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Clinical Investigations

Does Leptin Have an Effect on Bone in Adult Women?

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Abstract. Recent studies have implicated leptin in the modulation of bone mass during skeletal development. Whether leptin also exerts an influence on bone after growth has stopped is unknown at present. In this cross-sectional study on 94 women (60 premenopausal, 34 postmenopausal) aged 40–60 years, we analyzed the relationship between serum leptin and bone density and bone cortex geometry and bone metabolism. Total and trabecular bone density as well as total and cortical bone area were determined by quantitative computed tomography (QCT) at the distal radius. Bone metabolism was assessed by measuring bone-specific alkaline phosphatase, osteocalcin, procollagen type I C-terminal propeptide (PICP) and collagen type I C-terminal telopeptide in serum, and deoxypyridinoline in urine samples. None of the indices of bone density or geometry was significantly related to leptin serum concentrations ($P > 0.05$) before or after adjustment for body mass index (BMI). PICP was associated with serum leptin in the postmenopausal group only ($r = -0.40$ after adjustment for BMI; $P = 0.009$). Yet, as none of the other markers of bone metabolism exhibited a significant correlation with serum leptin in any of the menopausal groups, this association is likely to be due to the influence of extraskeletal factors on PICP serum levels. Thus, it appears that leptin has less influence on the mature than on the growing skeleton.

Key words: Bone — Bone density — Bone metabolism — Leptin — Obesity.

Leptin is a recently discovered hormone that is mostly synthesized and secreted by adipocytes [1]. Initial research focused on its role as a 'satiety signal' to the brain, but subsequently, leptin has been found to influence a variety of other physiological functions such as reproduction and hematopoiesis [2, 3]. Therefore, leptin is now regarded more generally as a systemic indicator of the adequacy of the body's energy stores [1, 4].

At present, very little is known about the effects of leptin on bone. In growing ob/ob mice, which are genetically leptin deficient, treatment with leptin has been shown to dramatically increase histological indices of bone formation, mainly at the endocortical level [5]. In a large longitudinal study on premenarcheal girls, leptin was associated with periosteal envelope expansion, leading to the hypothesis that leptin might mediate the effects of obesity on bone mass [6].

Whether leptin also has an effect on the adult skeleton is presently unknown. Therefore, we undertook the present study to analyze the relationship between serum leptin and bone density, bone geometry and bone metabolism in a cohort of healthy women from 40 to 60 years of age.

Subject and Methods

Subjects

The study population comprised healthy female volunteers who were recruited in cooperation with a health insurance company (BKK Deutsche Bank AG, Düsseldorf, Germany). These women form a subgroup of a larger cohort, which has been described in earlier reports [7, 8]. The age range was 40–60 years. The study protocol was approved by the local ethics committee and all subjects gave written informed consent.

A detailed questionnaire concerning health, diet, use of drugs, life-style, and gynecological history was obtained from each subject. Height and weight were measured and body mass index (BMI) was calculated as weight per square of height in meters (kg/m^2). A blood sample was obtained from each participant after an overnight fast, and each collected one 24-hour urine sample. On the same day, bone density and geometry measurements were taken. Complete data sets as well as blood and urine samples were available from 430 participants. For the purpose of the present study, those subjects who were on hormone replacement therapy, had irregular menses, or had a history of hysterectomy (as menopausal status was uncertain), ovariectomy, or diseases affecting bone metabolism were excluded. From the serum samples of the remaining 168 women, 94 were randomly selected for the determination of leptin levels. Menopausal status was defined as follows: premenopausal, women with regular menstrual cycles (n =

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Table 1. Clinical characteristics of study participants and serum leptin concentrations

	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m^2)	Years since menopause	Leptin (ng/ml)
Premenopausal (n = 60)	47.0 ± 3.3 (40.3–54.1)	165.2 ± 4.6 (156–175)	65.6 ± 10.1 (42–98)	24.1 ± 3.9 (15.8–34.7)	—	13.5 ± 8.2 (1.6–34.5)
Postmenopausal (n = 34)	56.4 ± 3.0 (48.5–60.9)	166.8 ± 6.2 (156–182)	67.7 ± 11.9 (48–95)	24.3 ± 3.9 (18.3–33.9)	6.1 ± 3.4 (2–16)	12.2 ± 7.2 (2.4–26.6)

Values are given as mean ± SD. Ranges are indicated in parentheses

Table 2. Results of quantitative computed tomographic analyses at the distal radius

	Total BMD (g/cm^3)	Trabecular BMD (g/cm^3)	Total bone area (mm^2)	Cortical bone area (mm^2)
Premenopausal	320 ± 56	143 ± 40	328 ± 57	180 ± 39
Postmenopausal	284 ± 53[a]	139 ± 43	345 ± 68	185 ± 44

Values are given as mean ± SD
[a] Significantly different from result in premenopausal group ($P < 0.05$)

Table 3. Coefficients of correlations between serum leptin and parameters of BMD and bone geometry

	Total BMD	Trabecular BMD	Total bone area	Cortical bone area
Premenopausal	−0.04	0.12	−0.15	0.07
adjusted for BMI	−0.14	−0.01	−0.19	−0.20
Postmenopausal	0.24	0.23	−0.04	0.14
adjusted for BMI	0.19	0.08	−0.17	−0.04

$P > 0.05$ for all correlations indicated

60); postmenopausal, women without menses for 6 months or more (n = 34).

Biochemical Measurements

Leptin serum concentrations were determined using a radioimmunoassay, which has been described in detail elsewhere [9]. The following serum markers of bone metabolism were determined using commercially available assays: bone-specific alkaline phosphatase (Tandem® -R Ostase™; Hybritech Inc., CA), osteocalcin (OSCAtest®, Henning Berlin GmbH, Berlin, Germany), procollagen type I C-terminal propeptide (PICP; Orion Diagnostica, Espoo, Finland), and collagen type I C-terminal telopeptide (Orion Diagnostica, Espoo, Finland). In urine samples, concentrations of immunogenic deoxypyridinoline (Pyrilinks-D™, Metra Biosystems Inc., Palo Alto, USA) were measured and results were expressed relative to urinary creatinine levels.

Bone Density and Bone Cortex Geometry

Bone mineral density (BMD) and parameters of bone geometry were determined by peripheral quantitative computed tomography (pQCT) (XCT-900; Stratec Inc.; Pforzheim, Germany) installed on a mobile densitometry unit, as described previously [7, 8]. In brief, computed tomographic single slice measurements (2.5 mm wide) were made at a site corresponding to 4% of the ulnar length proximal to the radial endplate. Cortical bone was separated from trabecular bone by a built in software algorithm; the central 45% area of the radial cross-section was considered as trabecular bone. Results were expressed as milligrams hydroxyapatite of calcium per cubic centimeter. Total and cortical bone area represent the area of the whole radial cross-section and the area attributed to cortical bone, respectively. These parameters were automatically calculated from the same slice using the iterative contour detection method, as described by Louis et al. [10].

Statistical Analysis

The Kolmogorov-Smirnov test was used to test for normal distribution of all data. Associations are given as Pearson's correlation coefficients. To account for the influence of body fat mass on both leptin levels and bone parameters, all correlation coefficients between leptin and these parameters were recalculated after adjustment for BMI. *T*-tests were used for comparisons between groups. All tests were two-tailed, and a 5% significance level was maintained. These calculations were performed using the SPSS software, version 6.0 for Windows.

Results

The clinical characteristics of the pre- and postmenopausal study participants are shown in Table 1. There was no significant difference in leptin levels between the two groups ($P = 0.44$). In the whole study population, leptin levels were associated with weight ($r = 0.53$; $P < 0.001$) and BMI ($r = 0.59$; $P < 0.001$), but not with age or height.

Results of the pQCT analyses at the distal radius are given in Table 2. Significant differences between the pre- and postmenopausal groups were only found for total BMD ($P = 0.003$). None of the indices of BMD or bone geometry

was significantly associated with leptin serum concentrations before of after adjustment for BMI (Table 3). Likewise, no significant association between serum leptin concentrations and levels of bone-specific alkaline phosphatase, osteocalcin, collagen type I C-terminal telopeptide, or the deoxypyridinoline/creatinine ratio was detected in the premenopausal and postmenopausal groups after adjustment for BMI ($P > 0.2$ each). PICP showed a significant (negative) correlation with serum leptin in postmenopausal women only ($r = -0.40$ after adjustment for BMI; $P = 0.009$).

Discussion

It is a well-known fact that obesity is associated with higher bone density [11]. As highlighted in a recent tutorial, this finding may be largely explained by the muscle-mediated mechanical effects of increased body weight on bone [12]. However, many other factors including hormones might modulate the response of bone to the higher strain associated with increased body weight [11, 12].

Leptin might be thought to be such a modulator, as it is implicated in a variety of hormonal feedback loops that profoundly affect bone metabolism, such as the ACTH/glucocorticoid and GHRH/GH axes [4]. Leptin might also influence bone metabolism through it effect on bone marrow cells [3], which are important regulators of bone metabolism [13]. Whether or not leptin has a direct effect on human bone cells has not been tested. In mice, the putative functional isoform of the leptin receptor appears to be expressed in bone during fetal development, but not postnatally [14, 15].

To analyze the relationship between leptin and the integrated activity of bone turnover in the entire skeleton, we determined a set of biochemical parameters of bone metabolism. These markers are thought to reflect either bone formation (bone-specific alkaline phosphatase, osteocalcin, PICP) or bone resorption (collagen type I C-terminal telopeptide, deoxypyridinoline) [16]. With the exception of PICP in the postmenopausal group, none of these parameters were related to leptin levels. Although PICP is generally regarded as a marker of bone formation, its serum levels are also influenced by collagen synthesis in skin and by the rate of uptake in the liver [16]. Therefore, it is possible that the negative association between PICP and leptin in postmenopausal women may be either a chance finding or due to an effect of leptin on skin or liver.

In addition to parameters of overall bone turnover, we determined indices of bone density and bone geometry at the distal radius, again, no relation to serum leptin levels was found. This is somewhat surprising, as both animal experiments and a study in girls have found an effect of leptin on cortical bone [5, 6]. However, these two studies analyzed the effect of leptin on the growing skeleton whereas we investigated adult individuals. It is therefore conceivable that leptin influences modeling of growing bones rather than remodeling of the mature skeleton. However, as our densitometric analyses were limited to the distal radius, we cannot exclude local effects of leptin at other sites.

In summary, neither indices of overall bone turnover nor results of bone densitometry at the distal radius were related to serum leptin in our group of adult women. These findings suggest that leptin's influence is less significant for the mature than for the growing skeleton.

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