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Fibroblast Growth Factor 21 Levels and Bone Mineral Density in Metabolically Healthy and Metabolically Unhealthy Obese Children

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What is already known on this topic?

Fibroblast growth factor 21 (FGF21) is produced by the liver and plays a unique role in the regulation of carbohydrate and lipid metabolism. FGF21 increases glucose uptake into fat cells, thermogenesis, energy expenditure, fat use, and insulin sensitivity. There are limited studies evaluating the role of FGF21 in obese children and its effects on bone metabolism, and the results of these studies are contradictory.

What this study adds?

Although FGF21 levels were higher in obese children compared to non-obese children, this difference was not statistically significant. No correlation was found between FGF21 levels and bone mineral density.

Abstract

Objective: The harmful or beneficial effect of obesity on bone mineral density (BMD) remains controversial in children and adolescents. Fibroblast growth factor 21 (FGF21) is a metabolic factor that plays a specific role in the regulation of carbohydrate and lipid metabolism. However, the role of FGF21 in bone metabolism appears paradoxical and is complex. To determine whether serum FGF21 level was associated with BMD in obese children and adolescents.

Methods: The study was conducted with the participation of children and adolescents aged 8-18 years. Ninety-eight obese children were included in the study group and 44 children were included in the control group. BMD, in addition to the routine obesity workup, which includes fasting blood glucose, fasting insulin levels, lipid profile, and liver enzymes; serum FGF21 levels have been analyzed.

Results: The mean age of the obese group (n = 98) was 13.34 ± 2.24 years and the mean age of controls (n = 44) was 13.48 ± 2.87 years. Based on International Diabetes Federation criteria, 15 of 98 (15.3%) patients were metabolically unhealthy. FGF21 levels were 193.54 ± 139.62 mg/dL in the obese group and 158.69 ± 151.81 mg/dL in the control group ($p = 0.06$). There was no difference between the FGF21 and BMD z-score values of girls and boys in the obese and control groups ($p > 0.05$).

Conclusion: BMD-z-score was increased in obese children compared to healthy control. Moreover, BMD-z-score tended to be higher when more metabolic risk factors were present. However, there was no significant relationship between FGF21 levels and BMD z-score values in obese children.

Keywords: Obesity, children, bone mineral density, FGF21

Introductıon

Since obesity is an important multifactorial problem caused by the interaction of eating behavior, physical activity, environmental conditions and genetic characteristics, it has proven challenging both to treat existing obesity and to design effective strategies for prevention (1). Obesity causes metabolic problems, such as insulin resistance, type 2 diabetes, atherosclerotic heart disease, non-alcoholic fatty liver disease, hypertension and hyperlipidemia (2).

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The association between obesity and healthy bone tissues has recently been highlighted as an area of concern (3). However, it is difficult to determine whether excess adipose tissue in the body is beneficial or harmful to growing bone tissue. While some studies reported increased bone mass in overweight children and adolescents compared to peers of normal weight (4,5,6), others concluded that obesity was associated with less bone mass (7,8,9). It has been suggested that bone mass gain may be reduced in adolescents with obesity-related metabolic disorders, and suboptimal peak bone mass gain in this period will bring the risk of osteoporosis in later life. It has been reported that bone mineral density (BMD) was decreased in obese adolescents, especially as the degree of hyperinsulinism increased and cardiometabolic risk factors accumulated (3).

Fibroblast growth factor (FGF) 21 (FGF21), a member of the family of FGF, is produced by the liver, and consists of 209 amino acids with a molecular weight of 23 kilodaltons. FGF21 plays a unique role in the regulation of carbohydrate and lipid metabolism (10). FGF21 increases glucose uptake into fat cells, thermogenesis, energy expenditure, fat use and insulin sensitivity. It has been suggested that FGF21 may be beneficialfor metabolic health by reducing blood glucose and lipid levels (11). The role of FGF21 in bone metabolism appears paradoxical because it can both improve metabolic health but also reduce bone formation. The relationship between various metabolic health parameters, including FGF21, insulin sensitivity and body composition in obese adolescents is not fully understood (12). Therefore, the aim of this study was to investigate differences in BMD between metabolically healthy obese (MHO) and metabolically unhealthy obese (MUO) children and the healthy controls and the association with metabolic parameters including serum FGF21 levels.

Methods

The study was conducted with the participation of children and adolescents who attended a single Pediatric Endocrinology outpatient clinic. Informed consent was obtained from the patients and their families. Ethical approval was obtained from Ankara University Faculty of Medicine Clinical Research Ethics Committee with the number 08-418-17 and made in accordance with the Helsinki Declaration (date: 24.04.2017). The study group included obese children, defined as body mass index (BMI) $>95^{th}$ percentile, aged between >8 and <18 years of age, who had no chronic systemic disease and did not use any medication. Patients with an active infection at the time of blood collection or who refused to give consent were excluded.

The weight of the participants was measured wearing light clothing. The height measurements were made with 1 mm spaced fixed meter with heels, hips and head against the wall without shoes, and measurements were evaluated according to the norms of Turkish children with age and are sex taken into consideration (13). Height standard deviation (SD) score (SDS), BMI, BMI %, BMI z-score, waist circumference, hip circumference, and waist/hip circumference ratios were determined. BMI was calculated with the formula of body weight (kg)/square of height (m^2) and children over the 95^{th} percentile were accepted as obese. Waist circumference was measured from the 10^{th} costa and iliac crest where the waist was the thinnest, while the children were standing upright while the abdomen was relaxed. Hip circumference was measured around the large trochanter while children were standing upright. Measurements were evaluated in accordance with previously published hip circumference reference values (14).

Blood pressure measurements were performed with a blood pressure measuring device having a suitable sleeve to cover the upper 2/3 of the arm using the upper right arm. Measurements were made in the morning before breakfast, after at least 30 minutes rest and in a sitting position. The patients were evaluated according to age, sex and height using the appropriate percentile curve. Patients with hypertension were identified. Physical examination was performed to identify acanthosis nigricans and puberty staging was undertaken according to the Tanner-Marshall classification (15,16).

Blood samples were taken between 8 am and 9 am after fasting for 12 hours at night. Fasting blood glucose, fasting insulin, lipid profile, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) levels were measured. In addition, after the blood sample was taken for FGF21 level, serum was separated and stored within an hour of collection at -80 °C. Biochemical evaluations were performed in Central Biochemistry and Endocrinology Laboratories.

Methodologies for testing were as follows.

Serum FGF21 levels were measured using a commercially available enzyme-linked immunosorbent assay (Biovendor Research and Diagnostic Products, Czech Republic). Absorbance measurements at 450 nm were performed on a microplate reading device ELx800 (Bio-Tek Instruments, Inc., USA). Serum samples were diluted 1:2 with buffer dilution prior to analysis to measure FGF21 levels according to manufacturer instruction. The standard curve range for analysis was 30-1920 pg/mL and sensitivity is reported as 7 pg/mL. Intra-assay and inter-assay coefficient of variation were 3.0-4.1% and 3.6-3.9%, respectively.

Glucose was tested by the glucose hexokinase method, total cholesterolwas determined enzymatically by an oxidase method while high-density lipoprotein-cholesterol (HDL-C) was measured directly by a non-immunological method. Triglyceride (TG) at concentrations of < 400 mg/dL was derived from the Friedewald formula but TG > 400 mg/dL was measured by homogeneous enzymatic method on a Roche Modular automatic biochemistry analyzer (Roche Diagnostics, Germany). Creatinine was determined by the Jaffe rate blanked method and fasting insulin levels were measured by radioimmunoassay.

Metabolic health status was assessed by both the presence of metabolic syndrome (MS) and assessment of cardiometabolic risk factors clustering (CMRFC).

The presence of MS in the study group was investigated according to the criteria by International Diabetes Federation (IDF) for children aged 10-18 years (17). MHO and MUO groups were determined according to IDF criteria. The IDF criteria for MS in obese children and adolescents are: waist circumference percentile ≥90th with at least two of the following:

1. Dyslipidemia: TG ≥150 mg/dL or HDL-C ≤40 mg/dL

2. Blood pressure: Systolic ≥130 mmHg/diastolic ≥85 mmHg systolic ≥130 mmHg or diastolic ≥85 mmHg-

3. Fasting blood glucose > 100 mg/dL or known type 2 diabetes history.

The presence of CMRFC was accepted if at least two of the following criteria were present (18).

1. Waist circumference percentile ≥90

2. TG ≥150 mg/dL

3. HDL-C ≤40 mg/dL

4. Blood pressure: Systolic and diastolic ≥90-95th

5. Fasting blood sugar > 100 mg/dL.

BMD measurements were performed by the DEXA Norland method on a Hologic Discovery Discovery DXA (Hologic) device. The children were supine with knees slightly bent to correct physiological lumbar lordosis. Hips were positioned in the supine decubitis position. The antero-posterior vertebral L2-4 was measured in three minutes, and evaluated for BMD, bone mineral content (BMC) and length (cm). Deviations in BMD were evaluated with z-score according to the reference values for each age and sex of the childhood age group. The z-scores of the subjects were calculated according to the following formula (19): BMD z-score = (measured BMD - age and gender matched control BMD)/ age and gender matched control standard deviation). If the z-score calculated according to this formula is lower than "-2 SD", the patient's BMD was considered low (the z-score calculated according to this formula that was considered to be less than "-2 SD" BMD).

Statistical Analysis

Statistical analysis of the data was performed using Statistical Package for the Social Sciences for Windows, version 20.0 (IBM Inc., Armonk, NY, USA). Descriptive statistics are presented as both mean ± SD and median (minimum-maximum) for continuous variables. Nominal changes were shown as number and frequency. Chi-square or Fisher's exact test were used to compare percentages between groups. In order to compare the continuous variables in two groups, the fit of the data to the normal distribution was tested (with chi-square), then the Mann-Whitney U test or t-test while for comparison of more than two groups ANOVA or Kruskal-Wallis variance analysis was used. Tukey test and Kruskal-Wallis multiple comparison tests were used to investigate which group or groups were the cause of any significant differences. Spearman correlation coefficient was used to investigate the correlation between continuous data. Statistical significance was assumed when $p < 0.05$.

Results

A total of 142 subjects, of whom 98 (69%) were obese and 44 (31%) were healthy controls, were included. The mean age of the obese group was 13.34 ± 2.24 years and the mean age of the control group was 13.48 ± 2.87 years. The sex dstribution in the two groups were 56.1% girls and 43.9% were boys in the obese group and 72.2% girls and 27.3% boys in the control group. There was no significant difference between the obese and control groups in terms of age and gender ($p > 0.05$).

There was no significant difference between obese and control groups in terms of diastolic and systolic blood pressure, height, and height SDS values. As expected bodyweight (BW), BW SDS, BMI, BMI%, BMI percentile, BMI SDS, waist circumference, hip circumference and waist/hip ratio values were significantly higher in the obese group compared to the control group ($p < 0.001$) (Table 1).

The mean values of fasting insulin, total cholesterol, TG, very low-density lipoprotein-cholesterol (VLDL-C), low-density lipoprotein-cholesterol (LDL-C), and ALT were significantly higher in the obese group compared to the control group ($p < 0.001$) (Table 2).

Based on IDF criteria, 15 of 98 (15.3%) patients in the obese group met the MS criteria, occurring at a rate of 16.3% in obese girls and 14% in obese boys. Thus the group was further stratified into 15 (15.3%) MUO and the remaining

Table 1. Clinical characteristics of all cases

	Obese (n = 98)	Control (n = 44)	p value
Female/male (n %)	55 (56.1 %)/43 (43.9 %)	32 (72.7 %)/712 (27.3 %)	0.060
Prepubertal/pubertal (n %)	9 (9.2 %)/89 (90.8 %)	3 (6.8 %)/741 (93.2 %)	0.754
Age (years)	13.34 ± 2.24 13.13 (9.06-17.7)	13.48 ± 2.87 14.08 (8.17-17.74)	0.771
Diastolic blood pressure (mmHg)	65.89 ± 10.18 60 (50-112)	63.84 ± 6.89 60 (60-85)	0.471
Systolic blood pressure	101.59 ± 15.81 100 (80-162)	103.25 ± 14.70 100 (80-135)	0.467
Height SDS	0.78 ± 1.18 0.70 (-2.11-3.95)	0.59 ± 1.23 0.61 (-1.69-3.88)	0.404
Weight SDS	2.17 ± 0.97 2.09 (0.39-5.32)	-0.20 ± 1.26 -0.41 (-2.55-3.12)	< 0.001
% BMI	139.94 ± 17.17 136.5 (107.4-200.36)	95.33 ± 1786 90.32 (67.74-152.69)	< 0.001
BMI (kg/m^2)	27.96 ± 3.79 27.78 (20.9-38.27)	18.82 ± 3.54 18.55 (12.16-29.05)	< 0.001
BMI percentile (%)	96.02 ± 5.12 97.35 (64.43-99.98)	36.16 ± 33.89 19.22 (0.18-99.06)	< 0.001
BMI SDS	2.08 ± 0.63 1.94 (0.37-3.78)	-0.55 ± 1.28 -0.87 (-2.91-2.35)	< 0.001
Waist circumference (cm)	92.47 ± 10.23 93 (70-115)	72.79 ± 11.54 75 (48-96)	< 0.001
Hip circumference (cm)	104.59 ± 10.49 105 (80-130)	86.14 ± 10.89 87.5 (60-104)	< 0.001
Waist/hip ratio	1.13 ± 0.14 1.13 (0.10-1.36)	1.17 ± 0.14 1.18 (0.58-1.47)	0.038

*Data are given as mean ± standard deviation and median (min-max) except for sex and puberty which are shown as frequency, given as n (%).
BMI SDS: body mass index standard deviation score, min-max: minimum-maximum

Table 2. Laboratory parameters of all cases

	Obese (98)	Control (44)	p
	Mean ± SD* Median (min-max)**	Mean ± SD Median (min-max)	
FBG (mg/dL)	87.78 ± 7.32 88 (60-104)	83.55 ± 5.71 83 (68-95)	0.001
Fasting insulin (mIU/mL)	25.19 ± 13.22 21.85 (10.1-112-4)	10.09 ± 3.01 10.20 (4-16)	< 0.001
Total cholesterol (mg/dL)	169.50 ± 30.97 171.5 (110-246)	152.43 ± 25.82 146 (102-204)	0.002
Triglyceride (mg/dL)	108.69 ± 45.02 102.5 (42-289)	74.52 ± 1.77 70.5 (36-136)	< 0.001
HDL-C (mg/dL)	45.57 ± 12.99 44 (30-129)	49.64 ± 10.13 48.5 (31-75)	0.045
LDL-C (mg/dL)	101.50 ± 24.71 98.5 (46-165)	89.66 ± 24.45 84 (55-154)	0.006
VLDL-C (mg/dL)	21.97 ± 8.98 21 (8-58)	14.82 ± 3.80 14 (7-27)	< 0.001
ALT (U/L)	18.39 ± 8.84 17 (4-69)	12.93 ± 4.55 12 (7-28)	< 0.001
AST (U/L)	21.26 ± 7.86 20 (11-75)	20.48 ± 5.19 19.5 (11-32)	0.815

*Mean ± SD. **Median (minimum-maximum).
HDL-C: high-density lipoprotein-cholesterol, LDL-C: low-density lipoprotein-cholesterol, VLDL-C: very low-density lipoprotein-cholesterol, ALT: alanine aminotransferase, AST: aspartate aminotransferase, FBG: fasting blood glucose, SD: standard deviation

83 (84.7%) were considered MHO. All patients with MS were over 10 years old. No patient met the MS criteria in the control group. CMRFC, another metabolic health parameter, was found in 57.1% of the obese group and 13.6% in the control group. As expected, the presence of MS and CMRFC were significantly higher in the obese group compared to the control group ($p < 0.06$ and $p < 0.001$, respectively). Fasting blood glucose, fasting insulin, total cholesterol, TG, LDL-C and ALT levels were significantly higher in the MUO group than in the control group (Table 3).

BMD z-score were 1.19 ± 1.48 (g/cm^2) in the obese group and 0.48 ± 1.75 g/cm^2 in the control group. BMD z-score values were significantly higher in the obese group compared to the control group ($p < 0.013$) (Table 4). The BMD z-score in the obese group was 1.28 ± 1.42 in girls [median 0.95 (-1.3-6.17)] and 1.09 ± 1.58 [median 0.89 (-1.44-5.6)] in boys. There was no difference between the BMD z-score values of girls and boys in the obese group ($p > 0.05$).

BMD z-score values were different between MUO and MHO and control groups. BMD z-score values were significantly higher in the MHO compared to the control group ($p < 0.044$) (Figure 1a). BMD z-score values were found to be significantly different between obese without CMRFC, obese with CMRFC, and control groups ($p < 0.016$). BMD z-score values were significantly higher in the obese patients without CMRFC group compared to the control group (Figure 1b). There was no significant correlation between BMD z-score and total cholesterol, TG, LDL, VLDL, HDL and ALT ($r = 0.09$ $p = 0.36$, $r = 0.09$ $p = 0.4$, $r = 0.09$ $p = 0.4$, $r = 0.1$ $p = 0.3$, $r = 0.02$ $p = 0.88$, and $r = 0.09$ $p = 0.37$, respectively).

No relation was found between FGF21 level and age, body weight, BW SDS, BMI, BMI%, BMI percentile, BMI SDS, waist circumference, hip circumference, fasting blood sugar, fasting insulin, total cholesterol, HDL-C, LDL-C, ALT, AST values and BMD z-score in obese and control groups ($p > 0.05$). There was a negative correlation between FGF21 and height SDS, and a positive correlation with TG and VLDL-C ($p < 0.045$, $p < 0.049$, $p < 0.025$, respectively) (Table 5). No significant correlation was found between HOMA-IR, BMD z-score and FGF21 ($r = -0.01$, $p = 0.90$ and $r = 0.04$, $p = 0.70$, respectively).

Table 3. Laboratory parameters and BMD z-scores of MHO, MUO and control groups (FGF21 values were not different between MHO, MUH and control groups. Fasting blood glucose, fasting insulin, total cholesterol, triglyceride, LDL-C and ALT levels were significantly higher in the MUO group than in the control group. BMD z-score values are significantly higher in the MHO group than in the control group)

	MHO (15)	MUO (83)	Control (44)	p value
	Mean ± SD* Median (min-max)**	Mean ± SD Median (min-max)	Mean ± SD Median (min-max)	
FGF21 (mg/dL)	196.69 ± 140.09 149.57 (32.48-735.79)	176.08 ± 140.47 141.92 (22.53-451.87)	158.69 ± 151.81 102.31 (11.82-853.65)	0.135
Fasting blood glucose (mg/dL)	87.18 ± 6.93 88 (60-104)	91.07 ± 8.74 89 (71-103)	83.55 ± 5.71 83 (68-95)	0.001
Fasting insulin (mIU/mL)	24.58 ± 13.20 20 (10.1-112.4)	28.55 ± 13.29 22.7 (14.9-63.8)	10.09 ± 3.01 10.20 (4-16)	< 0.001
Total cholesterol (mg/dL)	170.84 ± 31.55 175 (110-246)	162.07 ± 27.33 164 (114-214)	152.43 ± 25.82 146 (102-204)	0.004
Triglyceride (mg/dL)	104.65 ± 43.61 99 (42-289)	131.07 ± 47.64 127 (60-218)	74.52 ± 1.77 70.5 (36-136)	< 0.001
HDL-C (mg/dL)	47.96 ± 13.54 45 (30-129)	40.32 ± 6.62 38.8 (33-59)	49.64 ± 10.13 48.5 (31-75)	0.004
LDL-C (mg/dL)	102.59 ± 25.45 101 (46-165)	95.47 ± 19.68 94 (63-134)	89.66 ± 24.45 84 (55-154)	0.016
VLDL-C (mg/dL)	21.19 ± 8.70 20 (8-58)	26.27 ± 9.57 25 (12-44)	14.82 ± 3.80 14 (7-27)	< 0.001
ALT (U/L)	17.98 ± 7.54 17 (4-46)	20.67 ± 14.22 18 (8-69)	12.93 ± 4.55 12 (7-28)	< 0.001
AST (U/L)	21.34 ± 8.20 20 (11-75)	20.80 ± 5.85 20 (12-34)	20.48 ± 5.19 19.5 (11-32)	0.969
BMD z-scores	1.22 ± 1.53 0.97 (-1.44-6.170)	1.09 ± 1.25 0.56 (-0.13-3.31)	0.48 ± 1.75 0.13 (-2.67-5.76)	0.044

*Mean ± SD. **Median (min-max).
FGF21: fibroblast growth factor 21, HDL-C: high-density lipoprotein-cholesterol, LDL-C: low-density lipoprotein-cholesterol, VLDL-C: very low-density lipoprotein-cholesterol, ALT: alanine aminotransferase, AST: aspartate aminotransferase, BMD: bone mineral density, MHO: metabolically healthy obese, MUO: metabolically unhealthy obese, SD: standard deviation, min-max: minimum-maximum

It was demonstrated that FGF21 values were not different between obese without MS, obese with MS and control groups ($p > 0.05$). Similarly, FGF21 values were not found to be significantly correlated for obese without CMRFC, obese with CMRFC, and control groups (Figure 2a, 2b) ($p > 0.05$).

FGF21 levels were 193.54 ± 139.62 pg/mL in the obese group and 158.69 ± 151.81 pg/mL in the control group. The difference was not significant ($p > 0.06$) (Table 4).

Discussion

The prevalence of obesity in childhood can lead to psychological and social problems, as well as increasing the burden of chronic diseases, creating an important public health problem (20). The presence of MS in obese populations has been described for many years, identified by reference to a range of metabolic health parameters (21). MS refers to a combination of risk factors for cardiovascular diseases, including abdominal obesity, dyslipidemia, glucose intolerance and hypertension. The presence of these risk factors in childhood increases the likelihood of developing MS, type 2 diabetes and cardiovascular disease in adulthood. In addition to MS, CMRFC has been defined in recent years and accepted as another metabolic health parameter (18). In the present study both MS and CMRFC were compared and used for metabolic health evaluation. Since CMRFC has at least two parameters, its rate would be expected higher than MS, as in our cases. As expected, the presence of MS and CMRFC were significantly higher in the obese group compared to the control group ($p < 0.05$).

Different results have been obtained in different studies researching BMD values in adolescent obesity. In our study, BMD z-score levels were significantly higher in the

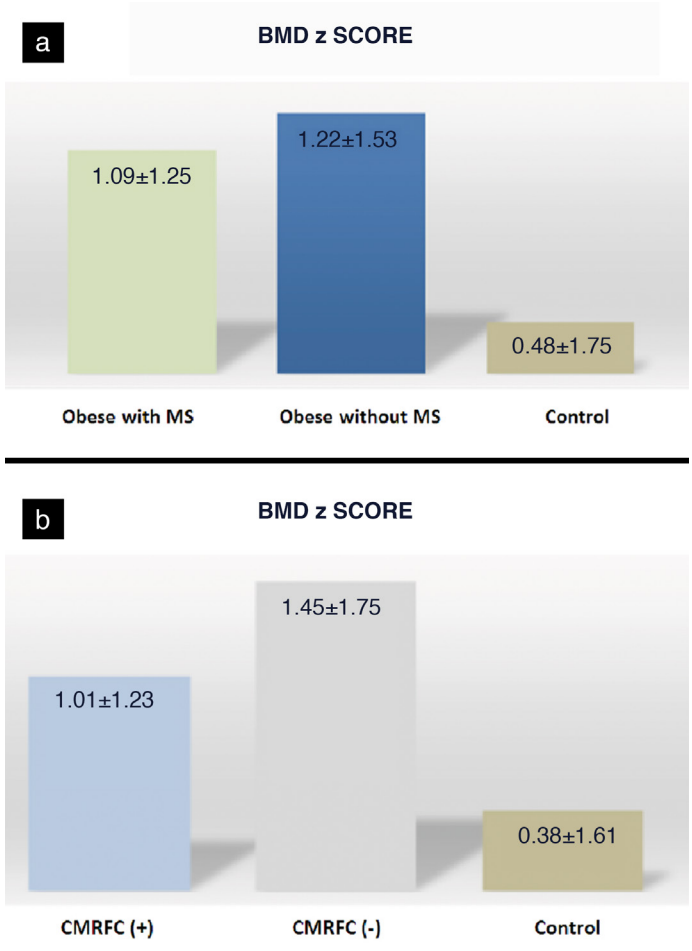

Figure 1. a) BMD z-score (g/cm^2) in obese with MS, obese without MS and control groups [BMD z-score values are significantly higher in the obese without MS group than in the control group ($p < 0.044$)]. **b)** BMD z-score (g/cm^2) in CMRFC (+) obese, CMRFC (-) obese and control groups [BMD z-score values were significantly higher in the CMRFC (-) obese group than in the control group ($p < 0.016$)]

BMD: bone mineral density, MS: metabolic syndrome, CMRFC: cardiometabolic risk factors clustering

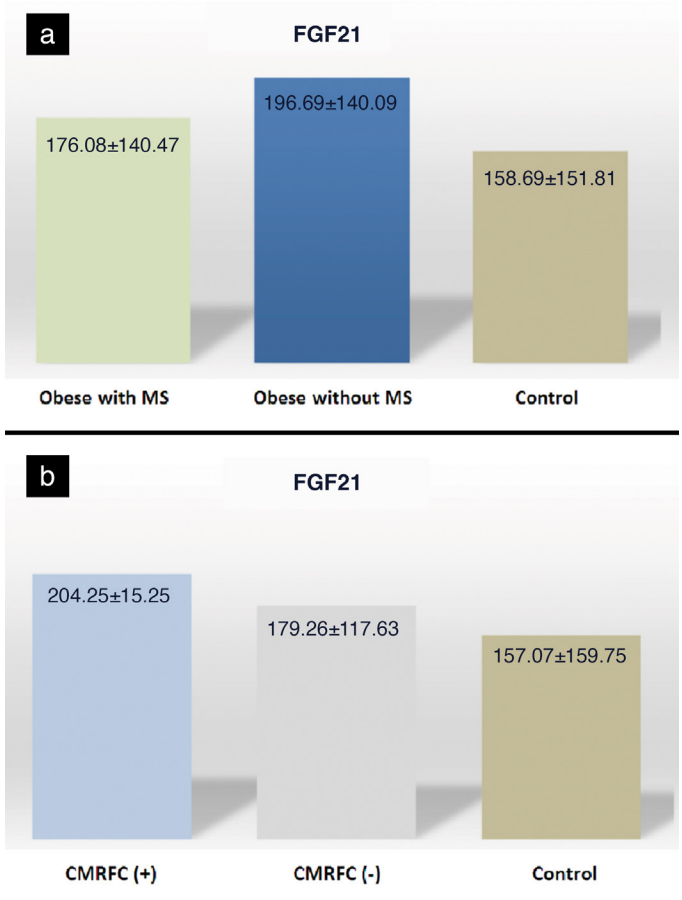

Figure 2. a) FGF21 (pg/mL) in obese with MS, obese without MS and control groups ($p > 0.05$). **b)** FGF21 (pg/mL) in CMRFC (+) obese, CMRFC (-) obese and control groups ($p > 0.05$)

MS: metabolic syndrome, CMRFC: cardiometabolic risk factors clustering, FGF21: fibroblast growth factor 21

Table 4. Comparison of obese and control group FGF21 (pg/mL) and BMD z-score (g/cm²) values

	Obese (98)	Control (44)	p value
	Mean ± SD Median (min-max)	Mean ± SD Median (min-max)	
FGF21	193.54 ± 139.62 147.66 (22.53-735.79)	158.69 ± 151.81 102.31 (11.82-853.65)	0.064
BMD z-score	1.19 ± 1.48 0.93 (-1.44-6.17)	0.48 ± 1.75 0.13 (-2.67-5.76)	0.013

FGF21: fibroblast growth factor 21, SD: standard deviation, min-max: minimum-maximum, BMD: bone mineral density

Table 5. Correlation between variables with FGF21 values in the obese and control groups

		FGF21	
		Obese	Control
Age (years)	r	0.094	-0.033
	p	0.358	0.833
Weight	r	0.095	-0.031
	p	0.353	0.841
Height	r	0.005	-0.159
	p	0.963	0.304
Height SDS	r	-0.051	-0.304
	p	0.616	0.045(*)
BMI (kg/m²)	r	0.132	0.017
	p	0.196	0.915
BMI percentile (%)	r	0.070	0.038
	p	0.493	0.807
BMI SDS	r	0.074	0.038
	p	0.473	0.807
Waist circumference (cm)	r	0.197	-0.075
	p	0.051	0.626
Hip circumference (cm)	r	0.019	-0.105
	p	0.856	0.499
Fasting blood glucose (mg/L)	r	-0.803	0.069
	p	0.416	0.657
Fasting insulin (mIU/mL)	r	0.056	0.053
	p	0.581	0.731
Total cholesterol (mg/dL)	r	-0.096	0.066
	p	0.349	0.669
Triglyceride (mg/dL)	r	-0.059	0.298
	p	0.562	0.049(*)
HDL-C (mg/dL)	r	0.103	-0.024
	p	0.314	0.875
LDL-C (mg/dL)	r	-0.109	0.036
	p	0.285	0.817
VLDL-C (mg/dL)	r	-0.078	0.338
	p	0.446	0.025(*)
ALT (U/L)	r	0.115	-0.197
	p	0.260	0.200
BMD z-scores (g/cm²)	r	-0.151	0.003
	p	0.137	0.985
BMD	r	-0.110	-0.010
	p	0.279	0.949

(*): $p < 0.05$.
HDL-C: high-density lipoprotein-cholesterol, LDL-C: low-density lipoprotein-cholesterol, VLDL-C: very low-density lipoprotein-cholesterol, ALT: alanine aminotransferase, AST: aspartate aminotransferase, BMD: bone mineral density, FGF21: fibroblast growth factor 21, BMI SDS: body mass index standard deviation score, min-max: minimum-maximum

obese group compared to the control group. Adolescence is a critical period for bone development and about 40% of adult skeletal calcium accumulates during this period (22). Therefore, maximizing BMD during this period may result in less osteoporosis and better protection against fracture in adulthood. Irreversible factors, such as gender, race, ethnicity, and genetics contribute to 60-80% of bone mass and environmental and lifestyle factors contribute to the remaining 20-40%. Diet and physical activity are the most widely studied factors to maximize BMD (23).

Bone regeneration and consequently skeletal homeostasis is governed by endocrine and/or humoral factors. Among anthropometric and metabolic factors, body weight is the main determinant of bone density (24). Many studies on the effect of excess adipose tissue on growing bone tissue have yielded variable results. Some studies reported more bone mass in overweight children and adolescents than in normal-weight peers, while other studies concluded that it was associated with less bone mass (4,25). Differences in these studies can be attributed to methodological limitations (3). The greater bone mass in obesity may be due to greater mechanical load on the bone. In addition, regional fat distribution may affect bone mass, independent of obesity (26). In our study, BMD z-score levels were significantly higher in the obese group compared to the control group, suggesting a positive association between obesity and increased BMD.

While a minimum level of fat is required for bone tissue to mature, excess adiposity is associated with increased bone size. However, this can have a negative effect on bone quality. During adolescence, body fat has been associated with larger bones in boys and larger and denser bones in girls (26). Baxter-Jones et al. (27), in a study of children aged 8-19 years, reported that total body and femoral neck BMD were higher in boys than in girls, but no gender differences were observed in vertebral BMD. In a study by Singhal et al. (28), which included 153 adolescent girls, parameters related to regional bone density and degree of obesity were higher in normal-weight adolescent girls compared to obese adolescent girls. In a study by Singhal et al. (28) involving 153 adolescent girls, bone density including areal BMD

z-scores from all regions was found to be higher in obese adolescent girls than in normal-weight adolescent girls. Other studies have shown that there is no difference in total BMD between boys and girls in the 9-11 age range (29,30). In the present study, there was no difference between BMD z-score values in obese girls and boys.

The metabolic effects of obesity may have an impact on bone development. Although some studies (31) have reported BMD increases in adults with MS, other studies have reported the opposite (32). These contradictory reports may partly depend on the heterogeneous samples examined but the findings suggest that the increase in BMD does not persist as metabolic health begins to deteriorate. The idea that insulin resistance due to obesity in children with MS may adversely affect BMD was first proposed by Afghani et al. (33). These authors found that BMD decreased in overweight children with insulin resistance. It has been suggested that the most important metabolic factor that may cause a negative assocaition between insulin levels and BMD is insulin resistance. Impaired glucose homeostasis has been shown to have a negative effect on the growing skeleton (34). Kindler et al. (35), conducted a study on children between the ages of 7-15 where body fat mass, waist circumference and insulin resistance were found to be negatively correlated with total body and vertebral BMD. In another study by Kindler et al. (36), insulin resistance was determined to be a potential inhibitor of IGF-I-dependent cortical bone development. Body fat mass and insulin resistance were found to be inversely associated with bone mass. Improvement in insulin resistance was seen to increase BMD in obesity (37). In addition, as the number of cardiometabolic risk factors increased, BMD decreased (3). In the present study the mean BMD z-score values were higher in the MHO obese group. In addition, no statistically significant relationship was found between insulin and HOMA-IR levels and BMD. However, there appears to be a decrease in BMD in the presence of MS or CMRFC. These results suggest a significant increase in BMD in simple obesity but only when MS or CMRFC has not yet occurred. However, a decrease in BMD was evident in the presence of MS or CMRFC. This suggests that obesity-related metabolic problems have negative effects on BMD and that a metabolic unhealthy state in obesity negatively affects BMD.

Adipokines secreted from adipose tissue and cytokines secreted from other tissues have a regulatory role in metabolism in obesity. One of these regulators is FGF21. It is produced in metabolically active tissues such as the pancreas, skeletal muscle, adipose tissue and placenta, but mainly in the liver. Experimental and clinical data revealed that FGF21 is a potent endocrine regulator with physiological effects on weight loss, insulin sensitivity, glucose and lipid metabolism (38). FGF21, which contributes to the regulation of insulin synthesis, inhibits β cell proliferation in pancreatic islet cells (11). FGF21 increases the effect of insulin as an insulin sensitizer and decreases glucose production during long post-starvation feeding or overeating (39). FGF21is also an important energy metabolism regulator with its beneficial effects on glucose and lipid metabolism (40). In animal studies, FGF21 has been shown to lower blood sugar levels and inhibit glucagon secretion (11). Animal studies have also shown that FGF21 improves hyperglycemia, hyperlipidemia and insulin resistance and thus it is suggested that FGF21 may ameliorate the development of type 2 diabetes (41). We did not find any relationship between FGF21 levels and anthropometric parameters, fasting blood sugar, fasting insulin, blood lipid profile, and ALT in both the obese group and the control group. These results suggest that not only FGF21 but also other factors may affect the mentioned parameters.

Although FGF21 acts as a protective molecule, most studies in adults reported that increased FGF21 levels are associated with an increased risk of obesity, MS and type 2 diabetes mellitus. This increases the likelihood of FGF21 resistance playing a role in the pathogenesis of some human metabolic disorders. However, to date, data specific to children is limited and remains controversial (42). For example, there are some studies in the literature that correlate circulating FGF21 levels with the amount of adipose tissue in the body (43) and there are other studies that do not detect this relationship (12). A similar situation exists for insulin resistance (42).

In a study by Reinehr et al. (43), comparing normal-weight children with obese children, FGF21 levels were significantly increased in obese children but this significant increase was not observed in the MS group. In a study by Korwutthikulrangsri et al. (44), serum FGF21 levels were high in obese children with insulin resistance or abnormal glucose tolerance. In another study, 210 children over nine years of age were evaluated, and no relationship was found between MS and FGF21 levels (45). The reason for these differences between studies may be due in part to the possibility of pubertal effects on small sample sizes and FGF21 levels (46). In addition, two studies showed no significant relationship between FGF21 levels and MS (43,45). FGF21 levels in obese children and adults were found to be significantly higher than normal-weight children and adults (43,47). In this study, we investigated whether FGF21 levels were different in obese children and their potential relationship with BMD in terms of metabolic health. Although FGF21 levels were higher in the obese

group compared to the control group, this difference was not significant (p = 0.06). Obese subjects did not differ from control groups according to their metabolic health status, when defined by either MS or CMRFC criteria. These results were similar to some alreay reported. Actually had similar results with the studies in the literature. We cannot rule out an effect of small sample size in our study as there were only 15 obese children who met the IDF criteria for being MUO. A further reason for the lack of significant differences may be the shorter duration of obesity compared to adult obesity. Finally, FGF21 might exert less effect on metabolic health for this age group.

FGF21 is a peptide that has also been reported to have a direct effect on bone as well as its metabolic effects. It has been suggested that FGF21 reduces osteoblast production from mesenchymal stem cells. However, it also increases fat cell production and ultimately reduces bone formation. Experimental animals treated with FGF21 were found to have bone loss, especially in the trabecular cortex of the bone. FGF21 is thought to directly disrupt bone formation (46). Data on FGF21 levels in obese children and adolescents are very limited. In our study, no statistically significant relationship was found that could relate changes in BMD and insulin levels with FGF21 levels. A recent study reported an inversely proportional relationship between FGF21 and lean muscle mass in girls aged 7-12 years. In addition, an inverse relationship was found between BMC and FGF21 in all cases (12). In another study of obesity accompanied by insulin resistance and hyperinsulinism, increased levels of FGF21 were reported. These authors reported that FGF21 receptor expression was decreased and FGF21 resistance, manifested as increased serum levels, was found (48).

Study Limitations

Limitations of our study should be noted. These include the number of patients in the control group being around 45% of the number in the obese group. Furthermore, the number of patients in the obese group who met the IDF criteria for MS was only 15 (15.3%) of the patients although none of the healthy controls met these criteria. The proportion of the obese group meeting the CMRFC criteria was higher at 57% but nearly 14% of controls also met these criteria.

Conclusion

Metabolic health parameters, such as hyperinsulinism and dyslipidemia were higher, and the frequency of MS and CMRFC was higher in the obese group, as expected. FGF21 levels did not differ between obese and healthy groups. In the control group, FGF21 level was negatively correlated with height SDS and positively correlated with TG and VLDL. Although BMD was significantly higher in the obese group than in the control group, it began to decrease with deterioration of metabolic health status. Changes in BMD in MUO obese children was shown to become more evident. This study suggests that metabolic changes should be considered together, without expecting isolated changes in insulin level or FGF21 level to be significant.

Ethics

Ethics Committee Approval: Ethical approval was obtained from Ankara University Faculty of Medicine Clinical Research Ethics Committee with the number 08-418-17 and made in accordance with the Helsinki Declaration (date: 24.04.2017).

Informed Consent: Informed consent was obtained from the patients and their families.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Filiz Akduman, Concept: Zeynep Şıklar, Merih Berberoğlu, Design: Zeynep Şıklar, Merih Berberoğlu, Data Collection or Processing: Filiz Akduman, Elif Özsu, Özlem Doğan, Metin Kemal Kır, Analysis or Interpretation: Zeynep Şıklar, Özlem Doğan, Metin Kemal Kır, Merih Berberoğlu, Literature Search: Filiz Akduman, Zeynep Şıklar, Elif Özsu, Writing: Filiz Akduman, Zeynep Şıklar, Merih Berberoğlu.

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