Serum leptin is correlated to high turnover in osteoporosis

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Abstract

OBJECTIVE: Clinical data have suggested that obesity protects against osteoporosis. Leptin, mainly secreted by white adipose tissue, might be involved by mediating an effect on bone metabolism. This study was conducted to investigate a possible relationship of leptin and bone turn-over in postmenopausal women with osteoporosis.

METHODS: We measured bone mineral density (BMD), serum leptin levels and markers of bone metabolism, including osteocalcin and cross-laps in 44 patients with osteoporosis. The main group consisted of 32 postmenopausal women.

RESULTS: Mean serum leptin was 13.1 µg/L and showed no statistically significant difference to the levels measured in a collective of normal persons adjusted for age and BMI. When related to serum cross-laps as markers of bone resorption, a positive correlation (p<0.05) was observed, whereas no correlation with osteocalcin could be seen.

CONCLUSIONS: A dual control of bone formation by leptin is assumed: This involves local mechanisms acting on osteoblasts and a central inhibitory effect on bone metabolism via a hypothalamic relay. Our data indicate that the net effect of circulating leptin may cause bone loss and is significantly related to high-turnover serum bone markers, at least in postmenopausal women with osteoporosis.

INTRODUCTION

Leptin, a 167 amino acid peptide hormone, is mainly secreted by white adipose tissue, but is also produced by human bone marrow adipocytes. Clinical data have been suggested that obesity protects against osteoporosis (Holloway et al. 2002) and that bone mass and serum leptin are related positively to body fat mass (Considine et al. 1996; Solin et al. 1997). Overweight people are thought to develop osteoporosis less frequently than lean persons. A number of factors such as mechanical loading and increased aromatisation of androgens to estrogens by adipose tissue have been proposed
to mediate this protective effect. In any case, the role of leptin in postmenopausal osteoporosis has not yet been established.

Bone mass is kept constant through the interaction of bone resorption by osteoclasts and bone formation by osteoblasts (Takeda et al. 2002). Findings in ovariectomy-induced osteoporosis in rodents suggest that leptin could modulate bone remodelling in favour of a better bone balance (Burguera et al. 2001). Leptin has also been shown to enhance differentiation to osteoblasts from marrow stromal cells (Thomas et al. 1999) and to inhibit osteoclast generation (Holloway et al. 2002). Neuroendocrine pathways and neurotransmitters also play a key role in the regulation of bone remodelling (Idris et al. 2005). The mediobasal hypothalamus has previously been shown to be a critical target of leptin action (Takeda et al. 2002). Leptin appears to influence bone formation by neuronal sympathetic regulation. Since a central control of bone mass is proposed, leptin may inhibit bone formation through a hypothalamic relay (Ducy et al. 2000). Leptin seems to be an underestimated player in bone metabolism; contradictory data may be related to the fact that leptin is involved in at least two different bone-controlling mechanisms (Reseland and Gordeladze 2002). To further investigate the clinical role of leptin in human osteoporosis we initiated our study. Its objective was to investigate a possible relationship between serum leptin concentration and bone metabolism in osteoporosis.

MATERIALS AND METHODS

Subjects

We investigated 44 patients with osteoporosis diagnosed at the Institute of Nuclear Medicine and Endocrinology at the General Hospital of Linz in 2003. The mean age of the population was 70 years (range 53–85 a). The criterion used was a bone mineral density less than a T-score of –2.5 in lumbar spine and/or hip measurement. The study included 32 postmenopausal women (73±10 a) who were all non-smokers treated at the Department of Orthopedics mainly for lumbar pain. BMD was measured on the same day that blood samples were drawn to analyze bone metabolism serum markers.

For comparison we also studied five patients from the Department of Urology (78±15a) with a clinical history of prostate cancer on whom resection of the prostate gland and chemocastration or gonadectomy have been performed. We also investigated seven patients (72±10 a, three women) with COPD who were hospitalised at the Department of Pneumonology, all of whom had a clinical history of cigarette smoking and systemic corticosteroid therapy lasting more than 6 months. None of the subjects were pregnant or were undergoing hormone replacement therapy. No study participant had ever received medical treatment for osteoporosis. Other secondary forms of osteoporosis were excluded. Laboratory tests in addition to leptin included parathormone, vitamin D, calcium, alkaline phosphatase, osteocalcin and cross-laps. This study was approved by the local Ethics Advisory Committee at the General Hospital Linz.

Measurements

Body weight and height were measured to the nearest 1.0 kg and 1.0 cm and body mass index (BMI) was calculated as weight/height² (kg/m²).

Bone mineral density

Dual-energy X-ray absorptiometry was performed using a HOLOGIC QDR 4500-fan beam X-ray bone densitometer. The bone mineral density (BMD) was measured at the spine (L1-4) and at the left hip and femoral neck.

Blood samples

Venous blood samples were collected in the morning from January to July 2003 after an overnight fast. Leptin samples were collected in serum tubes and separated out by centrifugation: they were stored at –75 °C until analysis in Málaga, Spain.

Serum leptin concentrations were determined by an commercial radioimmunoassay (RIA) with coated tubes for quantitative determination. For more information see on the homepage http://www.meddiagost.de (Mediagnost – Gesellschaft für Forschung und Herstellung von Diagnostika mbH ; Reutlingen, Germany, Aspenhausstrasse 25; D-72770 Reutlingen; company internal product number for leptin is LEP-R44 (DE/CA40/00809/4)). The bone turnover markers cross-laps and osteocalcin were measured at the Department of Nuclear Medicine and Endocrinology in Linz. The normal range in serum for postmenopausal women is 740 to 7 580 pmol/l for cross-laps and 9.6 to 48.3 ng/ml for osteocalcin.

For both we used an enzyme linked immuno sorbent assay (ELISA) from Nordic Bioscience Diagnostics, Denmark. The kit number for cross-laps is 4CR14000 and for osteocalcin it its 3OSC4000. Serum alkaline phosphatase was measured with a commercial kit commonly used in the laboratory at the General Hospital of Linz which was manufactured by Roche (Vienna/Austria) with the reagent number 2173107 and calibrator for automated systems number 10759350 (USA # 759350). Reference values for males are 44–145 U/l and for females 37–145 U/l. For calcium we used a kit manufactured by Roche (Vienna/Austria) with the reagent number 1730240 (calibrator 10759350 (USA # 759350). Reference values for calcium in both genders are 2.15 to 2.55 mmol/l. All assays were routinely performed according to the manufacturers’ instructions.

Statistics

All metric variables were initially tested for normality (Kolmogorov-Smirnov test with Lilliefors significance). Since the majority of the data was not normally
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distributed and/or did not have equal variances (Levene test) the comparisons of age, body mass index, cross-laps, osteocalcin and leptin between the three subgroups were performed by a non-parametric analysis of variance (Kruskall–Wallis followed by Nemenyi’s multiple comparisons). Since the leptin data exhibited logarithmic normal distribution this variable was compared between the three subgroups using a parametric analysis of covariance (ANCOVA) with the body mass index and gender as covariates. All tests are two-tailed with a confidence level of 95% ($p<0.05$). No adjustments for the $p$-values (such as Bonferroni-corrections) were made. Therefore all $p$-values are only descriptive. Comparisons of the patient leptin data and the data of normal persons provided by Mediagnost (leptin determination) were performed by using 95% confidence intervals (two tailed). Bravais-Pearson correlation coefficients were calculated.

RESULTS

From January to July 2003 we included 44 patients in our study. The group of postmenopausal women (N=32) from the Department of Orthopedics was the principal study population. The small number of patients forming the other subgroups permitted some general comparisons to be made, but a comprehensive statistical inter-group evaluation could not be undertaken and was not intended. Nevertheless, no statistically significant differences in age or BMI could be observed. Bone mineral density for the hip was T-score minus 2.5 (–0.7 to –5.6), for the lumbar spine it was T-score minus 2.9 (–0.5 to –5.0).

We compared the serum leptin values in the total study population and individually compared the three subgroups with the values of normal persons provided by the “Mediagnost normal collective” selected for BMI and sex (see above). All investigated patient groups did not yield a statistically significant difference ($p>0.05$) to the normal collective which is correlated for gender and BMI.

In the main group of the study population – the postmenopausal women (orthopedics) – a possible relationship between leptin and osteocalcin as a marker of bone formation and also cross-laps as a marker of turnover in bone metabolism was investigated by correlation analysis. A statistically significant correlation between leptin and osteocalcin could not be found. The Kendall Tau-b ($p=0.96$) and Spearmen-Rho ($p=0.98$) tests even suggested the presence of a merely random distrib-

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Correlating the values of leptin and cross-laps, we observed a statistically significant positive relationship between the two parameters by means of Spearman-Rho \((p=0.025)\) and Kendall Tau-b \((p=0.031)\) testing.

The median values of serum leptin \([13.1 \mu g/l (1.4–52.3 \mu g/l)]\), osteocalcin \([20.6 \text{ng/ml} (3.7–67.6 \text{ng/ml})]\) and cross-laps \([4484 \text{pmol/l} (530–11950 \text{pmol/l})]\) all fell within the normal range.

For reasons of exclusion and correlation, we measured serum calcium \([2.41 \text{mmol/l} (2.08–2.62 \text{mmol/l})]\), parathormone \([42 \text{U/l} (13–93 \text{U/l})]\), vitamin 1-OH-D3 \([17.6 \text{U/l} (3–46 \text{U/l})]\), vitamin 1,25-OH-D3 \([45.9 \text{U/l} (16–97 \text{U/l})]\) as well as alkaline phosphatase \([95 \text{U/l} (37–203 \text{U/l})]\). The median values of all these laboratory measurements were in the normal range.

The differences of serum markers between the various groups of osteoporosis patients did not achieve significance (leptin was additionally calculated using gender and BMI as parametric covariants) partially because of the small number of subjects. Nevertheless, it can be seen that persons with supposed corticoid-induced osteoporosis from the Department of Pulmonology had the lowest serum levels of osteocalcin and cross-laps (see Table 1). Median serum levels of leptin were rather low at 2.1 \mu g/ml (see Figure 1), but it must be considered that the majority of the subjects were male and that serum leptin levels in men are lower than in women. The androgen-deficient men with osteoporosis developed serum leptin levels comparable to those of postmenopausal women. The same constellation was observed for the serum markers of bone modulation (see Table 1).

 Principally we intended to show a possible relationship in between pathophysiologically relevant parameters of osteoporosis in postmenopausal women and serum leptin. A statistically significant difference of serum leptin levels could not be observed between patients’ and control data, which might be due to the small number of this study cohort. So our main finding consists of the positive correlation between serum leptin and cross-laps as a marker of high turnover in bone metabolism in patients with postmenopausal osteoporosis.

The small number of persons with androgen-deficient osteoporosis does not permit a proper statistical evaluation, but some trends can be observed. It must be kept in mind that an inverse relationship between leptin and BMD – after adjustment for BMI – has already been described in obese and non-obese men (Morberg et al. 2003). Although men have lower levels of circulating leptin than women our prostate cancer patient group had median serum leptin values of 12.1 \mu g/l that were comparable to those found in the group of postmenopausal women (Figure 1). The serum levels of osteocalcin and cross-laps also were comparable (Table 1). This may indicate that the effect of leptin on bone metabolism is similar in the two subgroups, as well as on the mechanisms of postmenopausal and postandropausal osteoporosis – at least our results do not contradict that. Again it has to be stated that any statistical comparison is limited by the small number of the study population and only a proof-of-principle concept was intended by investigating the two small subpopulations from the departments of urology and pulmonology.

On the contrary, the gender-mixed small subgroup of pulmonary patients with corticoid-induced osteoporosis presented rather low median levels of osteocalcin at 10.7 ng/ml, this is only about one-half the value measured in the patients of the other subpopulations (Table 1). This finding seems to be consistent with the suppression mechanism of new bone formation by glucocorticoids.

**DISCUSSION**

The relationship between obesity and osteoporosis has been widely studied, and epidemiological evidence has indicated that obesity correlates with increased bone mass. Nevertheless, the data obtained by Zhao et al. (2007) from a large cohort of human subjects contrasts with the data of previous studies. Their results indicate that, under the same conditions of mechanical loading, a higher fat mass tends to decrease bone mass (Zhao et al. 2007). Thomas et al. titled their article: “Is leptin the link between fat and bone mass?” (Thomas and Burguera 2002). Despite an accepted body of evidence
provided by preclinical data and investigation of the role of leptin in rodent bone formation, there may be no protective effect exerted by leptin on bone mass in human osteoporosis, or there may even be a contribution to bone loss.

Our objective was to investigate the role of serum leptin levels in women with postmenopausal osteoporosis. The group of postmenopausal women did not present statistically significant differences in serum leptin levels to normal data provided by the diagnostic kit’s manufacturer Mediagnost. This agrees with the published data. Blain et al. observed no correlation between circulating leptin and bone mineral content (Blain et al. 2002; Rauch et al. 1998) and found that none of the indices of bone density was significantly related to serum leptin concentrations in postmenopausal women (Rauch et al. 1998). Goulding et al. detected a positive correlation between BMD and plasma leptin concentrations. However, an adjustment for fat mass eliminated the significant correlation (Goulding and Taylor 1998). Similar relationships were also described by Iwamoto et al., who had examined premenopausal and postmenopausal women (Iwamoto et al. 2000). It must be considered that these study populations consisted of postmenopausal women with variable BMD values. In one available study of postmenopausal women with osteoporosis, a Turkish working group did not find significant differences in mean plasma leptin concentrations when compared to matched persons with osteopenia or normal BMD (Yilmazi et al. 2005). In conclusion, most authors think that it is unlikely that circulating leptin plays an important role in controlling bone cell activity in humans.

When investigating a possible correlation between plasma leptin and dynamic markers of bone resorption in healthy postmenopausal women, no significant correlations (Blain et al. 2002; Goulding and Taylor 1998) or only weakly negative correlations (Rauch et al. 1998; Iwamoto et al. 2000; Roux et al. 2003) were found. These findings stand in contrast to our data which show a significant positive correlation between cross-laps and serum leptin. This could indicate a possible role of circulating leptin in a pathologically high turnover rate of the skeleton. The explanation for these discrepancies may be that we only investigated patients with osteoporosis (before initiating specific treatment).

As a hypothesis, serum leptin levels might rise concordantly with cross-laps in osteoporosis indicating an antagonistic anabolic bone metabolism. Expression of functional leptin receptors in rat osteoblasts has been confirmed: this suggests a direct regulatory role of leptin in the bone formation process (Lee et al. 2002). However, this explanation is unlikely to account for our data, because the leptin levels in our study population were not correlated to osteocalcin as a biochemical marker of osteoblastic activity. A beneficial effect of leptin could be shown in rodents: the systemic administration of leptin to adult male mice reduced bone fragility (Cornish et al. 2002) and leptin was effective in reducing trabecular bone loss in ovariectomised rats (Burguera et al. 2001). In cell culture, leptin inhibits osteoclast generation. This inhibitory effect may be mediated by the RANKL/RANK/OPG system (Holloway et al. 2002).

Nevertheless, the results obtained by Ducy et al. (2000) indicate that leptin acting on the bone formation process in the entire animal does not require the binding of leptin to a receptor located on activated cells that are involved in bone metabolism. The authors showed that intracerebroventricular infusion of leptin causes bone-loss in mice (Ducy et al. 2000). Experimental evidence indicates that the anti-osteogenic function of leptin is a general regulatory pathway in vertebrate skeletal biology. Ewes given an ovariectomy and long-term intracerebroventricular application of leptin showed decreased bone formation and mineralising surface (Pogoda et al. 2006). Sheep may even constitute a more appropriate animal model for the study of human osteoporosis. In fact, it is assumed that leptin experts a dual control on bone formation which involves local mechanisms on pro-osteogenic cells and a central inhibitory effect via a hypothalamic relay. In agreement with these animal studies, our human data may indicate, that the central effect is dominant and that the net effect of circulating leptin causes significant bone loss related to high-turn over serum bone markers, at least in postmenopausal women with osteoporosis. In any case, the effect seems to be moderate since our study group did not exhibit significant serum level differences when compared to a normal control collective. Differences of serum leptin levels related to physical exercise and sports (Unal et al. 2005) which has a positive impact on prophylaxis of osteoporosis also have to be kept in mind. Studies including a larger number of patients with osteoporosis are recommended to clarify the role of leptin in bone metabolism.

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