Turkish Journal of Endocrinology and Metabolism, (2002) 4 : 141-144 **ORIGINAL ARTICLE**

Relationships Between Plasma Leptin and Bone Turnover During Short Term Weight Reduction in Obese Premenopausal Women

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The goal of this study is to assess the relationship between leptin and bone metabolism and to elucidate whether short term weight reduction influences this relationship. Twenty obese premenopausal women with a mean age of 32.8 years and a mean body mass index (BMI) of 32.8 kg/m ^{2} were followed for 4.25 (3-5.5) months. The patients followed a moderate energy restricted diet and regular exercise for weight reduction. BMI, plasma leptin, bone specific alkaline phosphatase (B-ALP), osteocalcin (OC), type 1 collagen (PICP) and urine deoxypyridinoline (DPD) levels were measured at baseline and at follow-up visit. Bone mineral density (BMD) measurements were done with dual energy X-ray absorptiometry. At the end of the study, the mean BMI decreased significantly to 31.2 kg/m ^{2} (p=0.006) and serum leptin levels had a tendency to decrease (p=0.168). B-ALP, OC, PICP levels, urine DPD levels and BMD of femur, lumbar vertebrae and radius did not change significantly at the end of the study. There was no relationship between leptin levels and BMI, markers of bone formation and resorption and BMD at baseline. Despite a reduction in BMI, changes in leptin did not correlate with any bone turnover marker. Our results suggest that leptin has no association with bone metabolism and bone density in obese premenopausal women.

Key words: **Leptin, obesity, premenopausal women, bone mineral density, bone turnover**

Introduction

Leptin is a 16 kDa polypeptide hormone encoded by the ob gene which is synthesized and secreted mostly by adipocytes (1-3). In addition to its role in food intake and energy expenditure (4), it participates in the modulation of reproductive (5), hematopoietic (6) and immune systems (7). It also has a role in angiogenesis (8), brain development (9) and carbohydrate metabolism (10,11).

Both serum leptin levels (12,13) and bone mass (14) are positively correlated with body fat content. The protective effect of obesity on bone has been ascribed to the mechanical loading on bone, or the presence of a mediator between bone and adipose tissue. In vitro studies have demonstrated a direct relationship between leptin and human marrow stromal cells, stimulating osteoblast differentiation (15). In a recent study, Ogueh et al. (16), have documented that leptin has an inhibitory effect on bone resorption in human fetus. However, Goulding et al. (12) have reported that there was no association between circulating plasma leptin and bone mineral content, nor was there a correlation between leptin and metabolic bone markers in postmenopausal women. In a recent study by Pasco et al. (17), it has been documented that serum leptin correlates positively with bone mineral content in nonobese women regardless of the menopausal status.

To understand further the effect of leptin on bone mass, we investigated the relationship between leptin bone mineral density, and metabolic bone markers.

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We also sought to elucidate whether weight reduction influences this relationship in premenopausal period.

Materials and Methods

Twenty obese premenopausal women aged between 17-43 years with a mean body mass index (BMI) of 32.8±3.1 kg/m^2 were recruited at the outpatient clinic. Obesity was defined as BMI greater than 27 kg/m^2. Subjects who had medical conditions that might affect bone metabolism were excluded from the study. Subjects previously treated with glucocorticoids, oral contraceptive pills, bisphosphonates and calcitonin were also excluded. None of the women were on a low-calorie diet or regular exercise at baseline. The subjects had regular menses. Height, weight, waist to hip ratio (WHR), blood pressure after 10 minutes rest and pulse rate were measured at baseline. BMI was calculated as weight per square of height in meters (kg/m^2). Informed consent was obtained from each subject at study entry.

Blood samples were obtained from each subject after an overnight fast, and all subjects collected a 24-hour urine sample. Leptin, bone specific alkaline phosphatase (B-ALP), osteocalcin (OC), procollagen type 1 C-terminal propeptide (PICP) were examined in blood samples, whereas creatinine and deoxypyridinoline (DPD) were examined in urine samples. B-ALP, OC and PICP were considered as bone formation markers and DPD was used as a bone resorption marker. Plasma leptin was measured by immunoradiometric assay; markers of bone turnover in serum were measured by enzyme-linked immunosorbent assay, and urine DPD was measured by chemiluminescent enzyme immunoassay. The normal ranges for B-ALP, OC and PICP were 11.6-30.6 U/L, 3.7-10.0 ng/ml and 69-147.0 ng/ml respectively. The normal range of DPD excretion as expressed per mmol of creatinine was 3.0-7.4 nmol. All subjects had bone mineral density (BMD) measurements at femur, spine and radius by dual energy X-ray absorptiometry (Hologic QDR –4500A, Bedford, MA).

All subjects followed a moderate energy restricted diet (1200 kcal/day) and were advised to do aerobic exercise at least three times weekly for a minimum of 20 minutes. After 3-5.5 months; height, weight and WHR were measured and BMI was calculated. Also, serum B-ALP, OC, PICP, urine DPD and BMD of femur, spine and radius were measured at the end of the study.

BMI, WHR, serum leptin, B-ALP, OC, PICP, urine DPD and BMD of femur, spine and radius at baseline and at the end of the study were compared by Wilcoxon Signed Ranks Test due to non-normal distribution of the data. Relationships of serum leptin levels and the changes in serum leptin levels with BMI, WHR, bone formation and resorption markers and BMD of femur, spine and radius were examined by Spearman rank correlation test. Statistical significance was set at $p<0.05$. All parameters were given as mean±SD. Data analysis was performed using the Statistical Package for Social Sciences (SPSS, Inc., Chicago, IL) for Windows Release 10.0.

Results

Clinical characteristics, bone turnover markers and BMD of femur, spine and radius of the subjects at baseline and at the end of the study are shown in Table 1. The changes in BMD and bone turnover markers did not reach a statistical level of significance at the end of the study (Table 1). The mean BMI decreased significantly from 32.8±3.1 kg/m^2 to 31.2±3.5 kg/m^2 ($p=0.006$). WHR did not change significantly at the end of the study ($p=0.196$). Despite a reduction in serum leptin levels (baseline: 39.3 ng/ml, at the end: 32.1 ng/ml), the difference did not reach a statistical level of significance ($p=0.168$) (Table 1). Leptin was not correlated

Table 1. Characteristics of the subjects at baseline and at the end of the study

Variable	Baseline	At the end	p value
Leptin (ng/ml)	39.3±19.9	32.1±13.0	0.168
BMI (kg/m^2)	32.8±3.1	31.2±3.5	0.006
WHR	0.8±0.06	0.8±0.05	0.196
B-ALP (U/L)	34.0±23.1	27.1±16.9	0.594
OC (ng/ml)	2.7±1.9	3.2±1.6	0.182
PICP (ng/ml)	111.2±55.5	90.0±27.9	0.071
Urine DPD (nmol)	11.0±9.9	12.1±5.2	0.101
BMD (g/cm^2)			
Femur total	1.0±0.1	1.0±0.2	0.213
Femur Ward's	0.8±0.1	0.8±0.1	0.515
Femur neck	0.9±0.1	0.8±0.1	0.953
Femur trochanter	0.7±0.1	0.7±0.1	0.510
Lumber spine	1.1±0.09	1.1±0.2	0.953
Radius	0.6±0.04	0.6±0.05	0.514

Data are mean±SD

with BMI, WHR, bone turnover markers and BMD of any skeletal site at baseline (Table 2). We found no significant correlations between the changes in leptin, and the changes in BMI and markers of bone turnover during the study (Table 3).

Table 2. Correlations of baseline features of the subjects with baseline leptin

Variable	r *(with leptin)	p value
BMI (kg/m^2)	0.095	0.736
WHR	-0.039	0.889
B-ALP (U/L)	0.073	0.805
OC (ng/ml)	0.176	0.547
PICP (ng/ml)	0.129	0.648
Urine DPD (nmol)	0.482	0.069
Femur BMD (g/cm^2)	-0.206	0.499
Spine BMD (g/cm^2)	-0.329	0.297
Radius BMD (g/cm^2)	-0.246	0.376

(*): Spearman correlation coefficient

Table 3. Correlations between the changes in leptin and the changes in body mass index and bone turnover markers

Variable	r*	p
Δ BMI (kg/m^2)	-0.272	0.418
Δ Type 1 collagen (ng/ml)	0.564	0.071
Δ Osteocalcin (ng/ml)	-0.091	0.790
Δ B-ALP (U/L)	0.018	0.958
Δ DPD (nmol)	0.145	0.570

Δ shows the changes in variables by time
(*): Spearman correlation coefficient

Discussion

In this study, we evaluated leptin and bone metabolism in obese premenopausal women. Leptin was neither correlated with bone turnover markers nor with BMD of femur, spine and radius at baseline. These results suggest that leptin may not be implicated in the well-known protective effect of obesity against bone loss. After significant weight loss at the end of the study, there was no correlation between the change in leptin levels and the change in bone turnover markers. The mean BMI reduced from 32.8 kg/m^2 to 31.2 kg/m^2 in our patients. Although this reduction was statistically significant at p=0.006, BMI was still in the obese range at the end, however. This fact might have influenced our results in terms of the association between leptin and bone metabolism.

In recent years, many reports have indicated that osteoporotic fractures are less frequent in obese subjects (14,18). The protective effect of obesity on bone may be due to high body fat content and mechanical loading of obesity on bone. Both bone mass and plasma leptin levels are positively correlated with body fat (12-14). So leptin may be a mediator between bone and adipose tissue and may play a role in the association between body fat and bone mass. In a recent study by Ogueh et al. (16), it has been documented that leptin has an inhibitory effect on bone resorption in the human fetus and Thomas et al. (15) have reported that leptin acts on human marrow stromal cells to enhance differentiation into osteoblasts. It was documented by Pasco et al. (17) that serum leptin levels are positively associated with bone mineral content in nonobese women regardless of the menopausal status. These findings suggest that there is an association between leptin and bone metabolism. However, the same findings can not be documented in postmenopausal women with osteoporosis. Goulding et al. (12) have not found any relationship between BMD, bone turnover markers and leptin concentration in postmenopausal women. Rauch et al. (13) have investigated the effect of leptin on bone after skeletal growth has stopped. They documented that leptin has less influence on the mature than the growing skeleton (13). In accordance with this finding, we also found no correlation between leptin and bone metabolism in obese premenopausal women.

There are several studies regarding the change in leptin levels during energy restriction and weight loss. Wadden et al. (19) reported that leptin levels decrease in response to short-term reductions in energy intake, as well as to long-term decreases in fat stores. Leptin reduction was greater with higher reduction of energy intake and body fat. Accordingly, leptin levels tended to decrease with weight reduction in our patients. Ricci et al. (20) have recently reported that moderate energy restriction increases bone resorption and decreases leptin levels significantly in obese postmenopausal women. Increased bone resorption might be due to changes in hormonal status after the menopause. Estrone is the most abundant estrogen in postmenopausal women and is protective against bone loss. Concentrations of estrone are greater in obese than in lean postmenopausal women. With weight reduction estrone levels may decrease and bone turnover may increase. In the study by Ricci et al. (20), there was a 40% decrease in leptin and a 19%

decrease in fat mass, whereas leptin reduction was not associated with any of the bone indices. To exclude the implication of postmenopausal hormonal changes, we studied the effect of weight reduction on leptin levels, bone turnover markers and BMD's in the premenopausal period. To the best of our knowledge, this is the first study investigating the relationship between leptin and bone turnover in obese premenopausal women. We could not demonstrate any association between the changes in leptin and the changes in bone turnover markers during short-term weight reduction in the group studied. Therefore, it seems plausible that leptin may not play an important role in the regulation of bone metabolism. However, controlled studies with greater sample size and longer follow-up might be useful to further elucidate the relationship between leptin and bone metabolism in the long term.

In summary, our results suggest that leptin levels are not associated with bone turnover markers and BMD in obese premenopausal women. During short term weight reduction, changes in leptin levels are not associated with changes in bone turnover markers.

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