

## CLINICAL STUDY

## Plasma leptin concentrations in postmenopausal women with osteoporosis

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### Abstract

**Background:** The obese are usually protected against osteoporosis and have increased bone mineral density and plasma leptin concentrations. A recent *in vitro* study demonstrated that leptin acts on human marrow stromal cells to enhance differentiation to osteoblasts, suggesting an influence of leptin on bone mass. However, little is known about the relationship between plasma leptin and bone mass in postmenopausal women with osteoporosis.

**Objective:** To investigate plasma leptin concentrations in postmenopausal women with osteoporosis to improve the understanding of the role of leptin in determining bone mass.

**Methods:** Fifty postmenopausal women with osteoporosis (ages  $61.18 \pm 6.51$  years; body mass index (BMI)  $28.91 \pm 3.44$  kg/m<sup>2</sup>, mean  $\pm$  s.d.) and 30 age- and BMI-matched healthy postmenopausal women were included in the study. Bone mineral densities (BMD) were measured by dual energy X-ray absorptiometry. Plasma leptin concentrations were determined using an immunoradiometric assay.

**Results:** The median spine BMD value in the patient group ( $0.695 \pm 8.26$  g/cm<sup>2</sup>, median  $\pm$  s.e.m.) was significantly lower than that in the control group ( $1.006 \pm 1.29$  g/cm<sup>2</sup>, median  $\pm$  s.e.m.;  $z = -7.454$ ,  $P < 0.001$ ). The median plasma leptin concentration in the patient group ( $18.70 \pm 1.78$  ng/ml, median  $\pm$  s.e.m.) was not significantly different from that in the control group ( $22.35 \pm 2.20$  ng/ml, median  $\pm$  s.e.m.;  $z = -1.630$ ,  $P = 0.103$ ). Plasma leptin concentrations were correlated with BMI in both groups ( $r_s = 0.394$ ,  $P = 0.031$  in controls and  $r_s = 0.404$ ,  $P = 0.004$  in the patient group). There was no correlation between plasma leptin concentrations and BMD values in controls ( $r_s = -0.107$ ,  $P = 0.575$ ) but a weak correlation was observed in the patient group ( $r_s = 0.285$ ,  $P = 0.045$ ).

**Conclusion:** Our data suggest that circulating plasma leptin does not have a significant direct influence on bone mass in postmenopausal women.

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### Introduction

Leptin, the hormonal product of the *OB* gene, plays an important part in the regulation of food intake, energy expenditure, and body weight (1). The leptin gene is expressed in adipose tissue, gastric epithelium and placenta (2–4). Plasma leptin concentrations correlate with body fat content; they are increased in obesity and decreased in anorexia nervosa (4, 5). Moreover, it has recently been shown that, in addition to its effects on food intake and energy expenditure, leptin influences the secretion of follicle-stimulating hormone, luteinizing hormone, adrenocorticotrophic hormone, cortisol and growth hormone (6–9).

The obese are usually protected against osteoporosis and have increased bone mineral density (10). This has been attributed to the mechanical effects of their

excessive weight on bone tissue. Obese postmenopausal women have a tendency to have increased bone mineral density compared with lean women (11–13). A recent *in vitro* study demonstrated that leptin acts on human marrow stromal cells to enhance differentiation to osteoblasts, suggesting a role of leptin in bone metabolism (14). These findings have prompted speculations on the possible role of leptin in the protective effect of obesity on bone. Moreover, there are few reports on the relationship between bone mineral density and plasma leptin concentrations; in those studies, no significant relationship between circulating plasma leptin and bone mass was reported (15, 16). To understand further the influence of leptin on bone mass, we have investigated the relationship between plasma leptin and bone mineral density in postmenopausal women with osteoporosis.

## Patients and methods

Fifty postmenopausal women with osteoporosis (ages (mean  $\pm$  s.d.)  $61.18 \pm 6.51$  years, range 50–76 years; body mass index (BMI) (mean  $\pm$  s.d.)  $28.91 \pm 3.44$  kg/m<sup>2</sup>, range 21.87–37.19 kg/m<sup>2</sup>) and 30 age- and BMI-matched healthy postmenopausal women (ages (mean  $\pm$  s.d.)  $58.30 \pm 6.02$  years, range 50–71 years; BMI (mean  $\pm$  s.d.)  $29.46 \pm 2.79$  kg/m<sup>2</sup>, range 25.64–36.25 kg/m<sup>2</sup>) were included in the study.

Patients who had vertebral fractures, surgical menopause, secondary osteoporosis or other medical conditions that may affect the skeleton or metabolism were excluded from the study. Patients treated previously with bisphosphonates, calcitonin, anabolic steroids or hormone replacement therapy at any time since menopause were also excluded. Patients with vertebral fractures were excluded from the study, to achieve homogeneity.

The diagnosis of osteoporosis was based on spine bone mineral density (BMD) measurements. Patients with spine BMD 2.5 standard deviations below a reference range established using our own data obtained from a Turkish population of normal healthy women using dual energy X-ray absorptiometry (*T* score less than  $-2.5$ ) were accepted as having osteoporosis. BMI was defined as weight in kilograms divided by (height in meters)<sup>2</sup>. BMD (g/cm<sup>2</sup>) was determined at the level of the second to fourth lumbar vertebrae (spine) by dual energy X-ray absorptiometry (Norland XR 36-WBL, Fort Atkinson, WI, USA). Plasma leptin concentrations were determined by using an immunoradiometric assay (Active Human Leptin IRMA, DSL-23100, Diagnostic System Laboratories Inc., Webster, TX, USA).

After overnight fasting, blood samples were collected in ethylenediamine tetra-acetate-coated venepuncture tubes. All samples were promptly centrifuged, separated and stored at  $-70^{\circ}\text{C}$  until required for the leptin assay. All plasma samples were run in the same assay.

All participants gave their informed consent to take part in the study, which was approved by the local ethics committee of Gülhane School of Medicine.

## Statistical analysis

The data for time since menopause, leptin, and BMD measurements were not distributed normally, therefore

the Mann–Whitney *U*-test was used for comparisons. The data for age, and BMI were distributed normally, and an unpaired *t*-test was used for comparisons. Spearman correlations were used to explore correlations between the variables. All statistical calculations were performed using a Windows-compatible statistical package. *P* values less than 0.05 were accepted as significant. Values are given as median  $\pm$  s.e.m. for non-normally distributed data and as mean  $\pm$  s.d. for normally distributed data.

## Results

Clinical and laboratory data are given in Table 1. Time since menopause was not significantly different between the two groups ((median  $\pm$  s.e.m.)  $14.00 \pm 1.01$  years, range 3–29 years for patients and  $12.00 \pm 1.43$  years, range 2–27 years for controls;  $z = -0.607$ ,  $P = 0.544$ ). Plasma leptin concentration in the patient group was not significantly different from that in the control group ( $18.70 \pm 1.78$  ng/ml and  $22.35 \pm 2.20$  ng/ml, respectively;  $z = -1.630$ ,  $P = 0.103$ ). Plasma leptin concentrations were correlated with BMI in both control and patient groups ( $r_s = 0.394$ ,  $P = 0.031$  and  $r_s = 0.404$ ,  $P = 0.004$ , respectively; Fig. 1). No correlation was observed between plasma leptin and BMD in controls, but there was a weak correlation in the patient group ( $r_s = -0.107$ ,  $P = 0.575$  and  $r_s = 0.285$ ,  $P = 0.045$ , respectively).

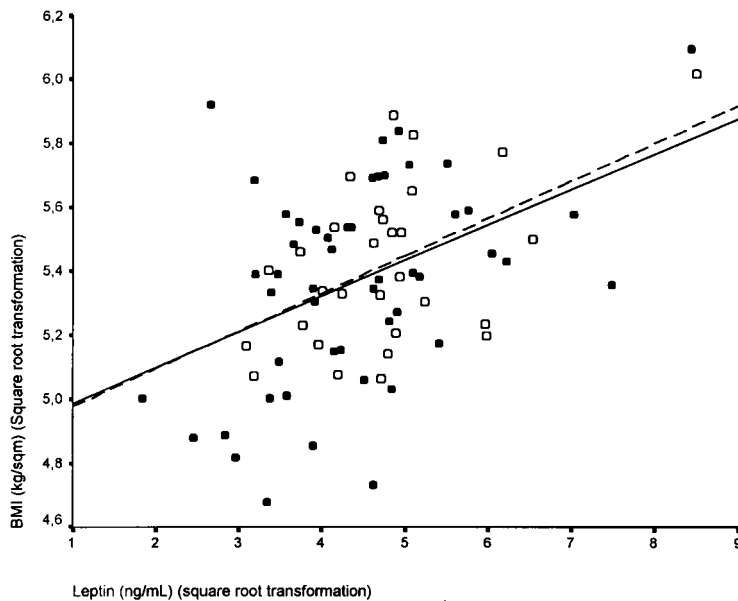
## Discussion

The main finding of this study was that there was no significant difference in plasma leptin concentrations between patient and control groups. Plasma leptin concentrations were not correlated with bone mass in the control group. Although a correlation between plasma leptin concentration and bone mass was observed in the patient group, the strength of this relationship ( $r_s^2 = 0.081$ ) was weak, in that only 8% of the variance in the BMD measurements could be accounted for by the leptin concentration. These findings suggest that circulating plasma leptin does not have a direct influence on bone mass in postmenopausal women.

**Table 1** Clinical and laboratory characteristics of the patient and control groups. Values are median  $\pm$  s.d. ( $\dagger$ ) or s.e.m.

	Patients ( <i>n</i> = 30)	Controls ( <i>n</i> = 50)	<i>P</i>
Age (years) $\dagger$	$61.18 \pm 6.51$	$58.30 \pm 6.02$	0.053 $\ddagger$
BMI (kg/m <sup>2</sup> ) $\dagger$	$28.91 \pm 3.44$	$29.46 \pm 2.79$	0.466 $\ddagger$
BMD (g/cm <sup>2</sup> )	$0.695 \pm 0.01$	$1.006 \pm 0.01$	<0.001 $\S$
Leptin (ng/ml)	$18.70 \pm 1.78$	$22.35 \pm 2.20$	0.103 $\S$
Time since menopause (years)	$14.00 \pm 1.013$	$12.00 \pm 1.43$	0.544 $\S$

$\ddagger$  Unpaired *t*-test;  $\S$  Mann–Whitney *U* test.



**Figure 1** Correlation between BMI and plasma leptin concentration in patients with osteoporosis ( $r_s = 0.404$ ,  $P = 0.004$ ) and in normal individuals ( $r_s = 0.394$ ,  $P = 0.031$ ).  $Y = 0.1115x + 4.8757$  for patients (—) and  $Y = 0.1178x + 4.8585$  for controls (---). ●, patients; ○, controls.

There are many reports indicating that osteoporosis and particularly hip fractures are less frequent in obese subjects (10–13). Obese and overweight women have a greater bone mass after menopause than lean women of the same age (10, 13). This protective effect appears to be related to both a high fat content and mechanical factors (13). Some studies suggest that greater concentrations of estrogen, decreased sex hormone binding globulin and increased free sex steroids, insulin-like growth factor-I and hyperinsulinemia are responsible for greater bone mass in obese women (10, 13). It is also interesting to note that both bone mass and plasma leptin concentrations are increased in obesity. A recent study indicates that leptin acts on human marrow stromal cells to enhance differentiation to osteoblasts, suggesting a role of leptin on bone metabolism (14). We have recently demonstrated that a genetically leptin-deficient obese patient had osteopenia, in spite of morbid obesity (17, 18). These findings suggest that leptin may have a role in bone and mineral metabolism.

There are few reports on the relationship between bone mineral density and plasma leptin concentrations. Klein *et al.* (19) reported that obese children were younger, taller, and had more advanced bone maturation than non-obese children of similar pubertal stage. However, they found similar BMD values in obese and non-obese children. Goulding & Taylor (15) did not find any relationship between BMD and plasma leptin concentrations in postmenopausal women, but they found a positive relationship between fat mass and bone mass. Similarly, Rauch *et al.* (16) also failed to find a relation between circulating plasma leptin and bone mass in adult women. In agreement with the findings of previous studies, we were unable to find a strong relationship between plasma leptin concentration and BMD in postmenopausal women with osteoporosis.

Plasma leptin concentrations were not significantly different between our patients with osteoporosis and the controls. Our data suggest that circulating plasma leptin concentrations may not act directly on bone in postmenopausal women.

Conversely, it is known that adipocytes participate in the microenvironment of the bone marrow and that these adipocytes are a secondary source of leptin production (20). Thomas *et al.* (14) recently demonstrated a direct osteogenic effect of leptin on a human marrow stromal cell line with the capability to differentiate to either osteoblasts or adipocytes. Thus it is possible that local production of leptin may play a part in bone metabolism.

Although effects of local leptin production in the bone microenvironment cannot be excluded, our data suggest that circulating plasma leptin does not have a significant direct influence on bone mass in postmenopausal women.

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