Rheumatol Int (2003) 23: 87–91
DOI 10.1007/s00296-002-0257-0

ORIGINAL ARTICLE

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Body composition, bone mineral density, and circulating leptin levels in postmenopausal Turkish women

Received: 15 May 2002 / Accepted: 26 August 2002 / Published online: 23 October 2002

Abstract *Objective* We analyzed the relationship between serum leptin levels and bone mineral density (BMD) values as well as the relationship between serum leptin levels and whole body composition, whether or not they were associated. In addition, we also investigated whether lean mass or fat mass is a better predictor of BMD in postmenopausal Turkish women. *Design and measurements* One hundred consecutive postmenopausal women with a mean age of 55.1 ± 6.3 years who visited our outpatient clinic for the evaluation of osteoporosis were recruited. Skin fold thickness at four sites and waist:hip ratio were measured. Body mass index (BMI) was calculated in kg/m^2. Serum concentrations of leptin, insulin, and estradiol were evaluated. Bone formation and resorption markers were also determined. The BMD values were measured by dual energy X-ray absorptiometry (DEXA) at the lumbar spine and femoral neck. Whole body composition (lean mass, fat mass, and percentage of fat), total bone mineral content (BMC) in g, and total BMD were also measured by DEXA. *Results* Serum leptin levels did not correlate with BMD values at all skeleton sites measured. Leptin correlated positively with fat mass, percentage of fat, and BMI ($r = 0.738$ and $P = 0.00$, $r = 0.536$ and $P = 0.00$, $r = 0.356$ and $P = 0.00$, respectively). Lean mass correlated with BMD at all sites measured ($r = 0.339$ and $P = 0.00$, $r = 0.312$ and $P = 0.01$, $r = 0.523$ and $P = 0.00$, $r = 0.636$ and $P = 0.00$). Lean mass correlated with BMI ($r = 0.636$, $P = 0.00$) but not with serum leptin ($r = -0.021$, $P = 0.881$), and it was an independent determinant of BMD at all skeleton sites measured. *Conclusion* Our study showed that lean mass is a better predictor than fat mass of bone mineral density and that serum leptin levels are not associated with BMD.

Keywords Bone mineral density · Fat mass · Lean mass · Leptin

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Introduction

Osteoporosis and obesity are significant health problems, especially in western societies. These two diseases correlate inversely [1]. Numerous studies have shown that obesity is related to increased bone mineral density [1]. Moreover, fat mass and body weight correlate with bone mineral density (BMD) in women, independently of their menopausal status [2]. Although body weight is one of the important determinants of bone mass and BMD, the nature of this relationship remains unknown [1, 2]. There is also controversy as to whether lean mass or fat mass is a better predictor of BMD [3]. However, Reid et al. found a significant relationship between fat mass and BMD in women [4]. Therefore, the relationship seems to be gender-dependent [4].

Several reports have suggested that the effect of fat mass on BMD may be mediated by hormonal factors such as sex hormones, leptin, and insulin. Although it is controversial, serum insulin levels have been shown to be related to BMD [3].

Leptin, the product of the ob gene, is secreted mainly by white adipose tissue and correlates with fat mass [5]. In addition to central effects, leptin also has effects on peripheral tissue [5]. Both serum leptin and bone mass positively correlate with body weight [6]. In vitro studies have demonstrated a direct effect of leptin on osteoblast differentiation and matrix mineralization [7]. Moreover, several clinical studies have shown that plasma leptin levels positively correlate with BMD in women.

In this study, we assessed plasma leptin and insulin concentrations, percentage of fat, lean mass, fat mass,

skin fold thickness, waist:hip ratio, body mass index (BMI), and BMD values. We also related bone formation and resorption markers in Turkish postmenopausal women and investigated whether serum leptin levels were associated with BMD and whole body composition. In addition, we also investigated whether lean mass or fat mass is a better predictor of BMD.

Methods

One hundred women with a mean age 55.1 ± 6.3 years who had been postmenopausal for at least 12 months and visited our clinic for the evaluation of osteoporosis were recruited into the study. The patients gave informed consent for the follow-up. We excluded subjects who had diabetes mellitus, thyroid disorder, or metabolic bone diseases. None were taking drugs or hormones influencing bone metabolism. The women had no vertebral compression fractures on lateral spine radiographs and no history of trauma, smoking, or alcohol abuse.

Anthropometric determination

Weight and height were measured by standard technique. The BMI was calculated as body weight (kg) divided by height squared (m^2). Waist circumference was measured at the midpoint between the lower rib margin and the iliac crest (normally at the umbilical level) and hip circumference at the level of the trocanter. Both circumferences were measured to the nearest 0.5 cm with a plastic tape, and the waist:hip ratio (WHR) was calculated. Skin fold thickness was measured by a calibrated caliper (Holstain) at four sites (biceps, triceps, subscapular, suprailiac).

Biochemical measurements

Sample preparation

Whole venous blood samples were taken. Blood samples were allowed to clot for 30 min at room temperature and centrifuged for 10 min at 5,000 rpm. Then serum samples were removed and stored at –20°C until used for assay.

The levels of osteocalcin were analyzed with quantitative determination of normal minimal inhibitory dose (N-MID) osteocalcin in serum. The concentrations of β crosslabs were quantitatively determined by analyzing the degradation products' type I collagen in serum. Electrochemiluminescence immunoassay (ECLIA) was used for determination of these tests. The levels of parathyroid hormone PTH were also analyzed with ECLIA (Electsys 2010 immunoassay analyzer) (Roche Diagnostics, Mannheim, Germany). The activity of alkaline phosphates was measured according to the recommended reference method of the International Foundation of Clinical Chemistry. Calcium levels were analyzed with method of Schwarzenbach with o-cresolphtalein complexion, and the concentrations of inorganic phosphate were analyzed with the direct phosphomolybdate method according to Daly and Ertingshausen (Cobas Integra 700 analyzer) (Roche Diagnostics). Urine specimens were collected in acid-washed bottles. Twenty-four-h specimens were collected in containers containing 5 ml of 6 mmol/L HCl. The urine specimens were mixed well prior to analysis.

Serum estradiol (E_2) was measured by radioimmunoassay (Roche Diagnostics).

Leptin assay

The levels of leptin were analyzed by competitive enzyme immunoassay measuring the natural and recombinant forms of the cytokine leptin. With this method, goat antirabbit antibodies were used to capture a specific leptin complex in each sample consisting of leptin antibody, biotinylated leptin, and sample or standard. The biotinylated leptin conjugates and samples or standards are compared for leptin-specific antibody binding sites. The assay is visualized using a streptavidin alkaline phosphatase conjugate and the ensuing chromogenic substrate reaction. The amount of leptin detected in each sample was compared to a leptin standard curve, which demonstrated an inverse relationship between absorbance and its concentration. Assay procedure was done according to suggestions of the manufacturer (Accucyte Human Leptin, lot no. AL010-DA) (Cytimmune Science, Md., USA).

Insulin

Blood samples were taken using standard sampling tubes, and serum samples were separated by centrifugation at 4,000 rpm for 10 min. Samples were stored at –20°C until analysis for insulin. Insulin levels were determined by ECLIA with a Roche modular analytics E170 immunoassay analyzer.

Bone mineral density and body composition measurements

The BMD values were measured by dual-energy X-ray absorptiometry (DEXA) using an XR-46 apparatus (Norland, Fort Atkinson, Wis., USA) at the lumbar spine (L2–L4) and femoral neck. Whole body composition, total bone mineral content (BMC), and total BMD were also evaluated by DEXA. Bone mineral density was automatically calculated from the bone area and BMC and expressed absolutely in g/cm^2.

Statistical analysis

The BMD values and demographic and biochemical parameters were expressed as mean ± SD. Statistical analysis was performed using SPSS 9.0 software. Simple linear regression analysis was used to assess the linear relationships between various parameters, and then Spearman's correlation coefficients were calculated. Multiple regression analysis was also employed. P values less than 0.05 were considered significant.

Results

The characteristics of the participants are summarized in Table 1 and Table 2.

Leptin correlated with fat mass and percent of fat ($r = 0.297$ and $P = 0.02$, $r = 0.359$ and $P = 0.00$, respectively) and with BMI ($r = 0.356$, $P = 0.00$) (Table 3).

Leptin levels correlated with biceps, scapular skin folds, and waist:hip ratio ($r = 0.302$ and $P = 0.03$, $r = 0.319$ and $P = 0.02$, $r = 0.273$ and $P = 0.05$, respectively). There was a positive correlation between leptin and insulin levels ($r = 0.45$, $P = 0.04$). There was no correlation between leptin and age ($r = 0.008$, $P = 0.940$). There was also no correlation between serum leptin levels and BMD at the lumbar spine and femoral neck regions, whole body BMD, or total BMC ($r = –0.132$ and $P = 0.232$, $r = –0.051$ and $P = 0.644$, $r = –0.098$ and $P = 0.359$, $r = –0.143$ and $P = 0.181$, respectively) (Table 4). In addition, there was no correlation between serum leptin levels and bone formation (osteocalcin) and resorption marker (β crosslab) ($r = 0.221$ and $P = 0.056$, $r = 0.104$ and $P = 0.367$).

Table 1 Background data of the participants

Characteristic	Mean SD
Age (years)	55.16.3
BMI (kgm^2)	24.44.4
Years since menopause	8.76.9
Age at menarche (years)	13.41.3
Leptin (ngml) ($n=60$)	10.37.3
Insulin (Uml)	10.56.1
Estradiol (pgml)	20.223.1
Lumbar (gcm^2)	0.8490.1
Femur neck (gcm^2)	0.7780.1
Total BMD (gcm^2)	0.9939.5
Total BMC (g)	2,294.13
Lean mass (g)	37,492.35
Fat mass (g)	34,903.318
% Fat (%)	44.65.2

Table 2 Bone metabolism markers and anthropometric determination in participants

	Mean ± SD
ALP (U/l)	203.9 ± 53.3
Ca (mg/dl)	9.4 ± 1.0
P (mg/dl)	3.6 ± 0.5
Osteocalcin (ng/ml)	29.4 ± 11.4
Beta-crosslab (ng/ml)	0.33 ± 0.1
PTH (pg/ml)	53.0 ± 21.4
Urinary Ca (mg/dl)	102.9 ± 1
Ca/Cre (mg/dl)	2.98 ± 12.2
Waist:hip ratio (cm)	0.88 ± 5.8
Biceps (cm)	16.2 ± 6.0
Triceps (cm)	23.9 ± 7.5
Subscapular (cm)	26.8 ± 7.3
Suprailiac (cm)	24.6 ± 7.8

No correlation was found between serum insulin levels and BMD at all skeletal sites measured.

Lean mass correlated with BMD at the lumbar spine and femoral neck, whole body BMD, and total BMC ($r=0.339$ and $P=0.00$, $r=0.312$ and $P=0.01$, $r=0.523$ and $P=0.00$, $r=0.636$ and $P=0.00$, respectively) (Fig. 1, Fig. 2). Lean mass also correlated with BMI ($r=0.636$, $P=0.00$) and with skin fold thickness at four sites and waist:hip ratio ($r=0.539$ and $P=0.00$, $r=0.519$ and $P=0.00$, $r=0.433$ and $P=0.00$, $r=0.529$ and $P=0.00$, $r=0.282$ and $P=0.01$) There was also a correlation between lean and fat mass ($r=0.456$, $P=0.00$).

Leptin did not correlate significantly with lean mass ($r=-0.021$, $P=0.881$).

Body mass index correlated with BMD at all skeletal sites measured ($r=0.292$ and $P=0.01$, $r=0.352$ and $P=0.002$, $r=0.444$ and $P=0.00$, $r=0.478$ and $P=0.00$). It correlated with skin fold thickness measured at four sites and also with waist:hip ratio ($r=0.558$ and $P=0.00$, $r=0.509$ and $P=0.00$, $r=0.521$ and $P=0.00$, $r=0.554$ and $P=0.00$, $r=0.421$ and $P=0.00$).

There was no correlation between BMD at all sites measured with fat mass or percentage of fat.

In linear regression analysis, lean mass was independently related to BMD at the lumbar and femoral neck sites and also to whole body BMD and total BMC.

Table 3 Correlation between variables and serum leptin (ng/ml) levels in participants

Variable	*R*	*P* value
Fat mass (g)	0.297	0.02a
Fat (%)	0.359	0.00[a]
BMI (kg/m^2)	0.356	0.00[a]
Lean mass (g)	–0.021	0.881
Lumbar BMD (g/cm^2)	–0.051	0.644
Total BMD (g/cm^2)	–0.098	0.359
Total BMC (g)	–0.143	0.181

[a] Significant

Table 4 Correlation of variables with lean tissue mass (g)

Variable	*R*	*P* value
Fat mass (g)	0.456	0.00[a]
Leptin (ng/ml)	–0.021	0.881
BMI (kg/m^2)	0.636	0.00[a]
Lumbar BMD (g/cm^2)	0.339	0.00[a]
Femur(neck) (g/cm^2)	0.312	0.01[a]
Total BMD (g/cm^2)	0.636	0.00[a]
Total BMC (g)	0.523	0.00[a]

[a] Significant

Discussion

Our findings indicate that BMD is not related to fat mass but to lean tissue mass. In addition, serum leptin levels were not associated with BMD in postmenopausal women. These findings suggest that circulating plasma leptin does not have a direct effect on bone mass in postmenopausal women.

The role of circulating leptin levels on BMD is unclear. However, serum leptin levels are increased in obesity and strongly and directly related to fat mass [8]. Yamauchi et al. reported strong positive correlation between percentage of fat and plasma leptin concentrations [9]. Isidori et al. reported that BMI was an independent contributor to serum leptin levels [10]. In the study, we found a significant relationship between leptin levels and fat mass, percentage of fat, and BMI. Leptin is also regulated by plasma insulin concentration [3]. We also found a significant positive relationship between serum leptin levels and circulating insulin levels. However, we could not find any correlation between serum insulin levels and BMD at all sites measured. Although serum insulin levels has been shown to be related to BMD, patients with type 2 diabetes mellitus have been reported to have increased, decreased, or unchanged BMD [3]. Martini et al. also found that not only leptin but also insulin-like growth factor 1 have no direct effect on bone mass and bone turnover [11].

Reid et al. indicated that BMD is strongly related to fat mass, but it remains controversial as to whether fat mass is better associated with BMD before or after menopause [4]. Yamauchi et al. found no correlation between percentage of fat and BMD [9]. Bedogni et al. concluded that BMC is associated more with lean tissue mass than

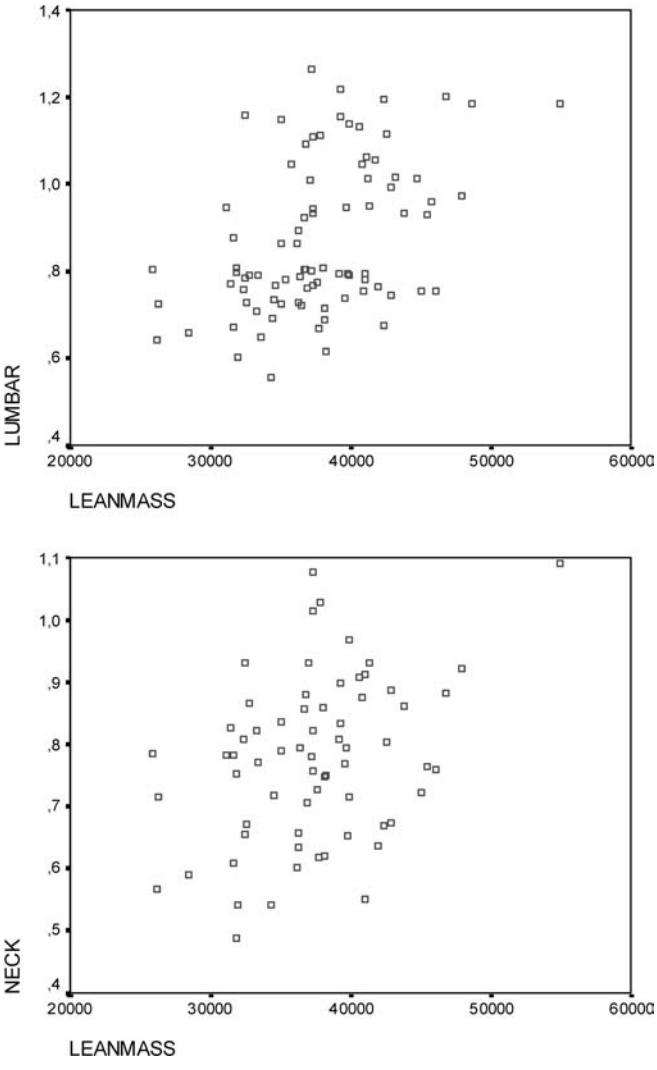

Fig. 1 **A, B** Relationships between L2–L4 BMD (g/cm^2) and lean mass (g) (**A**) and between femoral neck (g/cm^2) and lean mass (**B**)

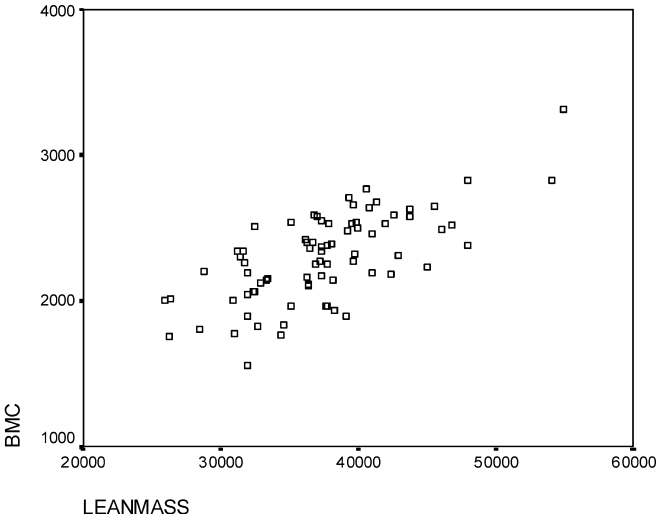

Fig. 2 Relationship between total BMC (g) and lean mass (g)

with fat mass [12]. It has also been suggested that significant loss in BMD and lean mass occur shortly after hip fracture, while increased body fat and lean tissue mass are the main determinants of bone mass [13]. Nakaoka et al. reported that lean mass was a useful marker which predicted the risk of reduced BMD and its cause of spinal fractures in postmenopausal Japanese women, and they found that it positively correlated with BMD at all sites [14]. In the present study, we found significant correlations between lean mass and BMD at all sites measured, but there was no correlation between lean tissue mass and serum leptin levels. Goulding et al. also showed that lean mass did not correlate with fat mass [15]. Lean mass may be more important than fat mass, especially in subjects with lower body weight [15]. Alternatively, body composition may effect BMD differently according to race [14]. The present study may reveal that lean mass has an effect on axial and total BMD.

As mentioned earlier, the exact role of leptin on bone mass remains controversial. Ducy et al. reported that leptin was a potent inhibitor of bone formation acting through the central nervous system [16]. Martini et al. concluded that serum insulin growth factor 1 and serum leptin have no direct effect on bone mass [17]. Iwamoto et al. also reported that leptin is not a key regulator of bone metabolism, although it may have some effects on bone metabolic markers and BMD regionally [18]. Goulding et al. revealed results that do not support the hypothesis that leptin mediates the bone-sparing effects of obesity [15]. Rauch et al. also failed to find a relationship between bone mass and serum leptin levels by examining total and trabecular bone density at the distal radius in adult women [19]. They also reported no correlation between plasma leptin level and bone metabolic markers in adult women [19]. Goulding et al. found no significant correlation between circulating leptin levels and biochemical markers of bone and thus speculated that leptin played no significant role in the regulation of bone cell activity [15]. Although our results relating to bone markers are consistent with the study of Goulding et al, biochemical bone markers are known to reflect current bone metabolism [15].

At a local level, bone marrow adipocytes produce leptin, which may enhance osteogenic activity and inhibit adipogenic activity [20]. Thus it is possible that local production of leptin may play a partial role in bone metabolism. However, the long-term effect of leptin on bone mass and on bone quality or geometry should be investigated.

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