Int J Endocrinol Metab. 2015 July; 13(3): e25542. DOI: 10.5812/ijem.25542v2
Published online 2015 July 1. **Research Article**

Relative Importance of Lean and Fat Mass on Bone Mineral Density in Iranian Children and Adolescents

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Received: November 25, 2014; **Revised:** February 16, 2015; **Accepted:** April 14, 2015

Background: Body weight is made up of lean and fat mass and both are involved in growth and development. Impression of these two components in bone density accrual has been controversial.
Objectives: The aim of this study was to evaluate the relationship between fat and lean mass and bone density in Iranian children and adolescents.
Patients and Methods: A cross-sectional study was performed on 472 subjects (235 girls, 237 boys) aged 9-18 years old in Fars Province. The participants' weight, height, waist circumference, stage of puberty, and level of physical activity were recorded. Bone Mineral Content (BMC), Bone Mineral Density (BMD), total body fat and lean mass were measured using dual-energy X-ray absorptiometry.
Results: Results showed that 12.2% of boys and 12.3% of girls were overweight and 5.5% of boys and 4.7% of girls were obese. Obese individuals had greater total body BMD (0.96 ± 0.11) than normal-weight ones (0.86 ± 0.11) ($P < 0.001$). We found the greatest correlation between total body BMD and total body lean mass ($R = 0.78$. $P < 0.001$) and the least correlation with total body fat percentage ($R = 0.03$, $P = 0.44$). Total lean mass in more active boys was 38.1 ± 10.9 and in less active boys was 32.3 ± 11.0 ($P < 0.001$). The results of multiple regression analysis showed that age and total body lean mass were independent factors of BMD in growing children and adolescents.
Conclusions: These findings suggest that lean mass was the most important predictor of BMD in both genders. Physical activity appears to positively impact on lean mass and needs to be considered in physical education and health-enhancing programs in Iranian school children.

Keywords: Child; Bone Density; Obesity; Body Fat; Lean Mass

1. Background

Osteoporosis is a worldwide public health concern especially in postmenopausal and old individuals and is defined as the low bone mass and microarchitectural changes in the bone. The most serious issue of this problem is the fragility fracture (1).

Maximum bone mass acquisition is established during childhood and adolescence, which is affected by environmental, hormonal and genetic factors (2, 3). After that, the bone mass decreases progressively since 35 years of age in both male and female sexes, and the rate of decline is very high in postmenopausal women (1). Peak bone mass and subsequent bone loss is two important determinants in osteoporosis development (1).

Now, childhood obesity is a global epidemic. The International Obesity Task Force (IOTF) reported that 1 in 10 children worldwide (a total of 150 million) is overweight and about 30 to 45 million of them are obese (4). Although most comorbidities are related to obesity, this metabolic problem seems to have been protective against osteoporosis in adulthood, because of the positive effect of mechanical loading conferred by body weight on bone formation (5). A new idea is that obesity results in bone mass accrual by increased mechanical loading and/or anabolic effect of adipokines or fatty acids (6). However, the actual effect of the adipose tissue on Bone Mineral Density (BMD) is very complex and unclear (7, 8).

Bone is a dynamic organ and bone mass at any time explains the balance between bone formation by osteoblasts and bone resorption by osteoclasts. On the basis of available data, the effect of obesity on bone metabolism is explained through several mechanisms (8). One of them is that obesity, by increasing adipogenesis, may decrease osteoblastogenesis because adipocytes and osteoblasts are derived from a common multipotential stem-cell (8). Secondly, obesity is associated with chronic inflammation, and proinflammatory cytokines are the main mediators of osteoclast differentiation, and bone resorption is increased in chronic inflammatory disorders (8). Finally, a high-fat diet may interfere with intestinal calcium absorption by insoluble calcium soaps produced from free fatty acids (8).

Although previous animal studies established the negative effect of adiposity on bone metabolism (8, 9), there is still controversy about the effect of fatness and obesity on the Bone Mineral Content (BMC) and density during human growth. Some previous studies are in favor of positive correlation between body fat and bone density (2, 10) and some other researchers found an inverse relationship between body fat and bone parameters (7, 11).

In a previous study, we showed that Body Mass Index (BMI) is a significant predictor of BMD in Iranian children and adolescents (12). We know that body weight is largely made up of two components, i.e. fat mass and lean mass. Generally, both lean and fat mass have a significant effect on bone mass, but in adult population lean mass is more important (13).

2. Objectives

In children, literature review showed a gap of knowledge and some controversy about the effect of body composition on BMD; thus, the aim of this study was to evaluate the relationship between fat and lean mass and bone density in Iranian children and adolescents.

3. Patients and Methods

This cross-sectional study was performed in Kawar, an urban community located 50 Km east of Shiraz, the capital of Fars Province, southern Iran, during 2012 - 2013.

3.1. Subjects

Our subjects were girls and boys aged 9 - 18 years who were pupils of elementary, guidance, or secondary schools. The participants were selected using systematic random sampling. An age-stratified systematic random sample of 7.5% was applied, and eventually 472 subjects (234 girls and 238 boys) participated in this study. Exclusion criteria were any chronic disease or medication that affects bone, such as rheumatologic and endocrinal disorders, renal failure, musculoskeletal disease, and usage of steroid and anticonvulsant drugs. The study was approved by the ethics committee of Shiraz University of Medical Sciences. The consent form was obtained from the parents of all participants.

3.2. Anthropometric Measurements and Tanner Stage

The participants' weight and height were measured by a physician. Weight was measured with a standard scale to the nearest 0.1 Kg (Seca, Germany), with the participant wearing light clothing and no shoes. Height was measured to the nearest 0.5 cm with a wall-mounted meter with the participant standing barefooted. Body mass index (BMI) was calculated by dividing weight (kg) by height per square meter.

We divided subjects into two groups based on their BMI: 1-Overweight (85th percentile $\leq$ BMI $<$ 95th percentile), 2-Obsess (BMI $\geq$ 95th percentile) (14). Based on our subjects' BMI cut-off points and also age-and sex-specific BMI cut-off points as defined by the IOTF cut-off points (15). The 5-stage puberty classification of Tanner was determined by an endocrinologist for each participant. Children at stages I and II of puberty were classified as pre-early puberty, children at stages III and IV as midpuberty, and children at stage V were classified as full puberty (maturity).

The participants were divided into two groups with fewer or more than three times physical activity per week according to the recommendation of the American College of Sports Medicine (16). The participants or their parents were asked how many days per week they did any physical activity including physical education classes, organized sports, recreational activity, regular walking, or cycling.

3.3. Bone Densitometry Assessment

The Hologic system (Discovery QDR, USA) was used to measure BMC (g), and BMD (g/cm^2). Bone mineral density was measured in the total body, lumbar spine and left femoral neck. Also, we measured Total Body Fat Mass (TBFM), total body lean mass, trunk fat mass and trunk lean mass in grams and also Total Body Fat Percentage (TBFP) and android gynoid ratio. Densitometry studies were done with the participants wearing special clothing and no footwear. To eliminate physiological lumbar lordosis during measurement of the lumbar spine, we elevated the participants' knee while they were in supine position. In accordance with international standards, all measurements of the femur were done on the left femur at the position of internal rotation (17). Scanner stability was checked throughout the course of the study with plots of daily spine phantom scans. We calculated Fat Mass Index (FMI) (Kg/m^2) by dividing total body fat mass (Kg) by height square (m^2). Based on preliminary measurements in 10 children, the Coefficient of Variation (CV) in our laboratory were 1% for the total body BMD, 0.51% for the lumbar spine BMD and 2.4% for the femoral neck BMD. The CV for fat mass was 0.7%, and for fat percentage and lean mass was 1.9%.

Estimated volumetric Bone Mineral Apparent Density (BMAD) was calculated for the Lumbar Spine (LSBMAD) and Femoral Neck (FNBMAD) according to the following Equations (12):

$$\text{LSBMAD} = \text{BMC} \quad \text{of} \quad L2 - \frac{L4}{\text{are}a^{1.5}} \tag{1}$$

$$\text{FNBMAD} = \text{BMC} \quad \text{of} \quad \text{femoral}\frac{\text{neck}}{\text{are}a^{2}} \tag{2}$$

3.4. Laboratory Data

Blood samples were taken by experienced technicians at the Shiraz Endocrinology Research Center and used to measure 25-hydroxide (OH) vitamin D by high performance liquid chromatography (Young Lee 9100, South Korea) in ng/mL.

3.5. Statistical Analysis

Statistical analysis included t-tests and ANOVA that was used for comparing mean levels of anthropometric, body composition and bone density parameters between two genders and between normal weight, overweight and obese individuals. Pearson's correlation coefficient was used to evaluate the relationship between body composition and bone density. To evaluate association between body composition and bone parameters, we used multiple regression analysis. The model was adjusted for age, stage of puberty, and level of 25 (OH) vitamin D. Data were analyzed using SPSS v. 18 software (Chicago, IL, USA). A P value < 0.05 was considered significant.

4. Results

Table 1 shows Mean and Standard Deviation (SD) for weight, height, waist circumference, BMI, TBFM, and TBFP in each age group in boys and girls (Table 1). We found that 12.2% of the boys and 12.3% of the girls were overweight (85 ≤ BMI < 95 percentile) and 5.5% of boys and 4.7% of girls were obese (BMI ≥ 95 percentile).

4.1. Bone Parameters in Overweight and Normal Individuals

Table 2 shows Mean and Standard Deviation (SD) for anthropometric and Dual-energy X-ray Absorptiometry (DXA) measures in boys and girls according to normal body weight, overweight and obese subjects. (Table 2) The mean age was not different between normal weight, overweight and obese subjects (P = 0.46). In the girls, only neck BMC, neck BMD and neck BMAD in overweight ones were more than normal subjects (P = 0.01, P = 0.03, P = 0.005); overweight boys had significantly higher total body BMC (P = 0.02), total body BMD (P = 0.02), and neck BMC (0.04) than the normal boys.

In comparison of obese and normal subjects, we found that all bone parameters in obese individuals were greater than those of normal subjects. Obese girls had greater total body BMC and BMD (P = 0.003), neck BMC, BMD and BMAD (all P = 0.001), lumbar spine BMC, BMD and BMAD (P = 0.002, P = 0.008, P = 0.001) than normal weight girls; however, obese boys only had greater lumbar spine and neck BMAD than the normal weight participants (both P = 0.04)

4.2. Correlation Between Bone Parameters and Body Composition

We studied the correlation between BMC and BMD with BMI, waist circumference, TBFM, TBFP, trunk fat mass, FMI, total body lean mass, trunk lean mass, and android gynoid ratio (Table 3).

We found the greatest correlations between bone parameters and lean mass (total lean mass and trunk lean mass) and the least correlation between bone parameters and total body fat percentage. Femoral neck BMC and BMD were negatively correlated with TBFP (P < 0.001 and P = 0.02). We evaluated this correlation in girls and boys separately and found that in both genders the greatest correlation was seen between bone parameters and total body lean mass. In boys, and not in girls, BMC and BMD in the lumbar spine, femoral neck and total body were negatively correlated with TBFP. (-0.18, -0.13, -0.16; P = 0.004, 0.03, 0.01, respectively). Also, we studied this correlation in different stages of puberty and again found out the greatest correlation for total body lean mass in all stages of growth.

4.3. Total Body Fat Percentage Versus Trunk Fat Percentage

We compared the correlation between bone parameters with TBFP and trunk fat percentage and found that lumbar spine BMC, total body BMC, and total body BMD didn't have a significant correlation with any of them. Lumbar spine BMD was positively correlated with TBFP (R = 0.18, P < 0.001), and trunk fat percentage (R = 0.19, P < 0.001). Femoral neck BMC had a negative association with TBFP (R = -0.20, P < 0.001), and trunk fat percentage (R = -0.15, P < 0.001). In the girls, the lumbar spine, femoral neck and total body BMC and BMD were positively correlated with TBFP and trunk fat percentage (P < 0.001). In the boys, the only significant correlation with trunk fat percentage was seen for the lumbar spine BMC (R = -0.14, P = 0.02) Moreover, the correlation between TBFP and all bone parameters was negative (P < 0.05).

4.4. Multiple Linear Regression Analysis

The relationship between fat and lean mass and bone measures was determined using multiple regression, adjusting for age, stage of puberty, and level of 25 (OH) vitamin D in the boys and girls (Tables 4 and 5).

We showed in these models that total body lean mass was the significant predictor of all bone parameters in both genders other than BMAD of the lumbar spine and femoral neck in the boys. On the other hand, total body fat mass had a significant association with femoral neck BMD and BMAD only in the girls. In all these models, we found the greatest effect for age and total body lean mass on the BMC and BMD. No significant difference was found between different stages of puberty in these models. However, in Tanner V the effect of lean mass was stronger than before (standardized coefficient 0.42 in Tanner stage I and 1.15 in Tanner stage V for total body lean mass in prediction of total body BMC, and standardized coefficient 0.31 and 0.78 for total body BMD) (has not shown in table).

Table 1. Anthropometric and Body Fat Measures in Girls and Boys in Each Age Group [a,b]

Age, y	Gender	Number	Weight, Kg	Height, m	BMI (Kg/m^2)	Waist Circumference, cm	25 (OH) vitamin D, ng/mL	Total body fat mass, Kg	Total body fat percentage, %	Total body lean mass, Kg
9										
	Female	9	26.4 ± 5.2	129 ± 6.0	15.7 ± 2.2	57.7 ± 6.1	14.8 ± 1.9	7.4 ± 3.9	25.3 ± 6.8	19.9 ± 2.2
	Male	11	26.0 ± 3.6	131.2 ± 4.1	15.0 ± 1.5	55.5 ± 3.9	16.8 ± 5.7	4.9 ± 1.7	18.1 ± 4.3	20.7 ± 2.5
10										
	Female	27	28.3 ± 4.7	134.6 ± 6.7	15.6 ± 1.9	58.9 ± 5.4	14.4 ± 5.0	7.3 ± 2.2	24.7 ± 4.0	20.3 ± 5.6
	Male	21	29.5 ± 4.2	136.2 ± 5.9	15.8 ± 1.5	59.6 ± 6.2	15.9 ± 3.9	6.4 ± 2.9	20.8 ± 6.3	22.4 ± 2.2
11										
	Female	26	29.6 ± 5.7	139.0 ± 8.6	15.1 ± 1.6	60.8 ± 5.4	15.3 ± 6.9	8.4 ± 3.7	25.3 ± 6.0	23.0 ± 4.8
	Male	23	30.7 ± 4.4	139.0 ± 6.3	15.9 ± 1.9	60.2 ± 6.9	15.8 ± 7.0	6.1 ± 2.5	19.1 ± 5.1	24.0 ± 3.0
12										
	Female	26	35.6 ± 6.5	148.9 ± 7.6	15.9 ± 2.0	65.0 ± 7.9	15.9 ± 5.0	9.6 ± 3.9	25.3 ± 6.2	26.5 ± 4.4
	Male	22	34.5 ± 5.3	145.5 ± 5.2	16.2 ± 2.2	60.6 ± 6.2	15.7 ± 4.8	6.6 ± 2.8	18.8 ± 5.4	26.4 ± 2.8
13										
	Female	25	42.5 ± 9.4	153.8 ± 5.5	17.8 ± 3.0	69.4 ± 6.5	14.6 ± 6.0	11.1 ± 4.2	25.3 ± 5.7	30.3 ± 4.2
	Males	25	40.4 ± 8.0	153.9 ± 7.7	16.9 ± 2.6	64.0 ± 8.4	17.6 ± 6.3	8.7 ± 4.7	19.8 ± 7.3	32.3 ± 5.9
14										
	Female	27	45.8 ± 8.7	154.9 ± 5.8	19.0 ± 2.7	72.2 ± 6.5	14.5 ± 4.7	13.6 ± 6.0	29.5 ± 6.6	31.2 ± 4.5
	Male	32	45.4 ± 11.2	160.7 ± 10.0	17.3 ± 2.8	66.0 ± 9.2	14.4 ± 0.0	8.6 ± 4.8	17.6 ± 5.6	36.9 ± 8.8
15	Female	21	48.4 ± 6.5	159.5 ± 4.7	19.0 ± 2.4	75.1 ± 8.5	13.6 ± 4.3	14.2 ± 3.7	28.8 ± 5.0	32.9 ± 4.4
	Male	32	52.3 ± 0.4	168.3 ± 7.6	18.4 ± 2.7	72.1 ± 9.0	14.5 ± 4.8	8.8 ± 5.2	16.0 ± 6.6	41.9 ± 6.0
16	Female	23	52.4 ± 9.1	158.5 ± 5.6	20.8 ± 3.3	79.5 ± 8.8	14.1 ± 4.2	16.3 ± 5.6	30.4 ± 5.4	34.1 ± 5.0
	Male	28	55.0 ± 11.2	169.7 ± 7.2	18.9 ± 2.9	75.8 ± 7.7	18.6 ± 7.8	8.3 ± 4.7	14.9 ± 4.9	42.1 ± 9.6
17										
	Female	21	48.2 ± 12.6	158.9 ± 5.5	19.5 ± 4.2	78.1 ± 9.1	15.1 ± 6.7	15.3 ± 6.6	30.1 ± 0.1	32.0 ± 5.3
	Male	19	61.0 ± 12.4	173.1 ± 4.6	20.3 ± 4.0	78.4 ± 7.0	15.0 ± 6.2	9.6 ± 5.5	15.2 ± 6.1	48.1 ± 7.4
18										
	Female	29	50.5 ± 7.6	159.6 ± 5.5	19.8 ± 2.7	73.2 ± 8.1	14.8 ± 0.3	15.1 ± 4.3	30.3 ± 6.3	33.8 ± 3.3
	Male	22	60.1 ± 11.3	175.8 ± 6.5	19.3 ± 3.0	75.4 ± 8.9	13.0 ± 4.9	8.6 ± 4.0	14.2 ± 4.9	48.3 ± 8.2
Total										
	Female	234	41.5 ± 11.9	150.7 ± 11.6	17.9 ± 3.3	69.4 ± 10.1	14.80 ± 5.30	12.05 ± 5.55	27.65 ± 6.23	28.89 ± 67.06
	Male	235	44.8 ± 14.5	157.4 ± 15.9	17.5 ± 3.0	67.5 ± 10.4	15.70 ± 5.78	7.90 ± 4.36	17.39 ± 6.11	35.38 ± 11.33

[a] Abbreviations: BMI, body mass index; OH, hydroxy.
[b] Values are presented as (Mean ± SD).

Table 2. Anthropometric and Dual-Energy X-ray Absorptiometry Measures in Normal, Overweight and Obese Subjects [a,b]

	Normal Weight			Overweight			Obese		
	Boys (195)	Girls (195)	Total (390)	Boys (29)	Girls (29)	Total (58)	Boys (13)	Girls (11)	Total (24)
Weight, Kg	42.1 ± 12.5	39.1 ± 10.0	40.6 ± 11.4	55.5 ± 15.7 [c]	48.9 ± 12.6 [c]	52.2 ± 14.5 [c]	61.0 ± 18.8 [c]	64.0 ± 10.1 [c]	62.4 ± 15.2 [c]
Height, m	156.9 ± 16.0	150.2 ± 11.5	153.6 ± 14.3	159.9 ± 15.9	150. 9± 12.7	155.4 ± 15.0	158.3 ± 15.6	157.2 ± 8.4	157.8 ± 12.6
BMI, Kg/m^2	16.6 ± 2.1	17.0 ± 2.5	16.3 ± 2.3	21.1 ± 2.4 [c]	20.9 ± 2.5 [c]	21.0 ± 2.4 [c]	23.6 ± 3.1 [c]	25.7 ± 2.4 [c]	24.6 ± 2.9 [c]
Waist Circumferences, cm	65.5 ± 9.3	67.7 ± 9.1	66.6 ± 9.2	76.1 ± 10.2 [c]	75.1 ± 10.9 [c]	75.6 ± 10.5 [c]	79.1 ± 11.0 [c]	85.0 ± 7.9 [c]	81.8 ± 9.9 [c]
Total body fat mass, Kg	6.5 ± 2.8	10.8 ± 4.3	8.7 ± 4.2	12.3 ± 3.3 [c]	16.4 ± 5.8 [c]	14.4 ± 5.1 [c]	18.0 ± 5.1 [c]	21.8 ± 8.78 [c]	19.8 ± 7.1 [c]
Total body fat percentage, %	15.6 ± 4.7	26.4 ± 5.6	21.0 ± 7.4	23.2 ± 5.1 [c]	32.1 ± 5.2 [c]	27.6 ± 6.8 [c]	29.8 ± 4.3 [c]	37.3 ± 4.1 [c]	33.2 ± 56 [c]
Total body lean mass, Kg	34.2 ± 10.8	27.9 ± 6.1	31.0 ± 9.3	40.5 ± 12.0 [c]	32.1 ± 7.5 [c]	36.3 ± 10.8 [c]	41.2 ± 13.1 [c]	37.7 ± 5.5 [c]	39.6 ± 10.3 [c]
Lumbar Spine BMC, g	39.7 ± 16.2	40.3 ± 15.0	39.8 ± 15.1	46.4 ± 22.7	42.8 ± 14.8	44.6 ± 19.1	43.8 ± 15.4	55.0 ± 18.0 [c]	48.9 ± 17.2 [c]
Lumbar Spine BMD, g/cm^2	0.80 ± 0.15	0.85 ± 0.17	0.83 ± 0.16	0.88 ± 0.21	0.90 ± 0.17	0.89 ± 0.19 [c]	0.85 ± 0.17	0.98 ± 0.12 [c]	0.91 ± 0.16 [c]
Lumbar spine BMAD, g/cm^3	0.19 ± 0.03	0.21 ± 0.03	0.20 ± 0.03	0.19 ± 0.05	0.22 ± 0.03	0.21 ± 0.04	0.21 ± 0.03 [c]	0.24 ± 0.02 [c]	0.22 ± 0.03 [c]
Neck BMC, g	3.6 ± 0.9	3.0 ± 0.6	3.3 ± 0.8	4.0 ± 1.0 [c]	3.3 ± 0.6 [c]	3.7 ± 0.9 [c]	4.0 ± 1.1	3.7 ± 0.8 [c]	3.9 ± 0.9 [c]
Neck BMD, g/cm^2	0.74 ± 0.13	0.65 ± 0.10	0.70 ± 0.12	0.78 ± 0.13	0.72 ± 0.12 [c]	0.75 ± 0.13 [c]	0.81 ± 0.13	0.80 ± 0.14 [c]	0.81 ± 0.13 [c]
Neck BMAD, g/cm^3	0.15 ± 0.02	0.14 ± 0.02	0.14 ± 0.02	0.15 ± 0.02	0.16 ± 0.02 [c]	0.15 ± 0.02 [c]	0.16 ± 0.02 [c]	0.17 ± 0.03 [c]	0.16 ± 0.02 [c]
Total Body BMC, g	1490.4 ± 467.2	1344.6 ± 353.9	1417.5 ± 420.3	1755.0 ± 575.0 [c]	1482.0 ± 388.1	1618.5 ± 505.3 [c]	1732.2 ± 608.1	1817.4 ± 399.7 [c]	1771.2 ± 514.0 [c]
Total Body BMD, g/cm^2	0.88 ± 0.11	0.87 ± 0.11	0.86 ± 0.11	0.93 ± 0.12 [c]	0.89 ± 0.11	0.91 ± 0.12 [c]	0.93 ± 0.11	0.99 ± 0.11 [c]	0.96 ± 0.11 [c]

[a] Abbreviations: BMI, body mass index; BMC, bone mineral content; BMD, bone mineral density; BMAD, bone mineral apparent density.
[b] Values are presented as Mean ± SD.
[c] $P < 0.05$ in comparison with normal weight individual.

Table 3. Correlation Between Bone Mineral Content, Bone Mineral Density, and Bone Mineral Apparent Density and Anthropometric and Dual-Energy X-ray Absorptiometry Measures in Iranian Children and Adolescents [a,b]

	Lumbar Spine						Femoral Neck						Total Body			
	BMC		BMD		BMAD		BMC		BMD		BMAD		BMC		BMD	
	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P
BMI, Kg/m²	0.60	< 0.001	0.61	< 0.001	0.52	< 0.001	0.55	< 0.001	0.56	< 0.001	0.38	< 0.001	0.67	< 0.001	0.65	< 0.001
Waist circumference, cm	0.65	< 0.001	0.66	< 0.001	0.54	< 0.001	0.58	< 0.001	0.54	< 0.001	0.29	< 0.001	0.71	< 0.001	0.67	< 0.001
TBFM, Kg	0.44	< 0.001	0.51	< 0.001	0.51	< 0.001	0.26	< 0.001	0.31	< 0.001	0.28	< 0.001	0.44	< 0.001	0.45	< 0.001
TBFP, %	0.06	0.19	0.18	< 0.001	0.32	< 0.001	-0.20	< 0.001	-0.10	0.02	0.06	0.14	-0.03	0.40	0.03	0.44
Trunk fat mass, Kg	0.42	< 0.001	0.49	< 0.001	0.51	< 0.001	0.25	< 0.001	0.30	< 0.001	0.27	< 0.001	0.42	< 0.001	0.43	< 0.001
FMI, Kg/m²	0.23	< 0.001	0.33	< 0.001	0.42	< 0.001	0.04	0.38	0.12	0.006	0.20	< 0.001	0.19	< 0.001	0.24	< 0.001
Total lean mass, Kg	0.74	< 0.001	0.68	< 0.001	0.42	< 0.001	0.84	< 0.001	0.75	< 0.001	0.35	< 0.001	0.90	< 0.001	0.78	< 0.001
Trunk lean mass,Kg	0.76	< 0.001	0.71	< 0.001	0.47	< 0.001	0.83	< 0.001	0.74	< 0.001	0.36	< 0.001	0.90	< 0.001	0.79	< 0.001
Android/Gynoid Ratio	0.39	< 0.001	0.34	< 0.001	0.25	< 0.001	0.37	< 0.001	0.34	< 0.001	0.21	< 0.001	0.39	< 0.001	0.38	< 0.001

[a] Abbreviations: BMI, body mass index; FMI, fat mass index; TBFM, total body fat mass; TBFP, total body fat percentage.
[b] Values are presented as (Mean ± SD).

Table 4. Association of Bone Parameters With Body Composition Adjusted for Age, Sex, Stage of Puberty, and Level of 25-Hydroxy Vitamin D in boys [a]

	Lumbar Spine BMC	Lumbar Spine BMD	Lumbar Spine BMAD	Femoral Neck BMC	Femoral Neck BMD	Femoral Neck BMAD	Total Body BMC	Total Body BMD
R Square	0.73	0.73	0.32	0.71	0.55	0.10	0.89	0.77
Age, y	1.20 ± 0.49 [b] (0.18)	0.015 ± 0.005 [b] (0.23)	0.005 ± 0.002 [b] (0.38)	0.003 ± 0.029 (0.008)	0.002 ± 0.005 (0.04)	0.001 ± 0.001 (0.09)	35.62 ± 9.50 [c] (0.18)	0.012 ± 0.003 [c] (0.26)
T1	-4.31 ± 3.07 (0.13)	-0.05 ± 0.03 (0.15)	-0.002 ± 0.010 (0.03)	-0.24 ± 0.18 (0.13)	-0.019 ± 0.032 (0.07)	0.003 ± 0.008 (0.06)	-17.8 ± 59.1 (0.01)	-0.005 ± 0.020 (0.02)
T2	-6.64 ± 1.86 [c] (0.20)	-0.04 ± 0.01 [b] (0.14)	-0.005 ± 0.006 (0.07)	-0.21 ± 0.10 [b] (0.11)	-0.007 ± 0.019 (0.02)	0.005 ± 0.005 (0.10)	-43.0 ± 33.8 (0.04)	-0.005 ± 0.011 (0.02)
25 (OH) Vit. D, ng/mL	-0.06 ± 0.10 (0.02)	0.0 (0.01)	0.0 (0.04)	-0.003 ± 0.006 (0.02)	-0.001 ± 0.001 (0.03)	0.0 (0.05)	-2.25 ± 1.97 (0.02)	0.0 (0.01)
TBFM, Kg	-0.21 ± 0.15 (0.06)	0.0 (0.009)	0.001 ± 0.001 (0.08)	-0.006 ± 0.009 (0.02)	0.001 ± 0.002 (0.02)	0.0 (0.09)	4.96 ± 3.05 (0.04)	0.001 ± 0.001 (0.02)
Total lean mass, Kg	0.95 ± 0.11 [c] (0.66)	0.008 ± 0.001 [c] (0.57)	0.0 (0.16)	0.064 ± 0.007 [c] (0.78)	0.007 ± 0.001 [c] (0.64)	0.0 (0.18)	33.03 ± 2.13 [c] (0.76)	0.006 ± 0.001 [c] (0.62)

[a] Data are expressed as B ± SE (standardized coefficient) in 9 - 18 years old Iranian male children and adolescents, (reference group for puberty is Tanner stage V, T1: Tanner stage I, II; T2: Tanner stage III, IV).
[b] $P < 0.05$.
[c] $P < 0.001$.

Table 5. Association of Bone Parameters With Body Composition Adjusted for Age, Sex, Stage of Puberty, and Level of 25-hydroxy Vitamin D in girls [a]

	Lumbar Spine BMC	Lumbar Spine BMD	Lumbar Spine BMAD	Femoral Neck BMC	Femoral Neck BMD	Femoral Neck BMAD	Total Body BMC	Total Body BMD
R Square	0.70	0.76	0.63	0.64	0.58	0.32	0.86	0.79
Age, y	1.15 ± 0.49 [b] (0.20)	0.01 ± 0.005 [b] (0.23)	0.004 ± 0.001 [b] (0.27)	0.04 ± 0.02 (0.17)	0.005 ± 0.004 (0.12)	0.0 (0.03)	38.76 ± 8.11 [c] (0.28)	0.014 ± 0.003 [c] (0.32)
T1	-8.90 ± 3.45 [b] (0.25)	-0.13 ± 0.03 [c] (0.32)	-0.025 ± 0.009 [b] (0.31)	0.04 ± 0.16 (0.03)	-0.002 ± 0.031 (0.007)	-0.001 ± 0.009 (0.02)	-27.8 ± 56.7 (0.03)	-0.017 ± 0.023 (0.06)
T2	-6.85 ± 2.21 [b] (0.21)	-0.06 ± 0.02 [b] (0.18)	-0.012 ± 0.006 [b] (0.15)	-0.09 ± 0.10 (0.06)	-0.036 ± 0.020 (0.14)	-0.011 ± 0.006 (0.19)	-96.6 ± 36.2 [b] (0.12)	-0.034 ± 0.014 [b] (0.13)
25 (OH) Vit. D, ng/mL	0.02 ± 0.11 (0.008)	0.001 ± 0.001 (0.04)	0.0 (0.007)	-0.001 ± 0.005 (0.006)	0.0 (0)	0.0 (0.01)	2.75 ± 1.87 (0.03)	0.001 ± 0.001 (0.02)
TBFM, Kg	-0.16 ± 0.15 (0.06)	0.001 ± 0.002 (0.01)	0.001 ± 0.0 (0.11)	0.011 ± 0.007 (0.09)	0.003 ± 0.001 [b] (0.16)	0.001 ± 0.0 [b] (0.23)	4.93 ± 2.54 (0.07)	0.0 (0.01)
Total lean mass, Kg	1.13 ± 0.16 [c] (0.50)	0.010 ± 0.002 [c] (0.39)	0.001 ± 0.0 [b] (0.21)	0.059 ± 0.008 [c] (0.61)	0.009 ± 0.001 [c] (0.52)	0.001 ± 0.0 [b] (0.29)	34.31 ± 2.67 [c] (0.62)	0.010 ± 0.001 [c] (0.56)

[a] Data are expressed as B ± SE (standardized coefficient) in 9 - 18 years old Iranian female, 250 children and adolescents, (reference group for puberty is Tanner stage V, T1: Tanner stage I, II; T2: Tanner stage III, IV).

[b] P < 0.05.

[c] P < 0.001.

4.5. Lean Mass and Physical Activity

About half of the boys (51.9%) participated in physical activities three times per week or more compared to 9.7% of the girls. We compared total body lean mass between individuals with more or less than three times physical activity per week and found that participants with more than three times physical activity had greater total lean mass than ones with less than three times (36.9 ± 10.8 and 30.0 ± 8.5) ($P < 0.001$). We evaluated lean body mass in the girls and boys and found 30.1 ± 7.7 in the girls with more than three times physical activity and 28.7 ± 6.5 in the girls with less than three times ($P = 0.3$). More active boys had total lean mass of 38.1 ± 10.9 in comparison to less active boys that had total body lean mass of 32.3 ± 11.0 ($P < 0.001$).

5. Discussion

In this cross-sectional study, we evaluated the effect of body composition on bone mineral density in Iranian children and adolescents. We showed that obese children and adolescents, especially obese girls had greater BMC and BMD than normal-weight subjects and also found that age and total body lean mass were the most important predictors of bone density.

To date, there is disagreement regarding the relative effect of fat mass on BMC and BMD (2, 7, 11, 18). We found a positive correlation between bone parameters and TBFP in the girls, but in boys, BMC and BMD in the lumbar spine, femoral neck and total body were negatively correlated with TBFP. El Hage and coworkers showed a negative association between fat mass and BMD in lumbar spine and total body in boys but in girls fat mass, in comparison to lean mass, was a better positive determinant of BMD (19).

Nagasaki and coworkers found a negative correlation between total body fat percentage and total body BMD in 12-15 year-old girls and in obese boys over 12 years of age (2). Goulding in 2008 demonstrated the fat mass as an independent predictor of bone mass in preschool children (20) although some previous studies reported a negative association between bone mass and fat mass in older obese children (21, 22).

The main mechanisms involved in the relationship between adipose tissue and bone are undetected (23). Adipokines or proinflammatory cytokines secreted from adipose tissue may have a role (24). IL-6 (Interleukin 6) and TNF-α (tumor necrosis factor α) can promote bone resorption by osteoclast differentiation (23). Some authors have reported the action of fat mass on BMD through estrogen, leptin (25), insulin or amylin (26). Leptin not only has a central role in energy expenditure, but also affects the bone metabolism and increases proliferation and differentiation of osteoblasts in adults (27). Leptin is secreted by adipocytes and increases in obese individuals. Maggio and coworkers showed that obese girls had a higher serum leptin concentration and higher BMD and a positive association between leptin and BMD has also been demonstrated in adult women (18). It seems that leptin is a mediator of adipose tissue hormonal effect on the bone mass (27). Another study presented leptin as a growth factor that affects the chondrocytes of growth plates via Insulin-like Growth Factor I (IGF-I) and in obese children it contributes to increase linear growth and bone mass. In adolescents, sex difference in BMD may be explained by differences in the serum leptin concentration, directly or indirectly via estrogen (18).

It seems that the effect of fat mass on bone accrual is dependent on gender and stage of puberty and is more significant in specific growth phases and during critical stages of growth and development (11); therefore, these different results could be related to various methodology, site of measurement and skeletal maturation in these study subjects.

There are some studies that compare the effect of lean versus fat mass on bone mineral density in children and adolescents; some advocated on the lean mass and some other on fat mass as a main indicator of BMC and BMD (2, 28, 29). We found the strong effect of total body lean mass on bone mineral content and density in multiple regression analysis.

Some mechanisms explain the correlation between lean mass and bone mineral density. These include mechanical load, and hormonal effects, such as increased conversion of androstenedione to estrogen and high circulating level of leptin (2). Although there is no evidence of bone response to static load (26), some studies suggest that the effect of fat mass on bone mass is mediated by increasing the muscle-mediated skeletal dynamic load (26) Schoenau et al. stated that great load and mechanical stress on the subperiosteal surface of long bones can stimulate bone formation, and in the obese children and adolescents, this biochemical loading is increased due to increased body weight and lean mass (30). Harold Frost's Mechanostat hypothesis stated "muscle-bone unit" in children and adolescents. On the base of this theory, increasing maximal muscle force during the growth period will increase the bone mass, size and strength, and physical activity plays an important role in maximizing bone mass during this period (31). Olmedillas et al. showed that leptin receptors are upregulated in hypertrophic muscles (32) and it seems that the effect of leptin on bone is mediated by muscles. In 2011, Ivana and coworkers in Prague showed a positive correlation between serum leptin and bone mineral density and between lean mass and IGF-I, claiming that low levels of lean mass and insufficient production of IGF-I or leptin could negatively influence the bone development in pubertal girls (10).

Using multiple regression analysis, we found that the most changes in the bone mineral density were attributed to the age and total body lean mass; however, the effect of total body fat mass in these models was not significant. It seems that the effect of fat mass on the bone is indirect and via lean mass and once lean mass is controlled, there is no significant relationship between fat and bone mass (33).

In some previous studies we showed greater lumbar spine and femoral neck BMD in more active boys and also found that fat-free mass (lean and bone mass) in the boys was greater than the girls (12, 34). In this study, we found that the main effect of body composition on BMD is related to total body lean mass in both genders. Also, the results showed that more active boys had a greater lean mass than less active ones. In animal studies, researchers have also found a greater response in males than in females to similar mechanical effects (35). Baptista and coworkers in 2012 found that lean mass was the most important predictor of BMD in boys and girls, while habitual weight-bearing physical activity has a positive effect on bone mineralization only in boys (36). In our study, it seems that girls not only are less active than the boys, but also may be less responsive to physical activity.

This study has a number of limitations. First, it is a cross-sectional survey and more prospective data are needed to evaluate the effect of lean mass and fat mass on BMD during growth. Second, in this study we did not measure hormonal factors and proinflammatory adipokines that seem to influence bone accrual.

In conclusion, the results of this study demonstrate more important associations between lean mass and BMD than fat mass. Thus, these findings support the recommendation that emphasis on physical activity and lean body mass in school children is more important than the exclusive concentration on fatness.

Acknowledgements

The authors would like to thank Dr. Nasrin Shokrpour from center for development of clinical research in Nemazee Hospital for her editorial assistance.

Authors' Contributions

Study concept and design: Mohammad Hossein Dabbaghmanesh, and Gholamhossein Ranjbar Omrani. Acquisition of data: Marjan Jeddi, and Marzieh Bakhshayeshkaram. Analysis and interpretation of data: Mohammad Hossein Dabbaghmanesh, Marjan Jeddi, and Zahra Bagheri. Drafting of the manuscript: Mohammad Hossein Dabbaghmanesh, Marjan Jeddi, and Marzieh Bakhshayeshkaram. Critical revision of the manuscript for important intellectual content: Mohammad Hossein Dabbaghmanesh, Gholamhossein Ranjbar Omrani, and Sayed Mohammad Taghi Ayatollahi. Statistical analysis: Sayed Mohammad Taghi Ayatollahi, and Zahra Bagheri. Administrative, technical, and material support: Mohammad Hossein Dabbaghmanesh, and Gholamhossein Ranjbar Omrani. Study supervision: Mohammad Hossein Dabbaghmanesh, and Gholamhossein Ranjbar Omrani.

References

1. Boot AM, de Ridder MA, Pols HA, Krenning EP, de Muinck Keizer-Schrama SM. Bone mineral density in children and adolescents: relation to puberty, calcium intake, and physical activity. *J Clin Endocrinol Metab*. 1997;**82**(1):57–62.
2. El Khayat HA, Emam EK, Hassan NE, Kandeel WA, Elagouza IA, Zaki ME, et al. Impact of Body Fat Mass on Bone Mineral Density and Content and on Serum Level of c-Terminal Telopeptide of Type 1 Collagen among Overweight and Obese Children and Adolescents. *J of Applied Sci Res*. 2013;**9**(1):770.
3. Ashouri E, Meimandi EM, Saki F, Dabbaghmanesh MH, Omrani GR, Bakhshayeshkaram M. The impact of LRP5 polymorphism (rs556442) on calcium homeostasis, bone mineral density, and body composition in Iranian children. *J Bone Miner Metab*. 2014.

4. Lobstein T, Baur L, Uauy R, Iaso International Obesity TaskForce . Obesity in children and young people: a crisis in public health. *Obes Rev.* 2004;**5 Suppl 1**:4–104.
5. Villareal DT, Apovian CM, Kushner RF, Klein S, American Society for N, Naaso TOS. Obesity in older adults: technical review and position statement of the American Society for Nutrition and NAASO, The Obesity Society. *Obes Res.* 2005;**13**(11):1849–63.
6. Lecka-Czernik B, Stechschulte LA, Czernik PJ, Dowling AR. High bone mass in adult mice with diet-induced obesity results from a combination of initial increase in bone mass followed by attenuation in bone formation; implications for high bone mass and decreased bone quality in obesity. *Mol Cell Endocrinol.* 2015.
7. Junior IF, Cardoso JR, Christofaro DG, Codogno JS, de Moraes AC, Fernandes RA. The relationship between visceral fat thickness and bone mineral density in sedentary obese children and adolescents. *BMC Pediatr.* 2013;**13**:37.
8. Cao JJ. Effects of obesity on bone metabolism. *J Orthop Surg Res.* 2011;**6**:30.
9. Cao JJ, Sun L, Gao H. Diet-induced obesity alters bone remodeling leading to decreased femoral trabecular bone mass in mice. *Ann N Y Acad Sci.* 2010;**1192**:292–7.
10. Ivana Z, Veronika C, Petr K, Vera L, Vaclav V, Petr M, et al. Relationship Between Hormonal Variables and Bone Mineral Density, Muscle Force, and Fat Mass in Peripubertal Girls. *Int J Endocrinol Metabol.* 2011;**9**(3):391–6.
11. Dimitri P, Wales JK, Bishop N. Fat and bone in children: differential effects of obesity on bone size and mass according to fracture history. *J Bone Miner Res.* 2010;**25**(3):527–36.
12. Jeddi M, Roosta MJ, Dabbaghmanesh MH, Omrani GR, Ayatollahi SM, Bagheri Z, et al. Normative data and percentile curves of bone mineral density in healthy Iranian children aged 9-18 years. *Arch Osteoporos.* 2013;**8**(1-2):114.
13. Ho-Pham LT, Nguyen UD, Nguyen TV. Association between lean mass, fat mass, and bone mineral density: a meta-analysis. *J Clin Endocrinol Metab.* 2014;**99**(1):30–8.
14. Barlow SE, Expert C. Expert committee recommendations regarding the prevention, assessment, and treatment of child and adolescent overweight and obesity: summary report. *Pediatrics.* 2007;**120 Suppl 4**:S164–92.
15. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ.* 2000;**320**(7244):1240–3.
16. Kohrt WM, Bloomfield SA, Little KD, Nelson ME, Yingling VR, American College of Sports M. American College of Sports Medicine Position Stand: physical activity and bone health. *Med Sci Sports Exerc.* 2004;**36**(11):1985–96.
17. Bianchi ML, Baim S, Bishop NJ, Gordon CM, Hans DB, Langman CB, et al. Official positions of the International Society for Clinical Densitometry (ISCD) on DXA evaluation in children and adolescents. *Pediatr Nephrol.* 2010;**25**(1):37–47.
18. Maggio AB, Belli DC, Puigdefabregas JW, Rizzoli R, Farpour-Lambert NJ, Beghetti M, et al. High bone density in adolescents with obesity is related to fat mass and serum leptin concentrations. *J Pediatr Gastroenterol Nutr.* 2014;**58**(6):723–8.
19. El Hage RP, Courteix D, Benhamou CL, Jacob C, Jaffre C. Relative importance of lean and fat mass on bone mineral density in a group of adolescent girls and boys. *Eur J Appl Physiol.* 2009;**105**(5):759–64.
20. Goulding A, Taylor RW, Grant AM, Murdoch L, Williams SM, Taylor BJ. Relationship of total body fat mass to bone area in New Zealand five-year-olds. *Calcif Tissue Int.* 2008;**82**(4):293–9.
21. Goulding A, Taylor RW, Jones IE, Manning PJ, Williams SM. Spinal overload: a concern for obese children and adolescents? *Osteoporos Int.* 2002;**13**(10):835–40.
22. Goulding A, Jones IE, Taylor RW, Williams SM, Manning PJ. Bone mineral density and body composition in boys with distal forearm fractures: a dual-energy x-ray absorptiometry study. *J Pediatr.* 2001;**139**(4):509–15.
23. Mosca LN, Goldberg TB, da Silva VN, da Silva CC, Kurokawa CS, Bisi Rizzo AC, et al. Excess body fat negatively affects bone mass in adolescents. *Nutrition.* 2014;**30**(7-8):847–52.
24. Viljakainen HT, Pekkinen M, Saarnio E, Karp H, Lamberg-Allardt C, Makitie O. Dual effect of adipose tissue on bone health during growth. *Bone.* 2011;**48**(2):212–7.
25. Elefteriou F, Takeda S, Ebihara K, Magre J, Patano N, Kim CA, et al. Serum leptin level is a regulator of bone mass. *Proc Natl Acad Sci U S A.* 2004;**101**(9):3258–63.
26. Gnudi S, Sitta E, Fiumi N. Relationship between body composition and bone mineral density in women with and without osteoporosis: relative contribution of lean and fat mass. *J Bone Miner Metab.* 2007;**25**(5):326–32.
27. Rhie YJ, Lee KH, Chung SC, Kim HS, Kim DH. Effects of body composition, leptin, and adiponectin on bone mineral density in prepubertal girls. *J Korean Med Sci.* 2010;**25**(8):1187–90.
28. Pietrobelli A, Faith MS, Wang J, Brambilla P, Chiumello G, Heymsfield SB. Association of lean tissue and fat mass with bone mineral content in children and adolescents. *Obes Res.* 2002;**10**(1):56–60.
29. Clark EM, Ness AR, Tobias JH. Adipose tissue stimulates bone growth in prepubertal children. *J Clin Endocrinol Metab.* 2006;**91**(7):2534–41.
30. Schoenau E, Neu CM, Rauch F, Manz F. The development of bone strength at the proximal radius during childhood and adolescence. *J Clin Endocrinol Metab.* 2001;**86**(2):613–8.
31. Kouda K, Fujita Y, Sato Y, Ohara K, Nakamura H, Uenishi K, et al. Fat mass is positively associated with bone mass in relatively thin adolescents: data from the Kitakata Kids Health Study. *Bone.* 2014;**64**:298–302.
32. Olmedillas H, Sanchis-Moysi J, Fuentes T, Guadalupe-Grau A, Ponce-Gonzalez JG, Morales-Alamo D, et al. Muscle hypertrophy and increased expression of leptin receptors in the musculus triceps brachii of the dominant arm in professional tennis players. *Eur J Appl Physiol.* 2010;**108**(4):749–58.
33. Gracia-Marco L, Ortega FB, Jimenez-Pavon D, Rodriguez G, Castillo MJ, Vicente-Rodriguez G, et al. Adiposity and bone health in Spanish adolescents. The HELENA study. *Osteoporos Int.* 2012;**23**(3):937–47.
34. Jeddi M, Dabbaghmanesh MH, Ranjbar Omrani G, Ayatollahi SM, Bagheri Z, Bakhshayeshkaram M. Body composition reference percentiles of healthy Iranian children and adolescents in southern Iran. *Arch Iran Med.* 2014;**17**(10):661–9.
35. Wallace JM, Rajachar RM, Allen MR, Bloomfield SA, Robey PG, Young MF, et al. Exercise-induced changes in the cortical bone of growing mice are bone- and gender-specific. *Bone.* 2007;**40**(4):1120–7.
36. Baptista F, Barrigas C, Vieira F, Santa-Clara H, Homens PM, Fragoso I, et al. The role of lean body mass and physical activity in bone health in children. *J Bone Miner Metab.* 2012;**30**(1):100–8.