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FİZİKSEL TIP

IS THERE ANY LINK BETWEEN CIRCULATING LEPTIN LEVEL, TOTAL BONE MINERAL DENSITY AND REGIONAL BONE MINERAL DENSITY IN POSTMENOPAUSAL HEALTHY WOMEN?: A PRELIMINARY STUDY

POST MENOPOZAL SAĞLIKLI KADINLARDA SERUM LEPTİN DÜZEYİ İLE TOTAL KEMİK MİNERAL YOĞUNLUĞU, BÖLGESEL KEMİK MİNERAL YOĞUNLUĞU ARASINDA İLİŞKİ VAR MI?: ÖN ÇALIŞMA

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SUMMARY

Background: *It has been suggested that leptin has an effect on bone metabolism. This study was performed to investigate the relationship of leptin to regional bone mineral densty, total bone mineral densty andbone metabolic markers in postmenopausal healthy women.*

Method: *The relationships between serum leptin level and bone mineral denstiy (BMD) of several skeletal sites of the body as well as whole body along with bone metabolic markers were examined in 70 postmenopausal healthy women.*

Results: *Serum leptin level was not correlated with BMD of the trunk, abdomen, leg and arm (r=0.009; p=0.951, r=0.115; p=0.413. r=0.100; p=0.476, r=0.064; p=0.647 respectively). Serum leptin level was not correlated with total BMD and total bone mineral content (r=0.011; p=0.937, r=0.127; p=0.360 respectively). Osteocalcin, beto cross Lab (CTX) and alkalen phosphatase (ALP) were tested in the postmenopausal women were not correlated with serum leptin level (r=0.060; p=0.693, r=0.057; p=0.706, r=0.045; p=0.053).*

Conclusion: *Serum leptin level does not act directly on total and regional bone mineral densty as well as bone metabolic markers. However, the effect of local leptin production on bone should be investigated in the bone microenviroment.*

Key Words: *Leptin, bone mineral denstiy, regional bone mineral denstiy.*

ÖZET

Amaç: *Leptinin kemik metabolizması üzerine etkis iolduğu öne sürülmüştür. Bu çalışma, postmenopozal sağlıklı kadınlarda bölgesel kemik mineral yoğunluğu, total kemik mineral yoğunluğu, ve serum leptini ile bazı kemik döngüsel belirleyicileri arasındaki ilişkiyi araştırmak için yapılmıştır.*

Metod: *Serum leptin seviyesi, total kemik mineral yoğunluğu, bölgesel kemik mineral yoğunluğu ve bazı kemik döngüsel belirleyicileri arasındaki ilişki, 70 post menopozal sağlıklı kadında incelenmiştir.*

Bulgular: *Serim leptin seviyesi, gövde, abdominal, bacak v ekol kemik mineral yoğunlukları ile korelasyon göstermemiştir. (r=0.009; p=0.951, r=0.115; p=0.413. r=0.100; p=0.476, r=0.064; p=0.647). Serum leptin seviyesi total kemik mineral yoğunluğu ve total kemik mineral içeriği ile korelasyon göstermemiştir (r=0.011; p=0.937, r=0.127; p=0.360 respectively). Postümenopozal sağlıklı kadınlarda serum osteocalsin, beta krosslab (CTX) düzeyi v eserum alkalen fosfataz (ALP) ile serum leptin düzeyi ile korelasyon bulunmamıştır (r=0.060; p=0.693, r=0.057; p=0.706, r=0.045; p=0.053).*

Sonuç: *Serum leptin düzeyinin bölgesel kemik mineral yoğunluğu ve total kemik mineral yoğunluğu üzerine direk etkisi yoktur. Bununla birlikte, kemik mikroçevresindeki lokal leptin üretiminin kemik üzerine olan etkisi araştırılamalıdır.*

Anahtar Kelimeler: *Leptin, kemik mineral yoğunluğu, bölgesel kemik mineral yoğunluğu.*

INTRODUCTION

Leptin, encoded by the *Lep* gene is synthesized in and secreted from adipose tissue and is a potential afferent signal of fat stores (1). Leptin circulates in plasma and after crossing the blood-brain barrier, acts in the hypothalamic nuclei to regulate food intake, energy expenditure and growth. In addition to its effects in the central nervous system, it also acts in the periphery (1,2).

The effects of leptin on bone metabolism are controversial (3). Ducy et al have recently shown that leptin deficient and leptin receptor deficient mice have increased bone mass despite hypogonadism and hypercortisolism (3). On the contrary, Steppan et al showed that, leptin administration led to a significant increase in total body bone area, bone mineral content and bone density in ob/ob mice (4). Goulding and Taylor reported that there was no significant correlation between leptin and bone mineral content, nor did they find any correlation of leptin with bone metabolic markers in postmenopausal women (5).

Masuzaki et al reported that the gene of leptin was expressed differently in regional adipose tissues (6). He suggests that there may be a difference of the effect of the leptin on bone between the segmental regions of the body (6). In addition, there are few studies in the literature, about the relationship between serum leptin level and regional bone mineral density (6).

In the present study, we examined the possible relationship between serum leptin level and bone mineral density of several regional sites of the body as well as whole body along with bone metabolic markers in postmenopausal healthy Turkish women.

METHODS

Seventy Turkish postmenopausal healthy women were recruited into the study.

Postmenopausal state was defined as the absence of menstruation for at least 12 months.

No subjects had medication or complications which affected bone metabolism. All participants were non smoker, non drinker, and led active lives but did not practice sport. All the subjects had no history of trauma and there was no any compression fracture on X-ray. Informed consent was obtained from each subject before study entry. Weight and height were measured by standard technique. Body mass index(BMI) was calculated as body weight (kg) divided by height squared (m^2).

A blood sample was obtained from each participant after an overnight fast. On the same day, each subject had a total body scan taken by DEXA (Norland XR-46, Norland Co, Fort Atkinson, WI, USA) to determine bone mineral density (BMD). BMD was measured at several regional sites of the body, that is arms, legs, trunk and abdomen and as well as whole body.

BIOCHEMICAL MEASUREMENTS

Sample Preparation:

Whole venous blood samples were taken from patients. Blood samples were allowed to clot for 30 min at room temperature and centrifuged for 10 min at 5000 rpm. Serum samples were removed and stored at –20 °C until used for assay.

The levels of osteocalcin were analyzed with quantitative determination N-MID osteocalcin in serum. The concentrations of b -crossLaps (CTX) were quantitatively determined by analyzing degradation products of type I collagen in serum. The electrochemiluminescence immunoassay (ECLIA) was used for determination of these tests. The level of PTH was also analyzed with electrohemiluminescence immunoassay method (Roche Electsys 2010 immunoassay analyzer, Mannheim, Germany). The activity of alkaline phosphatase was measured according to the recommended reference method of the IFCC (International Federation of Clinical Chemistry).

LEPTIN ASSAY

The level of leptin was analyzed by a competitive enzyme immunoassay (EIA) measuring the natural and recombinant forms of leptin in all subjects. With this assay method, goat anti-rabbit antibodies were used to capture a specific leptin complex in each sample consisting of leptin antibody and biotinylated leptin. The biotinylated leptin conjugates of samples are competed for leptin specific antibody binding sites. The assay is visualized using a streptavidin alkaline phosphatase conjugate and an ensuing chromogenic substrate reaction. The amount of leptin detected in each sample was compared to a leptin standard curve, which demonstrated an in-

verse relationship between absorbance and its concentration. The assay procedure was done according to the suggestions of the manufacturer (Accucyte Human Leptin, Lot. No. AL010-DA, Cytimmune Science Inc., MD, US).

Lateral radiographs of the thoracic and lumbar spine were taken for the evaluation of any compression fractures.

STATISTICAL ANALYSIS

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

RESULTS

Clinical characteristics of the study participants are shown in Table 1. BMD at several regional sites of the body , serum leptin and bone metabolic markers(osteocalcin, beta-cross-Lab; (CTX) of the participants are shown in Table 2.

Table 1. Clinical characteristics of the study participants

N=70	
Age (years)	54.1±6.0
Body mass index(kg/ m2)	29.1±4.5
Years since menopause	6.67±5.80
Age at menarche (years)	13.3±1.4

Data are shown as mean ± s.d.

Table 2. BMD at several regional sites of the body , serum leptin and bone metabolism markers.

N=70	
Leptin (ng/ml)	10.3±7.3
Osteocalcin(ng/ml)	28.53±10.67
Beta crossLap(CTX)(ng/ml)	0.334±17.6
ALP(IU/l)	201.6±57.7
Total BMD(g/cm2)	0.992±9.4
Total BMC(g)	2465.2±304.9
Trunk BMD(g/cm2)	0.846±9.1
Abdominal BMD(g/cm2)	0.981±0.1
Arm BMD(g/cm2)	0.874±0.1
Leg BMD(g/cm2)	20.90±145.1

Data are shown as mean ± s.d.

No correlation was found between serum leptin level and BMD at any regional site of the body and whole body BMD in the postmenopausal women.

There was no correlation between serum leptin level and trunk BMD, abdominal BMD, arm BMD and leg BMD (r= -0.009; P =0.951, r=-0.115;P=0.413, r= -0.100;P=0.476, r=-0.064; P=0.647 respectively).

Serum leptin level was not correlated with total BMD and total bone mineral content (r= -0.011; P=0.937, r=-0.127; P=0.360 respectively).

There was no correlation between serum leptin level and age in these subjects (r=0.008;P=0.940). Body mass index was correlated with serum leptin level (r= 0.356; P=0.00).

Bone metabolic markers (osteocalcin, beta-cross Lab and ALP) tested in the postmenopausal women were not correlated with serum leptin level in these subjects (r=0.060;P=0.693, r=0.057; P=0.706, r= 0.045; p= 0.053).

DISCUSSION

In the present study, we investigated the relationship of leptin to bone metabolism in detail by examining BMD at several regional sites of the body as well as whole body and bone metabolic markers in postmenopausal women.

We could not find significant correlations between serum leptin level and BMD at several sites of the body , as well as whole body BMD and total bone mineral content in these subjects. Therefore, we suggest that serum leptin level is not a major regulator of bone mineral density . Rauch et al 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 (7).They also reported in their study no correlation between plasma leptin level and bone metabolic markers in adult women (7). Early in life, leptin could stimulate bone growth and bone size through direct angiogenic and osteogenic effects on stromal precursor cells. Later, it may decrease bone remodeling in the mature skeleton when trabecular bone turnover is high (8).

Goulding et al found no significant correlation between circulating leptin levels and biochemical markers of bone and thus suggested that leptin played no significant role in the regulation of bone cell activity (5). In the present study, we also found no correlation between serum leptin level and osteocalcin, CTX and ALP in these patients.

Iwamoto et al investigated the relationships between serum leptin level and regional bone mineral density in healthy pre-

menopausal and postmenopausal women. They suggested that leptin is not a key regulator of bone metabolism and has no effects on BMD regionally in postmenopausal women (9). However, they found weak correlation between serum leptin level and BMDs of two regional sites of the body , pelvis and leg in premenopausal women.

Masuzaki et al reported that the gene of leptin was expressed differently in regional adipose tissues (6). If leptin receptors on bones, there may be a possibility that the expression of leptin receptors genes on bones differs between regional sites of the body (6). They speculate that serum leptin level has no effect on BMD of the regional site of the body in women especially at postmenopausal period (6). Leptin may have a different effect on BMD at premenopausal period.

In premenopausal women, whose estrogens are still present, these hormones have some effects on bone mass regardless of the presence of serum leptin status (9).

Conversely, it is known that adipocytes play a role in the microenvironment of bone marrow and these adipocytes are the source of leptin production (10). It has been demonstrated that stromal cells produce functional leptin receptors. Leptin exerted direct osteogenic effects by enhancing osteobastic differentiation and inhibiting late adipocytic differentiation of the human marrow stromal cells (10,11,12). Holloway et al reported that leptin may act locally to increase bone mass and may contribute to linkage of bone formation and resoption mediated by the RANKL/RANK/OPG system (13). In addition, leptin has peripheral and central effects (14). Negative effects of leptin on bone formation affected through the central nervous system pathway could counterbalance these peripheral positive effects (14). The central effect may be dominant when the blood brain barrier permeability decreases (14,15). Therefore, these central and peripheral effects should be taken into account in the postmenopausal period (14,15).

In conclusion, we suggest that leptin does not act directly on bone mass in post-menopausal healthy women. However, further studies investigating the local effect of leptin on bone tissue in the premenopausal and postmenopausal period are needed.

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