

## Bone mineral density in obese children with prediabetes

### Prediyabeti olan obez çocukların kemik mineral dansitometreleri

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#### Abstract

**Aim:** The aim of this study was to evaluate the relationships between bone mineral density (BMD) vs metabolic risk factors in obese adolescents with prediabetes.

**Materials and Methods:** A total of 131 obese children and adolescents, aged 6-18 years of age were enrolled the study. Prediabetes was determined by a fasting blood glucose level of  $\geq 100$  to 125 mg/dL or 2-hour oral glucose tolerance test value of  $\geq 140$  to 199 mg/dL. Five patients who were diagnosed as having type 2 diabetes were excluded and remaining participants were classified as normal glucose tolerance (NGT) and prediabetic. BMD was measured on calcaneus using quantitative ultrasound. BMD and metabolic parameters were investigated and compared in these two groups.

**Results:** There was no significant difference in BMD between children and adolescents with prediabetes (n = 37) or NGT (n = 89). The parameters of prediabetes did not affect BMD and these results did not change when we adjusted for weight, height, age, sex, pubertal status. The multivariate linear regression analysis revealed that the only independent factor associated with higher BMD was higher BMI (OR=0.007 95%CI 0.002-0.013 p=0.013). No significant associations were found between BMD and waist circumference or biochemical measurements.

**Conclusion:** Plasma glucose within the prediabetic range is not associated with bone mineral density in obese children. Thus, the positive association of BMI with BMD may be by means of alternative pathways.

**Keywords:** Bone mineral density, obesity, prediabetes, children.

#### Öz

**Amaç:** Çalışmanın amacı prediyabetik obez çocuk ve ergenlerde kemik mineral dansitometresinin (KMD) metabolik faktörlerle ilişkisini araştırmaktır.

**Gereç ve Yöntem:** Çalışmaya 6-18 yaş arasında 131 adet çocuk ve ergen dahil edildi. Prediyabet kriteri açlık kan glikozunun  $\geq 100$ -125 mg/dL aralığında olması yada glikoz yükleme testi 2. saat glikoz değerinin  $\geq 140$ -199 mg/dL arasında olması olarak belirlendi. Bu ölçütlere göre tip 2 diyabet tanısı alan 5 hasta çalışma dışı tutulurken geri kalanlar prediyabet ve normal glikoz toleranslı (NGT) hastalar olmak üzere iki guruba ayrıldılar. KMD kalkaneustan kantitatif ultrason ölçüm cihazı ile yapıldı. Her iki grupta KMD ve metabolik parametreler karşılaştırıldı.

**Bulgular:** Gruplar arasında KMD açısından anlamlı bir fark yoktu. Prediyabet ölçütleri KMD'yi etkilemiyor üstelik ağırlık, boy, yaş, cinsiyet ve ergenlik gibi KMD üzerine olası etkileri olabilecek parametrelere göre düzenleme sonrasında bu durum değişmiyordu. Lineer regresyon analizinde beden kitle indeksi (BKİ) ile KMD arasında pozitif ilişki bulundu. Bel çevresi ve diğer metabolik faktörler ile KMD arasında ise bir ilişki bulunamadı.

**Sonuç:** Prediyabetik aralıktaki glikoz değeri ile KMD arasında herhangi bir ilişki yoktur. Obez çocuklarda fazla BKİ ile KMD arasındaki pozitif korelasyonu açıklayacak bilinen metabolik parametreler dışında alternatif etkenler olabilir.

**Anahtar Sözcükler:** Kemik mineral dansitometresi, obezite, prediyabet.

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## Introduction

Bone mass tends to constantly increase during childhood and adolescence (1). Diseases or conditions associated with inadequate bone mineral accrual during childhood may lead to suboptimal peak bone mass and risk of fracture in adulthood (2). Childhood obesity increases the risk of metabolic disorders including prediabetes, which has increased in prevalence amongst overweight and obese children, bringing significant implications for long-standing health (3). It remains controversial in the literature as to whether bone disease is a complication of obesity as some studies indicate that greater adiposity is connected with increased bone mass accrual, whereas others suggest that increased fat mass is not associated with better bone health (4-7). These inconsistencies in the obesity-bone quality studies have been attributed to the negative effect of prediabetes, which is not observed in all obese patients, on bone mineral density (BMD) (8). However, this conflict also exists among prediabetic patients. Whereas some studies report a positive association between prediabetes and bone mineral content or (BMC) (9,10), others suggest an inverse association (11,12) or no significant relationship (13). We aimed to investigate the relation between BMD and childhood obesity, and assess the effect of prediabetes on this relationship.

## Materials and Methods

In this cross-sectional study, we enrolled obese children who presented to the outpatient clinic of the Department of Pediatric Endocrinology and Diabetes at the Necmettin Erbakan University Research Center in Konya, Turkey. The inclusion criteria were as follows: age 6-18 years and obesity (BMI  $\geq 95^{\text{th}}$  percentile for age and sex). Children were excluded if they were taking medications or had a previous history of type 1 or type 2 diabetes, or had a situation known to influence body composition, bone, or insulin metabolism (e.g. genetic, metabolic, or endocrine diseases). A total of 131 obese children and adolescents were enrolled into the study. A glucose tolerance test was used to determine glucose metabolism abnormalities. Five patients who were diagnosed as having type 2 diabetes were excluded and the remaining participants were classified as normal or prediabetic. This study was approved by the local ethics committee and was conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Pubertal maturity was evaluated by pediatric endocrinologists in accordance with the definition of Marshall and Tanner. Tanner stage one was defined as prepubertal and Tanner stage two or above were defined as pubertal. Height was measured to the nearest 0.1 cm

using a rigid stadiometer. Body weight was measured while the participants were in their underwear to the nearest 0.1 kg using a balance beam medical scale. Body mass index (BMI) was calculated by body weight (kg) divided by height (m) squared. Waist circumference (cm) was measured at the midpoint between the last rib and the iliac crest. Blood samples were obtained the morning after overnight fasting from participants for assessment of glucose, insulin, triglycerides, total cholesterol, HDL-cholesterol, and LDL-cholesterol. After an overnight fast, an oral glucose tolerance test (OGTT) was performed using 1.75 g oral glucose solution per kilogram of body weight (to a maximum of 75 g). Blood was sampled and assayed for glucose and insulin at baseline and at 30, 60, 90, and 120 min relative to the oral glucose ingestion. Glucose and insulin were measured using glucose oxidase and immunoassay (IMMULITE Diagnostic Products Corporation, Los Angeles, CA), respectively. Vitamin D was measured using tandem mass spectrometry (ZIVAC).

Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the formula: fasting insulin ( $\mu\text{U/mL}$ ) x fasting glucose (mg/dL) / 405. In accordance with the American Diabetes Association Expert Committee on Diagnosis and Classification of Diabetes Mellitus guidelines (14), children were classified as prediabetic if they had impaired fasting glucose (IFG; fasting plasma glucose  $\geq 100$  mg/dL and  $< 126$  mg/dL) and/or impaired glucose tolerance (IGT; 2-h glucose  $\geq 140$  mg/dL and  $< 200$  mg/dL). BMD was measured in the calcaneus with an ultrasonographic bone densitometer (Hologic's Sahara Sonometer). The results were reported as BMD in grams per square centimeter.

## Statistical analysis

Statistical analyses were performed using SPSS version 21 for Windows (IBM SPSS Inc. Chicago, IL, USA). Categorical variables were presented as counts and percentages. Normality of the distribution of the data was assessed using Kolmogorov-Smirnov test. Continuous data were presented with mean  $\pm$  standard deviations or median (minimum-maximum) according to normality of distribution of the data. Categorical variables were compared using Chi-square test and continuous variables were compared using Student's t or Mann-Whitney U tests as needed. Univariate correlation analyses were performed using Pearson or Spearman correlation tests depending on the normality of distribution of the data. Partial correlations between BMD and other factors were assessed with correction for age, sex, weight, height, pubertal state. After adjusting for covariates, multiple linear regression analysis was

performed to the entire sample to identify independent correlates of total body BMD.

## Results

A total of 126 participants were enrolled to the study. A summary of the patients' characteristics based on glycemic status is shown in Table-1.

**Table-1.** Descriptive Characteristics of the Participants.

Variable	Normal Glucose n=(89)	Prediabetic <sup>a</sup> n=(37)
Sex (M/F)	62/27	20/17
Age (years)	11.4±2.9	11.4±3.0
Puberty (positive%)	65/24	27/10
Height (cm)	148(125-182)	146(119.5-177.5)
Weight (kg)	54.75(32-110)	63.5(28-100)
BMD (gr/cm <sup>2</sup> )	0.47(0.26-0.97)	0.48(0.34-0.69)
BMI (kg/m <sup>2</sup> )	27.7±4.9	27.8±4.3
Birth weight (g)	3231±643	3273±537
Fasting glucose(mg/dL)	89.4±5.5	101.5±8.1*
2-h glucose(mg/dL)	111.2±14.6	133.1±28.0*
Fasting insulin(mU/mL)	12(2.6-50)	14(4.5-62)*
2-h insulin (mU/mL)	47(2.5-221)	67(10.8-675)*
HOMA-IR	2.5(0.6-11.7)	3.4(1-18.7)*
HbA1c, %	5.5(4.9-5.9)	5.7(5.6-6.4)*
Triglycerides (mg/dL)	100.5(35-352)	96(35-229)
Total cholesterol (mg/dL)	159(107-232)	163(118-215)
HDL-C (mg/dL)	45.2±10.0	41.2±9.1*
LDL-C (mg/dL)	99.1±26.5	104.6±21.7
Alt (U/L)	19(9-100)	18(6-117)
Waist circumference (cm)	96.6±13.2	96.2±12.1
25 OH vitamin D	12.4±5.6	12.5±3.9

BMD: Bone mineral density; BMI: Body mass index; HOMA-IR: Homeostatic model assessment of insulin resistance; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol.

<sup>a</sup>Pre-diabetes was defined as having a fasting plasma glucose ≥100 mg/dL but <126 mg/dL or 2-h values in the oral glucose tolerance test of ≥140 mg/dL but < 200 mg/dL. \*p<0.05 compared with Normal Glucose Group

Eighty-nine of the children were classified as normal and 37 were classified as prediabetic. Fasting glucose, 2-hr glucose, fasting insulin, 2-hr insulin, HBA1C, HOMA-IR were higher and HDL-cholesterol was lower in the prediabetic group compared with the normal group. No significant differences existed between the groups regarding age, sex, pubertal status, height, weight, BMI, birth weight, triglycerides, total cholesterol, LDL-cholesterol, ALT, uric acid, vitamin D level and waist circumference. There was no significant difference in BMD between the prediabetic and normal children. Table-2 shows partial correlations (adjusting for weight, height, age, sex, pubertal status) in the total sample between the glucose - insulin variables, lipids, and BMD.

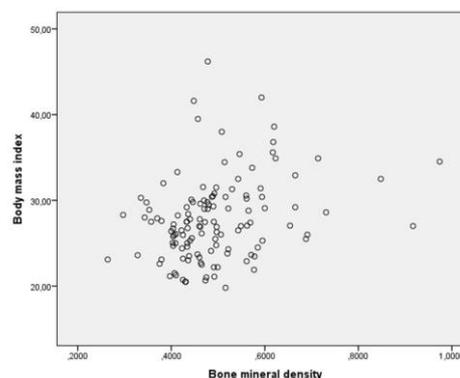
Levels of the these factors were not significantly correlated with BMD. The analyses were repeated based on prediabetes and the results did not change (Table-3). Multivariate linear regression analysis revealed that the independent factor associated with higher BMD was higher BMI (OR=0.007 95%CI 0.002-0.013 p=0.013) (data not shown). Figure-1 scatter/dot graph showing the correlation between BMI (kg/m<sup>2</sup>) and BMD (gr/cm<sup>2</sup>).

**Table-2.** Partial Correlations of BMD with Biochemical Parameters in the Total Sample (Adjusting For Weight, Height, Age, Sex, Pubertal Status).

Variable	r	p
Fasting glucose	-0,7	0,4
2-h glucose	0,1	0,3
Fasting insulin	-0,09	0,9
2-h insulin	0,15	0,11
HOMA IR	-0,16	0,86
Triglyceride	-0,4	0,6
HDL	-0,05	0,5
LDL	-0,12	0,9
Cholesterol	-0,04	0,6

**Table-3.** Partial correlations of BMD with biochemical parameters in obese children with normal glucose levels and with pre-diabetes (adjusting for weight, height, age, sex, pubertal status).

Variable	Normal glucose(n=89)	Prediabetes (n=39)
	r/p	r/p
Fasting glucose	-0.05/0.6	-0.1/ 0.5
2-h glucose	0.05/0.6	0.08/0.6
Fasting insulin	0.03/0.7	0.02/0.9
2-h insulin	0.03/0.7	0.3/0.1
HOMA-IR	0.02/0.8	0.02/0.9
Triglyceride	-0.12/0.9	-0.03/0.8
HDL	-0.14/0.2	0.2/0.1
LDL	0.013/0.9	0.04/0.9
Cholesterol	-0.042/0.7	0.02/0.9



**Figure-1.** Scatter/dot graph showing the correlation between BMI (kg/m<sup>2</sup>) and BMD (gr/cm<sup>2</sup>).

## Discussion

Rates of obesity and associated diabetes have increased for children of all age groups. As an example, when one considers the glucose metabolism among the youth in the United States of America, the prevalence of prediabetes / diabetes has recently increased from 9% to 23% (15). Few studies have reported on the effects of prediabetes on bone metabolism. The study of Afghani et al. (11) was the first in the pediatric population that sought the influence of prediabetes on bone in overweight children. After the study of Dimitri et al. (16) that suggested a relationship between childhood obesity and skeletal fractures, several studies were conducted regarding the relationship between obesity, prediabetes, and BMD (8,12,17,18).

Our study showed no results depicting a significant difference in BMD in prediabetic versus normal subjects. These results did not seem to change when the effect of sex and pubertal status were analyzed. Although these findings are in line with those of Afghani et al, they conducted their research in prepubertal children and defined the presence of prediabetes only with IGT (11). In our study fasting glucose, 2-h glucose, insulin, 2-h insulin, HOMA-IR, HbA1c, did not affect BMD and these results did not change when we adjusted for important covariates. This finding suggests that abnormal glucose regulation does not have major harmful effects on bone health in obese children. This result is consistent with some previous studies. Firstly, Lee et al. found no association between any prediabetes criteria and BMD in adult subjects (13). Another study in adolescent children with metabolic syndrome failed to show a relationship between glucose levels and BMD (18). However, several studies found a significant association between markers of glucose regulation and BMD, suggesting a detrimental effect of insulin resistance on bone health in children (8,11,12,17).

Among the factors that underlie these controversies, inhomogeneity between diagnostic criteria used to define prediabetes or glucose dysregulation may be important. Some of these studies only used the presence of IFG or IGT to indicate prediabetes and this may be considered an important limitation. However, Cheng et al suggested that fasting plasma glucose (FPG) is inadequate for viewing prediabetes in obese young people and the OGTT is the best way to detect the prediabetic state (19). Another study that investigated screening markers of IGT revealed that HbA1C was the only predictor of IGT (20). This data can explain why all markers of glucose regulation have not been consistently associated with BMD in these or other studies.

Studies in adults have shown that triglycerides and HDL were negatively correlated with BMD (21). Pollock et al's (8) studies found no association between lipids and BMD in prepubertal children. Silva (18) et al found that the association between lipids and BMD changes were dependent on sex; triglycerides were inversely correlated with BMD only in female adolescents. We observed no association between lipid levels and BMD, and this did not change according to the pubertal status or sex.

We found one significant relationship in this study, there was a positive correlation between BMI and BMD. Body size has been suggested as the strongest sole determinant of BMD. This association was attributed weight bearing and to the endocrine properties of adipose tissue, which forms estrogen from androstenedione (11). The study of Lorentzon and et al. (21) suggested that cortical bone parameters at the tibia were correlated with fat mass while this was not valid for the radius. This finding supports the positive effect of weight bearing on BMD. This could also explain the positive correlation that we found between BMI and the BMD of the calcaneus.

Visceral adiposity has been shown to be deleterious for bone health (17,18,22). This relationship is attributed to the strong association between metabolic disturbance and visceral rather than subcutaneous adiposity (18). Although we did not directly measure visceral adiposity, we assessed this parameter through measurement of waist circumference, which is often used as a substitute for visceral adiposity, and found no correlation between BMD and waist circumference. As with the direct effect of insulin resistance parameters, the indirect effects of these parameters such as waist circumference were also not correlated with BMD.

Despite high BMD in patients with type 2 diabetes (23), diabetes has been associated with an increased risk of bone fracture, possibly reflecting the effect of falls due to impairment of visual sharpness or proprioception caused by diabetes (24).

Obesity has been suggested to be a risk factor for Vitamin D deficiency. It is estimated that as much as 87.5% of obese children have vitamin D insufficiency / deficiency (25). We could not analyze the influence of vitamin D status on BMD between the two groups because of our small sample size, similar levels of vitamin D in the normal and prediabetic groups. Furthermore, the results of all patients whose vitamin D levels were studied were recorded as insufficient. It may be important to consider vitamin D status when analyzing the effect of obesity and the prediabetes on BMD. Vitamin D status has not been taken into account

by earlier studies that have investigated the relationship between BMD and prediabetes.

Our study has some limitations. Firstly this is a cross-sectional study. Secondly, ultrasonography was used to determine BMD in place of dual-energy X-ray absorptiometry (DEXA). However, ultrasonography is a reliable diagnostic tool for identifying osteoporosis (26) and quantitative ultrasound is commonly used in clinical practice because it is less costly and transportable than DEXA (27,28). Obesity is associated with elevated systemic inflammatory markers. A relation has been reported between rising inflammatory markers in obese patients and osteocalcin, a bone turnover marker (29).

On that basis, we might have obtained more enlightening data concerning BMD if we had investigated inflammatory markers and bone turnover markers in our study. Furthermore, data regarding dietary habits and physical exercise, the major risk factors for low BMD were not available in this study.

### Conclusion

Obesity-associated glucose metabolism abnormalities that serve as indicators for prediabetes had no influence on BMD. The relationship between obesity and BMD seems because of generalized rather than central obesity.

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