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Serum leptin and bone metabolism parameters in obese children.

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Abstract

Aim: Complex correlations exist between leptin, obesity and bone formation. Leptin is thought to affect growth and bone formation as well as osteoblastic and osteoclastic activities. Additionally, leptin increases bone mineral content. The aim of this study was to evaluate the correlation between serum leptin levels and osteoblastic and osteoclastic parameters and Bone Mineral Density (BMD) in children with exogenous obesity.

Patients and method: Forty-five children with exogenous obesity, aged 6-17 years (27 girls and 18 boys) who were followed up at the Eskisehir Osmangazi University Faculty of Medicine, Department of Pediatrics, were enrolled. Thirty-nine healthy children, aged 6-17 years (17 girls and 22 boys), were assigned to the control group. In addition to serum leptin, serum osteocalcin was measured as an indicator of osteoblastic activity, and urine Deoxypyridinoline/Creatinine ratio (DPD/Cr) was measured as an indicator of osteoclastic activity. BMD was assessed in all children by DEXA screening.

Results: Serum leptin levels and osteocalcin levels were significantly higher in obese children than in the controls (both $p<0.001$). The urine DPD/Cr in the group of obese children was significantly lower than that in the control group ($p<0.001$). BMD in obese children was also higher than that in the controls ($p<0.001$). No significant differences in serum leptin levels, osteocalcin levels and urine DPD/Crs were observed between obese boys and girls in the study group ($p>0.05$); however, BMD in obese girls was higher than that in obese boys ($p<0.05$). Positive correlations were found between serum leptin concentrations, serum osteocalcin concentrations and BMD values in obese children ($p<0.05$).

Discussion: In the present study, we identified an increase in BMD and serum osteocalcin levels in obese children. Accordingly, such an increase in osteoblastic activity is considered to be associated with an increase in serum leptin levels. More comprehensive studies are required to assess the central and peripheral effects of leptin on bone metabolism.

Keywords: Obesity, Child, Leptin, Bone, Osteocalcin, Bone mineral density.

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Introduction

Obesity is a chronic disease that causes an increase in fatty tissue in the body and is associated with high mortality and morbidity due to complications. The incidence of obesity has increased worldwide and the majority of childhood obesity is primary (exogenous) obesity [1,2]. Obesity is a health problem that, if observed during childhood and adolescence, requires early attention because it can continue throughout adulthood and could lead to the above-mentioned complications [1,3,4].

Clinical studies of obesity have shown that it has protective effects against osteoporosis. Some studies have demonstrated that bone density and osteoblastic and osteoclastic balance are regulated by body weight [5]. In studies aimed at identifying the protective mechanisms, leptin has been defined as a mediator that plays a role in the protective effect of fat mass on bones [6]. Fat mass, serum leptin concentration and bone mass are associated with each other in many aspects. High serum leptin concentration and high bone mass associated with increased fat mass are associated with a lower risk of osteoporosis [7]. Additionally, experimental studies have shown that leptin has useful effects on bone mass and that it increases osteoblastic differentiation and decreases osteoclastic development and function. Leptin stimulates osteoblasts peripherally, however inhibits osteoblasts by means of a central mechanism [8].

Accordingly, *in vivo* and *in vitro* findings also indicate that leptin modulates both osteoblastic and osteoclastic activity and that it affects bone remodeling directly. However, no direct evidence in human-related studies has confirmed this result. Various studies have reported positive correlations [9], negative correlations [10] or no correlations between serum leptin levels and Bone Mineral Density (BMD) in humans [11,12]. The literature primarily includes tests/experiments carried out on animals and studies carried out on adult humans, particularly postmenopausal women [9,10]. However, to date,

no study analysing the biochemical measurements of bone turnover in obese children has been conducted. Therefore, the aim of this study is to assess the correlations between serum leptin levels and osteoblastic and osteoclastic parameters and BMD in children with exogenous obesity.

Materials and Methods

In this study, children who were diagnosed with exogenous obesity in the polyclinics of the Department of Pediatrics of the Medical Faculty of Eskisehir Osmangazi University were assigned to the study group. Healthy children who were the same age and gender as the obese children were assigned to the control group. Parents of all children in both groups were provided information about the study, and they provided consent for study participation. The study protocol was approved by the Ethics Committee of Eskisehir Osmangazi University.

All children in the study group underwent a physical examination, and their body weight and height were measured. Female and male children with no pubertal maturation, who were younger than 10 and 11, respectively, were considered to be in the prepubertal phase. The body weights of all children in the study group were measured using a scale, and their heights were measured using a ruler attached to the scale. Data provided by the National Center for Health Statistics (NCHS) were used to analyze body weight and height measurements [13]. Body Weight-For-Height (BWFH) of the study and control groups was calculated as percentage standard using the following formula:

BWFH (st (%))=Measured weight of the patient/Weight of a healthy child of the same height and gender at the 50^{th} percentile × 100

Body Mass Index (BMI) was calculated using the following formula:

Body Mass Index (BMI)=Weight (kg)/Height $(m)^2$

Children whose BWFH was greater than 120% of the standard value and whose BMI was 95% and above were considered obese. The inclusion criteria for exogenous obese children were as follows: no pathological findings other than obesity upon physical examination, no suggestive findings of endocrine disease, and no medication history. Blood samples were collected from all participants between 08:00 and 09:00 following 12 hours of fasting. These samples were used to determine calcium, phosphor, alkaline phosphatase, leptin, Insulin like Growth Factor (IGF-I), Inulin like Growth Factor Binding Protein-3 (IGFBP-3), and osteocalcin levels. Calcium, phosphor, and alkaline phosphatase values were assessed by spectrophotometric analysis. A 5 ml blood sample was collected for determination of serum leptin and osteocalcin, for which the serum was separated. The second urine sample was collected in the morning for determination of deoxypyridinoline levels and was stored at -70°C prior to analysis. Leptin was measured by ELISA using a DSL-Human Leptin kit; osteocalcin and deoxypyridinoline were measured by chemiluminescence using an Immulite One Analyser. All assessments were carried out at the Biochemistry Laboratory of the Medical Faculty of Eskisehir Osmangazi University.

BMD of the children in the study group was assessed by Dual Energy X-Ray Absorptiometry (DEXA). Assessments for lumbar area (L2-L5) were measured in g/cm^2. According to the World Health Organization, a BMD T score measuring below -1 SD using the DEXA method indicates osteopenia, while a score above -2.5 SD indicates osteoporosis. However, Z scores are used rather than T scores because it is not possible to compare the values obtained from children with those obtained from adults. The Z score is associated with age. The assessments took 10-20 minutes based on the cooperation and the weight and height measurements of the patients. The dose of radiation administered to patients during a period of one drawing was 1-3 mrem. BMD was assessed using a LUNAR DPX-L densitometer (Hologic DQR-1000/W) at the Department of Nuclear Medicine of the Medical Faculty of Osmangazi University.

SPSS for Windows 13.0 was used for statistical analysis of the data. Normally distributed data are presented as the mean ± standard deviation, and non-normally distributed data are presented as the median (minimum-maximum). Independent samples were compared by t-test, and Pearson's correlation was used to determine correlations. A p-value below 0.05 was considered statistically significant.

Results

The present study included 45 children (27 girls and 18 boys; 12 prepubertal and 33 pubertal) with exogenous obesity, aged 6-17 years, and 39 healthy children (17 girls and 22 boys; 15 prepubertal and 24 pubertal), aged 6-17 years.

The body weights, BMI and BWFH levels of obese children in the study group were higher than those of children in the control group, as expected ($p<0.001$ for all). No significant differences were found between the obese children in the study group and the children in the control group with respect to age, gender and height (Table 1).

Serum calcium, phosphor and Alkaline Phosphatase (ALP) levels of obese patients were higher than those of healthy controls ($p<0.002$, $p<0.0001$, and $p<0.0001$, respectively). Osteocalcin levels of obese patients were statistically higher than those of healthy controls (67.8 ± 17.5 ng/ml *vs.* 36.1 ± 18.2 ng/ml, $p<0.0001$). Leptin levels of obese patients were significantly higher than those of healthy controls (72.1 ± 32.0 ng/ml *vs.* 7.82 ± 6.57 ng/ml, $p<0.001$). The urine Deoxypyridinoline/Creatinine ratio (DPD/Cr) of obese children was significantly lower than that of healthy controls (15.4 ± 7.2 nM/mM *vs.* 24.9 ± 14.7 nM/mM, $p<0.001$). The BMD of obese children was higher than that of healthy controls (0.80 ± 0.18 g/cm^2 *vs.* 0.60 ± 0.19 g/cm^2, $p<0.0001$), and no significant differences in IGF-1 and IGFBP-3 levels were observed between obese children and healthy controls ($p>0.05$) (Table 1).

Among obese children, no significant differences in urine DPD/Cr, IGF-1, IGFBP-3, and serum calcium, phosphor, alkaline phosphatase, osteocalcin, and leptin levels were observed between girls and boys. Only BMD was higher in female obese children than in male obese children (0.85 ± 0.18 g/cm^2 *vs.* 0.73 ± 0.18 g/cm^2, p<0.05). Also, there were no significant differences in urine DPD/Cr, IGF-1, BMD, or serum calcium, phosphor, alkaline phosphatase, osteocalcin, and leptin levels regarding to pubertal status of obese children (p>0.05).

No significant differences in serum calcium, phosphor, alkaline phosphatase, IGF-1, IGFBP-3 levels, and urine DPD/Crs were observed between obese and non-obese girls (p>0.05 for all). Serum leptin levels of female obese children were significantly higher than those of female non-obese children (75.5 ± 34.8 ng/ml *vs.* 11.4 ± 6.8 ng/ml, p<0.001). The osteocalcin levels of female obese children were statistically higher than those of female non-obese children (67.5 ± 17.4 ng/ml *vs.* 30.9 ± 16.8 ng/ml, p<0.001). The BMD of female obese children was higher than that of female non-obese children (0.85 ± 0.18 g/cm^2 *vs.* 0.64 ± 0.21 g/cm^2, p<0.001).

No significant differences in serum calcium, phosphor and alkaline phosphatase, IGF-1 and IGFBP-3 levels were observed between obese and non-obese boys (p>0.05 for all). Serum leptin levels of male obese children were significantly higher than those of male non-obese children (67.2 ± 27.5 ng/ml *vs.* 5.02 ± 4.87 ng/ml, p<0.001). The osteocalcin levels of male obese children were statistically higher than those of male non-obese children (68.1 ± 18.0 ng/ml *vs.* 40.0 ± 18.6 ng/ml, p<00001). The urine DPD/Crs of male obese children were significantly lower than those of male non-obese children (15.3 ± 4.8 nM/mM *vs.* 25.2 ± 9.1 nM/mM, p<0.001). The BMD of male obese children was higher than that of male non-obese children (0.73 ± 0.18 g/cm^2 *vs.* 0.58 ± 0.18 g/cm^2, p<0.05).

In the present study, a positive correlation was found between serum leptin concentrations with BMD values and osteocalcin concentrations in obese children (r=0.38, p<0.01 and r=0.498, p<0.05, consecutively). Additionally, in obese children, positive correlations were found between DPD/Crs and serum IGF-1 concentrations (r=0.374, p<0.05). In obese children, negative correlations were found between DPD/Crs and serum IGFBP-3 concentrations (r=-0.374, p<0.05) and between DPD/Crs and serum osteocalcin concentrations (r=-0.314, p=0.05).

Table 1. *Anthropometric measures; serum and urinary bone markers; serum leptin, IGF-1, and IGFBP-3 levels; and bone mineral density results in children with exogenous obesity and healthy controls.*

	Obesity group (n=45)	Controls (n=39)	p
Age (months)	150.5 ± 36.8	138.6 ± 32.4	p>0.05
Gender(Girls/Boys)	27/18	17/22	p>0.05
Weight (kg)	67.3 ± 9.6	38.1 ± 14.2	p<0.001
Height (cm)	148.6 ± 18.6	149.2 ± 18.1	p>0.05
Weight for height (%)	156.6 ± 24.0	97.4 ± 5.59	p<0.001
BMI (kg/m^2)	28.4 ± 4.18	17.2 ± 2.29	p<0.001
Calcium (mg/dl)	9.88 ± 0.42	9.62 ± 0.32	p<0.01
Phosphor (mg/dl)	4.9 ± 0.71	4.2 ± 0.61	p<0.001
ALP (IU/L)	383.9 ± 149.5	217.4 ± 98.0	p<0.001
Osteocalcin (ng/ml)	67.8 ± 17.5	36.1 ± 18.2	p<0.001
DPD/Cr (nM/mM)	15.4 ± 7.2	24.9 ± 14.7	p<0.001
BMD (g/cm^2)	0.80 ± 0.18	0.60 ± 0.19	p<0.001
Leptin (ng/ml)	72.1 ± 32.0	7.82 ± 6.57	p<0.001
IGF-1 (ng/ml)	273.0 ± 121.1	280.9 ± 174.2	p>0.05
IGFBP-3 (mg/l)	5.02 ± 1.02	5.18 ± 1.51	p>0.05

Discussion

In this study, serum leptin levels of obese children were found to be significantly higher than those of healthy controls, like studies on humans which have found that leptin levels are higher in obese people than in non-obese people [14,15]. Because leptin levels are higher and the incidence of osteoporosis is lower in obese people, studies investigating the correlation between leptin levels and bone metabolism have been carried out and have shown as the serum leptin level increases, bone mass increases [16-17]. In our study, BMD in exogenous obese children was found to be higher than that in healthy controls, and a positive correlation between serum leptin levels and BMD values was observed in obese children. Some researchers have identified positive correlations between BMD and serum leptin levels, while other researchers have failed to show any correlations and still others have found negative correlations [9,11,12,18].

New bone growth and skeletal homeostasis are regulated by endocrine and/or humoral factors. Among the anthropometric and metabolic factors, body weight is the key determinant of bone mass. In obese people, the bone formation rate is denser during obese years, and the rate of bone loss is slower in subsequent years of life [19,20]. It is expected that being overweight increases the mechanical burden on bones and leads to an increase in density; however, the increase in density is observed in the metacarpus and radius, which do not suffer from overload associated with excess weight. The mechanism of this effect is unknown, although positive correlations have been identified between bone mass and leptin levels released from fatty tissue in adolescents [21,22]. Similarly, we found a positive correlation between serum leptin levels and BMD in children with exogenous obesity. A study by Hasanoglu et al. [23] of children with exogenous obesity in Turkey revealed that BMD values in pubertal obese children are high and that BMD values in girls are higher. Similarly, in the present study, BMD values in girls were higher than those in healthy controls. BMD values in prepubertal and pubertal obese children were found to be higher than those in healthy controls; however,

there were no differences in BMD values between prepubertal and pubertal obese children.

Leptin is thought to have two different effects on bone metabolism: a direct stimulating effect on osteoblastic differentiation, growth and mineralization and an indirect suppressive effect on bone development via the hypothalamus [24,25]. In this study, we identified correlations between serum leptin levels and osteoblastic and osteoclastic parameters in children with exogenous obesity. Serum osteocalcin levels are measured to assess the osteoblastic activity of the bone structure in particular. In this study, serum osteocalcin levels of children with exogenous obesity were found to be significantly higher than those of healthy children and also observed a positive correlation between serum leptin concentrations and serum osteocalcin concentrations in obese children. Research has shown that cells with osteoblastic activity in the human stromal cell pathway are targeted by leptin, and such cells express both the short and long forms of leptin receptors [19,26]. In a study by Thomas et al. [19], osteoblastic differentiation increased upon administration of leptin to human bone marrow cells, and this resulted in an increase in extracellular matrix mineralization. In the present study, we concluded that the correlation between increased leptin levels and increased osteocalcin levels in children with exogenous obesity may be associated with the increase in osteoblastic activity and higher BMD levels. In studies of leptin-free mice, leptin administration stimulated bone growth, and a dramatic increase in cortical bone formation was observed [27]. In fact, intraperitoneal administration of leptin to older ob/ob mice for a period of three weeks corrected the osteopenia and defects in bone growth that were observed by DEXA and peripheral quantitative CT despite a 40% decrease in food intake and a 17% decrease in body mass [27].

In this study, DPD/Cr levels were measured as an indicator of osteoclastic activity in children with exogenous obesity, urine DPD/Cr in the obese children was found to be significantly lower. Leptin inhibits the expression of the nuclear factor κB receptor activator (RANK), which is an important cytokine that regulates osteoclastogenesis in human stromal cells [28]. Moreover, leptin stimulates the expression of Osteoprotegerin (OPG), which is a potent inhibitor of osteoclastogenesis [28]. Our results indicate a decrease in osteoclastic activity in children with exogenous obesity; however, there was no correlation between serum leptin levels and osteoclastic activity.

Accordingly, *in vivo* and *in vitro* findings also suggest that leptin modulates both osteoblastic and osteoclastic activities and affects bone remodeling directly, although this was not demonstrated directly. Farooqi et al. [29] found no differences in bone mineral content and density in three leptin-free morbidly obese children when compared with their peers, despite highly advanced body weight and increased bone maturation. Body mass and fat mass decreased dramatically after subcutaneous administration of leptin for a period of four years, whereas BMD and skeletal structure increased normally, and this was attributed to the beneficial effects of leptin on bones. In this study, high levels of serum leptin in patients with exogenous obesity and high levels of indicators of osteoblastic activity and BMD support the correlation between increased serum leptin levels and bone structure in such children. In advanced studies carried out by Ducy et al. [30] on changes in the bone metabolism of ob/ob mice, leptin was shown to affect the skeleton indirectly through the hypothalamus. They also demonstrated that intracerebrovascular administration of leptin to 4-month-old ob/ob mice returns the bone changes to normal [30]. Intracerebrovascular administration of leptin did not result in a change in serum leptin levels, suggesting that the effect occurs through a central mechanism. Therefore, it is thought that leptin has two different effects on bone metabolism in animals: an indirect negative effect via the hypothalamic pathway when administered centrally and a direct positive effect when administered peripherally. Physiologically, the net effect of leptin on bones is based on the serum leptin concentration and the combination of anabolic peripheral and catabolic central effects arising from central transportation of leptin [8].

In addition, leptin increases the proliferation and differentiation of chondrocyte populations in skeletal growth centers in organ cultures [31,32]. Some of these effects may occur due to the regulation of IGF receptor expression by the IGF system [31]. IGF-1 has anabolic effects on muscle and bone tissue and has catabolic effects on fatty tissue. IGF-1 plays an important role in bone formation, and there is a positive correlation between serum IGF-1 levels and BMD [33]. Additionally, serum leptin levels and IGF-1 levels affect each other. Leptin increases IGF-1 receptor mRNA and protein in chondrocytes. In this study, no differences in IGF-1 levels in the exogenous obesity and control groups were observed, and no correlation was found between serum leptin and IGF-1 levels. However, a positive correlation was found between DPD/Crs and serum IGF-1 concentrations in the group with exogenous obesity. A correlation between serum leptin, IGF-1 and osteoclastic activity was not clearly demonstrated in this study.

In conclusion, leptin has important effect(s) on bone metabolism; however, such effect(s) could not be clarified with clinical studies. Briefly, the effect of leptin on bones varies based on age, gender and bone region. Leptin increases bone formation and has a positive effect on BMD when administered peripherally, whereas it has a negative effect when administered centrally. The peripheral positive effect may become prominent when obesity-induced leptin resistance occurs, and BMD is affected positively by the peripheral effect. In the present study, we reported an increase in BMD and serum osteocalcin levels; accordingly, such an increase in osteoblastic activity is associated with the increase in serum leptin levels. More comprehensive studies are required to evaluate the central and peripheral effects of leptin on bone metabolism.

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