:512–6; doi:10.3899/jrheum.080196)

Key Indexing Terms:
LEPTIN RHEUMATOID ARTHRITIS OSTEOPOROSIS BONE MINERAL DENSITY

Osteoporosis is a well recognized complication in patients with rheumatoid arthritis (RA)[1]. The low bone mineral density (BMD) in these patients is accompanied by a high risk of fracture[2], leading to increased healthcare costs, and high rates of disability and mortality. Multiple factors are implicated in the bone mass loss in these patients[3]. Recent evidence from studies performed mainly in patients with primary osteoporosis suggests that some adipokines may have a significant influence on bone remodeling[4]. Of these adipokines, leptin is a major candidate for the protective effects of adipose tissue on bone mass[5]. Leptin is an adipocyte-derived hormone that is essential for adequate regulation of body weight[6]. Multiple functions of leptin have been discovered, including effects on the immune sys-

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tem[7-10]. Receptors for leptin are located in polymorphonuclear leukocytes, monocytes, macrophages, and lymphocytes[8-10]. Leptin can increase the activation of T cells, macrophages, and neutrophils and induce the release of tumor necrosis factor-α (TNF-α) and interleukin 6 (IL-6)[9-11]. In vitro studies have shown that leptin can inhibit osteoclast generation[12] and enhance the proliferation, differentiation, and mineralization of osteoblasts[13,14]. These effects induced by leptin in bone may influence changes in BMD in patients where the leptin levels are abnormal. It remains unknown whether the effect of leptin on the BMD in patients with RA is clinically significant.

Studies in patients with RA have identified important variations in serum leptin concentrations[15-19], but the significance of these differences for the BMD is poorly understood. To date, only 3 studies have evaluated the association between leptin and BMD in RA patients and the results were inconsistent[20-22]. These differences could be explained by an inappropriate control of confounders. Because serum leptin and BMD are influenced by a number of conditions including gender, body mass index (BMI), menopause, and medications, among others, it is important to evaluate the effect of leptin in BMD using an adequate control for these variables. We designed a study to evaluate the association between leptin and BMD in RA patients, controlling for confounders in a multivariate analysis.

MATERIALS AND METHODS

Patients. From March 2005 to March 2007, 130 women diagnosed with RA according to the 1987 American College of Rheumatology (ACR) criteria[23] were included. All patients were ≥ 40 years of age at study entry. Participants were selected from an outpatient rheumatology clinic in a secondary-care center (Hospital General Regional 110, IMSS, Guadalajara, Mexico). Exclusion criteria included diabetes mellitus, hypertension, thyroid disease, chronic renal failure, overlapping syndrome, active infections, pregnancy, treatment with prednisone ≥ 15 mg/day, and antiresorptive or anabolic bone therapy.

Clinical assessment. Two investigators assessed the clinical characteristics including those related to RA and its treatment. Evaluation included counts of joint tenderness and joint swelling (0–28 scale). Visual analog scales (100 mm) were used to assess morning stiffness and the patient and physician perceptions of disease activity. Functioning was evaluated using the Spanish modified version of Health Assessment Questionnaire-Damage Index[24]. Global functional status was assessed according to the 1992 revised criteria of the ACR[25].

Leptin determination. Blood samples were taken from a peripheral vein after an overnight fast. All samples were centrifuged immediately after being drawn and stored at –20°C until measurement. The maximum sample storage was 4 months. The measurement of serum leptin levels (ng/ml) was carried out using an ELISA technique with a commercial kit (R&D Systems). One researcher, blinded to the clinical variables, performed the measurement of leptin levels. The coefficient of intra-plate variability for leptin in our laboratory was 0.7%.

Additional laboratory measurements. Rheumatoid factor (RF) and C-reactive protein (CRP) were measured by nephelometry using commercial kits. Erythrocyte sedimentation rate (ESR, mm/h) was measured using the Wintrobe method.

Bone mineral density. BMD (g/cm^2) was measured by central dual-energy x-ray absorptiometry (DEXA; GE Lunar Prodigy densitometer, GE Medical Systems; software version 8.8) at the lumbar spine in the posterior-anterior projection (L1–L4) and total hip. The coefficient of variation during measurement of a standard phantom in our laboratory was 0.7%. According to the BMD results patients were classified into one of the following categories proposed by the World Health Organization: (1) normal BMD, with T score > –1 standard deviation (SD); (2) osteopenia, with T score between –1 and –2.4 SD; and (3) osteoporosis, with T score ≤ –2.5 SD[26].

Statistical analyses. Considering that leptin levels are abnormally distributed, we used medians and ranges to describe the data distribution. Kruskal-Wallis test was used for comparisons of quantitative variables between the groups of patients with osteoporosis, osteopenia, and normal BMD. Spearman's correlation coefficient (rho) was used to evaluate the strength of association between BMD and other clinical variables. A multiple regression analysis (stepwise method) was used to adjust for confounders. In the final model, the dependent variable was the BMD in total hip and lumbar spine, and the covariates were those with significance < 0.1 in the univariate analysis. Statistical significance was considered as $p \leq 0.05$. All statistical analyses were performed using SPSS, version 8.0.

The hospital ethics committee approved the study (no. 2005-1303-082). All patients were informed about the study objectives and signed a voluntary consent prior to inclusion. The study was performed following the guidelines of the Declaration of Helsinki.

RESULTS

One-hundred eighty patients with RA were tested for eligibility. Of these, 45 were excluded by the following causes: under-age = 15, comorbid disease = 18, treatment with antiresorptive therapy = 10, and diagnosis of overlapping syndrome = 2. From 135 patients who were potentially eligible, 5 refused to participate. The results from 130 women with RA were evaluated in the final analysis. The median age of these patients was 52 years and they had a median serum leptin concentration of 22.6 ng/ml (range 2–128 ng/ml). The median duration of RA was 8 years (range 1–40 yrs). After the bone densitometry, 25% were diagnosed with osteoporosis and 38% with osteopenia; the remaining women had normal BMD. Table 1 compares the clinical characteristics between the groups according to the BMD results. Patients with osteoporosis were older ($p < 0.001$) and had longer time since menopause ($p < 0.001$) and lower functional status ($p = 0.01$). A trend was observed for longer duration of RA in patients with osteoporosis ($p = 0.08$). Other variables including joint swelling or joint tenderness count or prednisone doses did not differ significantly between groups. Leptin levels did not differ among patients with osteoporosis, osteopenia, and normal BMD.

At the time of the study all patients were receiving disease controlling antirheumatic therapy (DC-ART): 101 (78%) received methotrexate, 50 (39%) chloroquine, 49 (37%) sulfasalazine, 14 (11%) azathioprine, and 2 (1.5%) D-penicillamine. Eighty patients (62%) received combined therapy with DC-ART. Only 9 patients received anti-TNF agents (6 etanercept and 3 infliximab). In a subanalysis, the use of DC-ART or anti-TNF agents was not associated with a difference in BMD of the lumbar spine or total hip, nor with differences in leptin concentrations. Sixty-five patients

Table 1. Comparison of patients' characteristics according to bone mineral density (BMD) results.

Characteristic	Total Patients, n = 130	Normal BMD, n = 47	Osteopenia, n = 50	Osteoporosis, n = 33	p*
Age, yrs median (range)	52 (40–75)	48 (41–72)	53 (40–73)	61 (41–75)	< 0.001
Body mass index, kg/m^2 median (range)	27 (17–47)	28 (17–39)	27 (18–47)	26 (17–39)	0.10
Duration since menopause, yrs median (range)	5 (0–44)	1 (0–23)	7 (0–41)	11 (0–44)	< 0.001
Duration of RA, yrs median (range)	8 (1–40)	6.5 (1–35)	6 (2–35)	11 (1–40)	0.08
Global functional status III-IV, n (%)	24 (19)	7 (15)	8 (16)	9 (27)	0.01
HAQ-DI score, median (range)	0.59 (0–2.3)	0.5 (0–2.3)	0.4 (0–2.2)	0.6 (0.2–2.5)	0.10
Joint tenderness count, median (range)	6 (0–40)	6 (0–40)	5 (0–40)	6 (0–30)	0.50
Joint swelling count, median (range)	3 (0–20)	4 (0–20)	2 (0–18)	2 (0–16)	0.20
Prednisone doses, mg/day median (range)	2.5 (0–12.5)	0 (0–12.5)	0 (0–12.5)	5 (0–10)	0.80
Serum leptin, ng/ml median (range)	23 (2–128)	26 (2–82)	24 (5–128)	17 (4–77)	0.10

* Comparisons between quantitative variables were by Kruskal-Wallis test. HAQ-DI: Health Assessment Questionnaire-Damage Index.

(51%) used prednisone; use of prednisone was associated with lower BMD of the total hip (p = 0.006) but not with lower BMD of the lumbar spine or leptin levels (data not shown).

Table 2 shows the correlations between BMD and clinical variables. An inverse correlation was found between BMD of the lumbar spine and age (rho = –0.48, p < 0.001) and years since menopause (rho = –0.47, p < 0.001). On the other hand, an increment in the BMD of the lumbar spine was correlated with higher BMI (rho = 0.17, p = 0.05), joint swelling count (rho = 0.17, p = 0.05), ESR (rho = 0.24, p = 0.01), and serum leptin levels (rho = 0.17, p = 0.04). BMD of the total hip was inversely correlated with age (rho = –0.44, p < 0.001), duration of menopause (rho = –0.40, p < 0.001), and functional status (rho = –0.21, p = 0.01). An increment of BMD in the total hip was correlated with higher BMI (rho = 0.33, p < 0.001), longer disease duration (rho = 0.19, p = 0.03), RF titers (rho = 0.23, p = 0.02), ESR (rho = 0.21, p = 0.01), and serum leptin levels (rho = 0.21, p = 0.01). A weak but significant correlation was observed between leptin levels and BMI (rho = 0.47, p < 0.001).

Table 3 shows the factors associated with BMD in the multivariate analysis. After adjustment with other covariates, the factors associated with BMD in the total hip in the final model were age (p = 0.021) and BMI (p = 0.003). BMD in the lumbar spine was associated with BMI (p = 0.032) and ESR (p = 0.011), and had a borderline significant correlation with age (p = 0.07). Leptin was not associated with BMD after adjustment for confounders.

DISCUSSION

Our results showed that although leptin concentrations were weakly correlated with BMD in the bivariate analysis, this association did not remain significant after adjustment for confounding factors. On the other hand, BMI was one of the main determinants for BMD in the multivariate model, independent of age or leptin levels.

This finding is interesting because leptin has been con-

Table 2. Correlation between bone mineral density (BMD; g/cm^2) and selected characteristics.

Characteristic	Bone Mineral Density: Lumbar Spine rho	Lumbar Spine p	Total Hip rho	Total Hip p
Age, yrs	–0.48	< 0.001	–0.44	< 0.001
Body mass index, kg/m^2	0.17	0.05	0.33	< 0.001
Years since menopause	–0.47	< 0.001	–0.40	< 0.001*
Duration of RA, yrs	–0.12	0.10	0.19	0.03*
Global functional status (range I-IV)	–0.06	0.40	–0.21	0.01*
HAQ-DI score (range 0–3)	0.00	0.9	–0.12	0.10
Joint tenderness count (range 0–28)	0.12	0.10	0.01	0.80
Joint swelling count (range 0–28)	0.17	0.05*	0.01	0.80
Prednisone, mg/day	–0.06	0.40	–0.17	0.05
Rheumatoid factor, IU/ml	–0.10	0.30	0.23	0.02*
C-reactive protein, mg/l	0.13	0.20	0.04	0.70
Erythrocyte sedimentation rate, mm/h	0.24	0.01*	0.21	0.01*
Serum leptin, ng/ml	0.17*	0.04*	0.21*	0.01*

Correlations were computed using Spearman's correlation test (rho).

Table 3. Determinants of bone mineral density (BMD) in the adjusted analysis.

Variable	Total Hip			Lumbar Spine		
	ß Coefficient	Partial r^2	p	ß Coefficient	Partial r^2	p
Age, yrs	–0.00801	0.158	0.021	–0.00604	0.209	0.071
Body mass index, kg/m^2	0.01118	0.108	0.003	0.00776	0.043	0.032
Years since menopause	–0.000980	0.001	0.745	–0.00407	0.008	0.171
Duration of rheumatoid arthritis, yrs	0.001234	0.003	0.553	–0.000326	0.001	0.861
Joint tenderness count	0.001994	0.004	0.380	0.0007992	0.006	0.717
HAQ-DI	–0.0172	0.006	0.579	0.02498	0.001	0.408
Prednisone doses, mg	–0.00643	0.019	0.169	–0.00189	0.004	0.676
Leptin levels, ng/ml	0.0005161	0.002	0.568	–0.000582	0.003	0.508
Erythrocyte sedimentation rate, mm/h	–0.00121	0.007	0.332	–0.00310	0.045	0.011

Significance obtained by multiple regression analysis using the BMD (g/cm^2) for each site as dependent variable.

sidered a major factor to explain the protective effect of adipose tissue on the BMD of normal individuals[5]. Experimental studies have reported a direct effect of leptin on bone metabolism mediated by modulating osteoblast recruitment, differentiation, and bone remodeling[14]. Nevertheless, the information to support this hypothesis is inconsistent, even in nonrheumatic patients. Blain, *et al* observed that leptin levels can explain around 7% of the whole-body BMD and 3.7% of femoral neck BMD variances in postmenopausal women without arthritis, whereas it seems to be irrelevant for the BMD in lumbar spine[27]. On the other hand, Pasco, *et al* observed only a weak association in their multivariate analysis between leptin concentrations and BMD exclusively in the lateral lumbar spine in premenopausal and postmenopausal nonobese women[28].

In RA, the effects of leptin are also more complex and may influence other pathways that affect bone mass. Leptin is an adipokine with proinflammatory effects that enhances the activation of monocytes, macrophages, and T cells, and induces the production of cytokines including TNF-α and IL-6[11,28-30]. These cytokines can increase the bone remodeling leading to osteoporosis in patients with RA. Therefore, leptin could increase bone mineral formation or bone resorption in patients with RA depending on the circumstances, but not directly related to the serum concentrations of this hormone. On the other hand, disease activity can contribute to osteoporosis and can be a confounder in the relationship between leptin and BMD. In this regard, we found a weak but significant correlation between the lumbar spine BMD and the joint swelling count, although this relationship was not observed with any other measure of disease activity.

To date, there are a limited number of studies evaluating the relationship between leptin and BMD in patients with RA[20-22]. Toussirot, *et al* evaluated 38 corticosteroid-treated patients with RA, and found no correlation between leptin levels and BMD[20]. Wislowska, *et al* also found no correlation between leptin and BMD in RA comparing 30 women with RA to 30 women with osteoarthritis[21]. Canhao, *et al* found a paradoxical association between low levels of leptin and higher BMD in a study of 32 women with RA, all of whom had osteoporosis and active disease[22]. These studies had several limitations in their aim to establish definitive conclusions about whether serum leptin is associated with BMD. One of these limitations was related to differences in the inclusion criteria among these studies, which limit comparisons between them. Additionally, all these studies had a small sample size that affected their statistical power, and none of the studies performed an adjusted analysis; thus there is uncertainty as to whether confounders may have biased their results.

In comparison with the previous reports, our study offers several advantages including a larger sample and the inclusion of only postmenopausal women in order to avoid the confounders of sex and hormonal status. The main advantage of our study was the use of a multivariate analysis to control for confounders that may influence BMD and lead to misinterpretation of the relevance of serum leptin in BMD. Based on this multivariate analysis, our results do not support a major role for serum leptin in the bone density in patients with RA. A major effect was observed for age and BMI on the BMD in these patients. These results strongly suggest that some effects of BMI on bone density might be attributed by other factors independent of leptin.

Our study has several limitations, including its cross-sectional design, making it unable to determine whether leptin levels influence future changes in BMD (positively or negatively). Therefore, followup studies to evaluate if variations in leptin levels can precede the changes in BMD in patients with RA are still required. Leptin has a complex effect on bone, promoting the bone mineralization mediated by direct anabolic effects within the bone microenvironment, or instead, decreasing the mineralization under other circumstances, mediated by interaction of hypothalamic neuropeptides. In RA the influence of leptin on cytokine production can contribute to a more complex influence in bone.

Our results showed a significant correlation between leptin and BMD in the bivariate analysis. However, after adjustment for confounders, this association did not remain significant. These data suggest that serum leptin concentrations

have only a small role in osteoporosis in patients with established RA, and other factors may be more strongly associated. Nevertheless, our data do not exclude leptin as a mediator of bone response in RA. Because this was a cross-sectional study, our results do not provide predictive data for future consequences in patients with low or high levels of this hormone. Further followup studies are required to evaluate a possible link between abnormalities in serum leptin concentrations and future osteoporotic fractures.

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