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ORIGINAL ARTICLES

Effect Of Diet Quality On Bone Mineralization In Obese Egyptian Children And Adolescents

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ABSTRACT

Objective: To assess bone mineral content and density in relation to dietary and some non- dietary factors in a group of obese Egyptian children and adolescents. **Methods:** Ninety obese boys and girls of age 7.6 to16 years were recruited for the study. Anthropometric measurements including weight, height and BMI in addition to the Tanner stage were assessed. Bone mineral content, (BMC), and bone mineral density, (BMD) of the lumbar spine (L2-4) were determined by dual-energy-X-ray absorptiometry (DXA). The food intake of three days was recorded for each individual. **Results:** Girls had higher BMD and BMC than boys. The diet of both obese boys and girls was deficient in calcium and vitamin D. A strong positive association of total and vegetable protein with BMC and BMD was evident in boys only. Vitamins and minerals were positively related to the lumbar spine measurements for boys and girls but positive correlation with calcium was evident in obese boys only. The potential renal acid load (PRAL) for obese boys and girls was negatively related to the lumbar spine BMC and BMD. Multiple regression analysis showed that the increase in BMI, calcium and vitamin D intake can predict the increase in spine BMC and BMD for boys. While the increase in vitamin D intake and the reduction in PRAL plus the more advanced Tanner stage can predict the increase in spine BMC and BMD for girls. **Conclusion:** Multiple dietary and non- dietary factors contribute to development of normal bone mass. Consuming a well-balanced diet on a daily basis, an appropriate intake of calcium accompanied by sources of alkali reducing PRAL and vitamin D supplementation are recommended in this age group. Obesity and overweight presented positive effect on bone mass of the study sample; however, the long-term effect on bone resistance in adulthood must be followed up.

Key words: Obesity, bone density, children, adolescent, food intake.

Introduction

There is greater awareness and recognition of osteoporosis (OP) in children both as a primary problem and as a harbinger of OP in adulthood. Although it was once thought to afflict mainly adults with chronic diseases and elderly patients, in recent years OP has been recognized as a pediatric problem as well (Uziel *et al*., 2009).

Osteoporosis was defined as a skeletal disorder characterized by compromised bone strength that predisposes to an increased risk of fracture (NIH, 2000). Pediatric osteoporosis was defined as bone density Z score below -2, in combination with a fracture (Gordon *et al*., 2008).

Bone mass increases progressively during childhood, but mainly during adolescence when approximately 40% of total bone mass is accumulated. Peak bone mass is reached in late adolescence, and is a well recognized risk factor for osteoporosis later in life. Thus, increasing peak bone mass can prevent osteoporosis. Osteoporosis prevention is a key factor and it should begin in childhood (Baroncelli *et al*, 2005).

Multiple factors contribute to normal skeletal development and attainment of normal BMD (Henwood and Binkovitz, 2009). Apart from genetics and hormonal influences, factors associated with lifestyle—such as obesity (Leonard *et al*., 2004), and diet (Tylavsky *et al*., 2004)—also affect variables of bone mass and bone dimension.

Childhood dietary intake influences both adiposity and bone mass, and these intake behaviors may track into later years (Nicklas *et al*.1991 and Boulton *et al*., 1995). Well-balanced nutrition is essential, but adequate amounts of calcium and vitamin D in the diet are especially important, particularly in adolescence because puberty is a critical time for accruing bone mass. Other nutrients and dietary components, such as potassium, magnesium, vitamin K, and fruit and vegetables, have also shown beneficial effects (Nicklas *et al*., 1991). Moreover, beneficial effects have been hypothesized for protein, saturated fat, phosphorus, vitamin C, sodium, and dietary isoflavone (Boulton *et al*.,1995).

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However, there is a notable scarcity of studies that evaluated dietary intakes that affect bone mass in obese children (Wosje *et al*., 2010).

In this study, bone mineral content and density were assessed in relation to dietary and some non- dietary factors in a group of obese Egyptian children and adolescents.

Subjects and Methods:

Ninety obese children and adolescents of age range (7.6-16 years) were selected from Endocrinology Clinic in 6th October Medical Insurance Hospital. All studied children provided signed written informed consent to participate in assessments. Those with organic causes of obesity were excluded, only those with simple obesity were included in this study. Exclusion was based on laboratory data (serum T3, T4, ACTH, etc.). Children were considered obese when they had Body Mass Index (BMI) ≥ 95th percentile according to WHO Growth reference data for 5-19 years old children and adolescents (de Onis and Lobstein 2010). None of them had followed a weight reduction regimen prior to this study, nor had they received vitamin D or calcium supplements. Apart from their obesity they were in good general condition with normal sun light exposure and had no other known metabolic or endocrine disorders. All had normal serum creatinine, total protein and albumin. All subjects had a negative history for any disorders known to affect bone metabolism, including renal disease, liver disease, chronic diarrhea and gastric or bowel surgery. Children receiving a high dose of vitamins within 6 months of the study and those taking medications to affect bone metabolism such as antiepileptic drugs, rifampicin, cholestyramine, or chronic steroid therapy were also excluded.

Anthropometry and Tanner staging:

Anthropometric measurements including weight and height were assessed at the National Research Centre. The studied children were dressed in physical training uniforms during the measurement sessions. Height was measured to the nearest 0.5 cm without shoes using wall-mounted stadiometer. Body weight was measured to the nearest 0.1kg by a standard clinical balance. All measurements were made according to techniques described in the Anthropometric Standardization Reference Manual (Lohman *et al*., 1988). Each measurement was performed twice. The mean of the two trials was used in all statistical analyses. Body mass index (BMI) was calculated by dividing weight by height squared (kg/m2), which was plotted on WHO reference curves for 5-19 years children and adolescents (WHO, 2009) (http://www.who.int/growthref/tools/en/). Children from 5th up to 85th percentiles were considered within normal weight, those above the 85th percentile were considered overweight (85th to 95th percentile), whereas 95th or above were considered obese cases. Weight-for-age z-scores (WAZ), height-for age (HAZ) z-scores among all children and body mass index for age z- score (BAZ) were calculated based on the WHO growth standards (WHO, 2009). Pubertal status was determined by physical examination and classified according to the method of Tanner (1998).

Lumbar spine DXA scan:

Bone mineral content, (BMC), and bone mineral density, (BMD) of the anterior-posterior lumbar spine (L2-4) were determined by dual-energy-X-ray absorptiometry (DXA, Lunar model, with pediatric software version 3.8). All subjects were measured on the same machine. The measurements were performed by using standard positioning techniques.

Food intake and analysis:

The food intake of three days was recorded for each individual on a nutritional sheet. This included every single item eaten during this day, its frequency, size and portion. The data was collected and filled by the same researcher to assure accuracy. Computer program (Nutrisurvey, 2007) was used to analyze the data. The mean of the 3 days dietary intake was calculated to present a more accurate intake of 24 hours dietary intake. Total caloric intake in 24 hours was calculated, the percentage of protein, carbohydrates and fats in relation to total calories were calculated to determine the nutritional pattern of each individual. Analysis of mineral and vitamin content of the different food items was also calculated. The daily intakes of calories and protein were compared with Required Daily Allowances (RDAs) according to Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (DRI, 2002/2005), while vitamins and minerals were compared with RDAs according to Dietary Reference Intakes for Calcium and Vitamin D (DRI, 2011).

The dietary potential renal acid load (PRAL) was calculated for each individual according to Remer *et al*., 2003 using the following equation:

PRAL = 0.4888 x protein (g/d) + 0.0366 x phosphorus (mg/d) - 0.0205 x potassium (mg/d) - 0.0263 x magnesium (mg/d)

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Statistical Analysis:

Descriptive statistics including means ± SD for numeric variables were measured. Data was compared using t test and correlated using Pearson Correlations. Multiple regression models were performed to predict factors affecting bone mineral content and density. Statistical analysis was performed with SPSS for windows version 10. Significant values were considered when $P<0.05$.

Results:

The study included 90 obese children. The age, anthropometric measurements including weight, height, HAZ (height for age z-score), BMI in addition to the Tanner stage, were not significantly different between boys and girls ($P>0.05$). The WAZ (weight for age z-score); BAZ (body mass index for age z- score) for boys were greater than girls with high significant difference ($P<0.01$) (Table 1).

The DXA results for the lumbar spine (L2-4) measurements showed that girls had higher age matched Z-score than boys. The lumbar spine measurements including the BMC and BMD were greater in girls than boys with high significant difference ($P< 0.01$). The length was greater in girls while the area was greater in boys but these differences were insignificant ($P>0.05$) (Table 2). The value of Lumbar spine Z-score was negative in 40% of cases, but none of Z-score values was less than -2 Z-score.

Food quality analysis showed that obese boys consumed more calories, protein and carbohydrates but less fat than obese girls. Obese boys had high amounts of calcium, phosphorus, magnesium, vitamin D and vitamin k than obese girls. The potential renal acid load (PRAL) for obese boys was more than girls (Table 3).

Both obese boys and girls had more energy intake and more percentage calories from protein than the recommended dietary allowance (RDAs) for the same age. They consumed the relative average percentage of calories from carbohydrates. However obese girls had higher % calories from fat than boys and that was higher than RDA. The diet of both obese boys and girls was deficient of calcium and vitamin D. However they had higher amounts of phosphorus, magnesium, potassium and vitamin k than RDA (Table 3).

Correlation between the consumed food components and bone characteristics showed that the total protein and the amount of vegetable protein as percentage of calories of the daily consumed food was positively related to lumbar spine measurements including the BMC and BMD for obese boys. While the amounts of carbohydrates as percentage of calories of the daily consumed food was negatively related to the lumbar spine measurements including the BMC and BMD for obese boys (Table 4).

Positive but not significant correlation was found between the total protein and the amounts of vegetable and animal protein as percentage of calories of the daily consumed food and the lumbar spine BMC and BMD for obese girls (Table 4).

The correlation between the consumed food composition of some vitamins and minerals showed that phosphorus, magnesium, potassium, vitamin D and vitamin k were positively related to the lumbar spine measurements including the BMC and BMD for obese boys and girls.

Calcium was positively related to the lumbar spine measurements including the BMC and BMD for obese boys, while this relation was not evident for obese girls. The potential renal acid load (PRAL) for obese boys and girls was negatively related to the lumbar spine measurements including the BMC and BMD.

Multiple regressions of lumbar spine bone measurements with all different variables were done. The increase in BMI and calcium can predict the increase in spine BMC and BMD for boys ($P<0.01$). The increase in age and the more advanced Tanner stage can predict the increase in spine BMC and BMD for girls ($P<0.01$) (Table 5).

After adjustment for the non-dietary variables, multiple regressions of lumbar spine bone measurements with only dietary daily intake were done. The increase of calcium and vitamin D intake can predict the increase in spine BMC and BMD for boys ($P<0.01$). The decrease in PRAL and the increase in vitamin D intake can predict the increase in spine BMC and BMD for girls ($P<0.01$) (Table 6).

Table 1: Baseline characteristics (age, Anthropometric measurements and Tanner stages) of the study population according to sex.

Variable	Boys (n=45)	Girls (n=45)	P-value
Age (y)	12.83±3.18	13.86±4.10	NS
Weight (kg)	74.30±19.61	72.40±27.61	NS
WAZ	2.26±0.17	1.83±0.42	<0.001
Height (cm)	152.26±16.35	146.80±12.57	NS
HAZ	-0.31±0.77	-0.69±0.81	NS
BMI (kg/m^2)	31.34±3.28	32.34±7.12	NS
BAZ	2.36±0.19	2.12±0.18	<0.001
Tanner Stage	2±1	3±1	NS

WAZ: weight for age z-score
HAZ: height for age z-score
BAZ: body mass index for age z- score.

Table 2: Bone characteristics of the study population according to sex.

Variable	Boys (n=45)	Girls (n=45)	P-value
L2-4 BMC (g)	24.33±7.18	31.57±12.69	.002
L2-4 BMD(g/cm^2)	0.63±0.13	0.84±0.21	<0.001
L2-4 BMD Z- score	-0.42±0.62	0.22±0.24	<0.001
L2-4 length(cm)	8.64±1.01	8.85±1.20	NS
L2-4 area(cm^2)	37.55±5.47	35.90±6.79	NS

Table 3: Dietary daily intake of the study population compared to RDAs for corresponding age and sex.

Dietary daily intake	Boys(n=45)	RDA for boys	Girls(n=45)	RDA for girls	P-value
Energy intake (kcal)	4466.18±822.77	2279-3152	4034.88±1118.40	2071-2368	NS
Total protein(g/d)	139.56±28.02	34-52	118.90±36.34	34-46	.001
Total protein(% of energy)	12.86±2.77	10-30	12.20±2.75	10-30	.030
Animal protein (g/d)	53.40±26.41	ND	43.34±26.71	ND	NS
Animal protein (% of energy)	4.96±2.19	ND	4.52±2.30	ND	NS
Vegetable protein (g/d)	85.98±10.91	ND	75.56±19.28	ND	.001
Vegetable protein (% of energy)	8.24±1.77	ND	7.68±1.15	ND	NS
Carbohydrate (g/d)	600.84±86.65	130	470.50±158.11	130	<0.001
Carbohydrate (% of energy)	55.40±7.15	45-65	47±5.75	45-65	<0.001
Fat (g/d)	164.02±59.13	ND	184.56±51.77	ND	NS
Fat (% of energy)	31.40±5.98	25-35	40.80±3.66	25-35	.000
Calcium (mg)	819.72±224.63	1300	726.6±408.46	1300	.015
Phosphorous (mg)	3239.24±637.52	1250	2484.34±810.28	1250	.000
Magnesium (mg)	788.12±108.50	240-410	624.7±198.97	240-360	.000
Potassium (g/d)	5.3±0.659	4.7	5.3±0.166	4.7	NS
Vitamin D (µg)	1.46±1.56	15	1.14±1.37	15	NS
Vitamin K (µg)	50.75±10.36	60-75	42.74±12.57	60-75	.000
PRAL (mEq/d)	57.42±36.58		24.89±15.90		.000

RDAs: Recommended dietary allowances. PRAL: Potential renal acid load.
ND: Not determined. NS: Not significant. P-value: Gender difference by independent t- test.

Table 4: Pearson correlation coefficient (r) between dietary daily intake and bone characteristics for boys and girls.

Dietary daily intake	L2-4 BMC- boys	L2-4 BMD-boys	L2-4 BMC- girls	L2-4 BMD- girls
Total protein(% of energy)	0.653**	0.286*	0.320	0.217
Animal protein (% of energy)	-0.252	-0.163	0.348	0.118
Vegetable protein (% of energy)	0.680**	0.539**	0.194	0.047
Carbohydrate (% of energy)	-0.500**	-0.372*	0.429*	0.216
Fat (% of energy)	0.274	0.275	-0.208	0.052
Calcium	0.920**	0.820**	0.077	0.246
Phosphorous	0.836**	0.715**	0.630**	0.689**
Magnesium	0.407*	0.318	0.659**	0.645**
Potassium	0.533**	0.607**	0.558**	0.553**
Vitamin D	0.894**	0.763**	0.745**	0.818**
Vitamin K	0.665**	0.538**	0.573**	0.553**
PRAL	-0.951**	-0.932**	-0.507**	-0.247

** Significant P<0.01
* Significant P<0.05

Table 5: Sex-specific linear regression (stepwise method) models of spine BMC and BMD in relation to studied variables (Anthropometry, Tanner stages & dietary daily intake).

Dependent Variable	Predictors	Co-efficient (b1)	Std. Error	t	P-value	Adjusted R Square
L2-4 BMC- boys	Constant Calcium BMI	-28.767 .278 1.323	1.642 .015 .001	-17.522 18.707 12.242	.000 .000 .000	.988
L2-4 BMD- boys	Constant BMI	0.224 5.026	.056 .000	3.974 7.571	.000 .000	.660
L2-4 BMC- girls	Constant Age Tanner stage	-7.149 .268 1.391	.319 .006 .043	-22.409 41.581 32.025	.000 .000 .000	.999
L2-4 BMD- girls	Constant Tanner stage	.149 5.042	.025 .002	6.018 29.403	.000 .000	.968

Discussion:

Peak bone mass, a major determinant of risk for osteoporosis later in life, is achieved by early adulthood. Because prevention of osteoporosis by maximizing peak bone mineral mass during childhood and adolescence

is superior to treatment in adulthood, it is critical to understand the factors that influence bone mineral accrual during growth (Cashman, 2007).

Table 6: Sex-specific linear regression (stepwise method) models of spine BMC and BMD in relation to dietary daily intake after adjustment for non-dietary variables

Dependent Variable	Predictors	Co-efficient (b1)	Std. Error	t	P-value	Adjusted R Square
L2-4 BMC- boys	Constant	10.122	.833	12.148	.000	.986
	Calcium	5.411	.002	33.656	.000	
	Vitamin D	0.594	.035	17.049	.000	
L2-4 BMD- boys	Constant	0.224	.056	3.974	.000	.660
	Calcium	5.026	.000	7.571	.000	
L2-4 BMC- girls	Constant	50.469	1.365	36.985	.000	.902
	PRAL	-.759	.046	-16.354	.000	
L2-4 BMD- girls	Constant	1.155	.027	43.431	.000	.864
	Vitamin D	1.23	.001	13.629	.000	

In this study, bone mineral content and density were assessed in relation to dietary factors in a group of obese Egyptian children and adolescents.

The results of our study indicated that girls had higher BMD and BMC than boys (table 2), this finding is in agreement with other studies (Hasanoğlu *et al*., 2000; Bailey *et al*., 2000) most probably because of the earlier onset of puberty in females. During puberty, growth hormone (GH) as well as sex steroid levels increase, and both have a positive influence on BMD (Schoenau, 2006). The influence of puberty on BMD is higher in girls than in boys. Studying clinical syndromes showed a more important role of estrogen than of androgen in mineralization of the skeleton (Frank, 2003). Several previous human studies showed that Lumbar spine BMD increased significantly with higher Tanner stages (Boot *et al*., 1997; Arabi *et al*., 2004).

In our study Lumbar spine Z-score was negative in 40% of the children's BMD. However, none of them had values less than -2 Z-score. This means that, none of these obese children and adolescents had "low for age" BMD or BMC. The Pediatric Position Development Conference (PDC) of the International Society of Clinical Densitometry guidelines suggested that the diagnosis of osteoporosis in children be made only when both low bone mass (BMC or BMD Z-scores of less than-2) and a clinically significant fracture history (defined previously) are present (Rauch *et al*., 2008).

A previous national study for the determination of bone mass density among Egyptian adolescents recorded osteopenia in 18% of male adolescents and11% of Female adolescents and showed lower mean values of BMD T-scores than our study (-1.38$\pm$1.06 and 0.56$\pm$0.92 for male and female adolescents respectively). This difference may be due to the diversity of body mass index (BMI) among subjects of the previous study which included underweight, normal, overweight and obese subjects. Subjects having low BMI were having low BMD (Hassan *et al*., 2004). In addition, they used T-score, while we used Z-score which is more accurate in children and adolescents.

In our study, a highly significant positive correlation was found between BMI and BMC and BMD of lumbar spines (table 4), indicating the positive effect of obesity on bone mass of the studied subjects.

As regards the effect of childhood obesity on bone mass, findings are inconsistent. Some studies have found that obese children have presented with normal BMD (Schepper *et al*., 1995), even in studies adjusted BMD values for weight, lean mass or fat mass (Rawad *et al*.,2009).Others have attributed greater bone mass to excess body weight in the growing years (Leonard *et al*., 2004; Wetzsteon *et al*., 2008) but other studies have also been suggested that obese children have lower bone mass for a given weight (Goulding *et al*., 2000; Petit *et al*., 2008).

Dietary intake with principal components analysis has been done, and the association between the quality of diets consumed by obese children and adolescents and their bone mass has been evaluated. Data analysis revealed that mean energy intake of all subjects of our study was higher than requirements recommended for their ages and sex (>100% of RDA) with no significant sex difference (table 3). This finding is in agreement with other Egyptian study (Hassan *et al*., 2010).

The food adequacy data from National Nutrition Institute (NNI) national surveys show that the percentage of children receiving more than 100 percent of their energy RDAs increased from about 14 percent in 1995 to about 46.9 percent in 2000. This may be attributed to changing life styles, with more consumption of high energy-dense foods, less physical activity, and changing eating habits (FAO, 2006)

Our results showed that the percentage of calories derived from proteins, carbohydrates and fats satisfied the age and sex requirements of the study population. Even, the percentage of calories from fats consumed by girls was more than 100% of RDA (table 3). It is apparent that, girls consumed more energy from fats while boys consumed more energy from carbohydrates.

Studies to date of the association between protein intake and BMD report inconsistent results. Some have found beneficial associations (Teegarden *et al*., 1998; Alexy *et al*., 2005), others reporting no association

(Ballard *et al*., 2006), and others finding adverse associations (Barzel and Massey, 1998; Spence and Weaver, 2003).

In our study, a strong positive association of total and vegetable protein with BMC and BMD was evident in boys. On the contrary, a negative association was observed with animal protein, and positive but not significant association was reported in girls (table 4).

Protein intake affects bone in several ways: it provides the structural matrix of bone, optimizes IGF-1 levels (IGF-I is a major determinant of bone growth and mineral content), increases urinary calcium, and increases intestinal calcium absorption (Heaney and Layman 2008).These beneficial and detrimental effects of protein on bone health depend on a variety of factors, including the level of protein in the diet, the protein source, calcium intake, and the acid/base balance of the diet (Bonjour, 2005).

The relation between protein intake and bone is complicated by the potential negative effect of overall dietary acid-base balance (FAO/WHO/UNU 2007). Dietary acid load was characterized as potential renal acid load (PRAL) by using an algorithm including dietary protein, phosphorus, magnesium, and potassium.

In our study, there was a strong negative association between BMC, BMD of lumbar spines and PRAL in both sexes. However, the value of PRAL was statistically significantly higher in boys which may contribute to having lower BMD than girls. A high PRAL indicates an inadequate intake of alkalizing minerals and can at least partly negate an osteotrophic protein effect (Alexy *et al* 2005).

Heaney and Layman, 2008 suggested that more concern should be focused on increasing fruit and vegetable intake (having alkalinizing effects) rather than reducing protein sources.

The high consumption of carbohydrates in our study sample was negatively associated with bone variables in boys, while weak positive association was evident in girls. This may depend on the type of consumed carbohydrates, whether fine or complex.

Diets high in refined sugar as soft drinks have been shown to badly affect bone growth and mechanical strength. (Whiting *et al*., 2001; Whiting *et al*., 2004). This might increase the risk of fractures in childhood and osteoporosis in later life.

On the other hand recent research suggests that complex carbohydrates, such as fruits and vegetables, in addition to omega-3 fatty acids, may actually improve bone mass density and increase calcium absorption. Fruits and vegetables contain non-digestible carbohydrates, like inulin-type fructans, that cannot be digested by the small intestine. Hence, as they move toward the large intestine and begin to be processed, they produce organic acids that enhance the distribution of calcium throughout the body (Lorincz *et al*., 2009).

Well-balanced nutrition is essential for normal skeletal development and attainment of normal BMD, but adequate amounts of calcium and vitamin D in the diet are especially important (Henwood and Binkovitz 2009) .Calcium is the largest component of bone minerals. Children aged 9 to 19 years need 1,300 milligrams of calcium, the highest of any age group, to optimize the calcium deposited in their bones during this time of rapid growth (DRI, 2011).

Calcium intake level among our sample is below the recommended international figure especially in girls. The majority of Egyptian children and teens do not consume enough dietary calcium on a daily basis, as reported in the national survey carried out by the National nutrition Institute, Egypt (DNPCNCD 2008).

The positive relationship between bone mass and Ca intake that was observed in boys in our study coincides with results from clinical trials in pre-teens and teens (Nowson *et al*., 1997; Rozen *et al*., 2003). A lack of significant association between Ca intake and BMC in females may reflect the powerful effect of other factors, such as puberty, on bone health; therefore, it can hide the effects of calcium on bone mass. Another possibility is the cross-sectional design of our study which may be imprecise, and may not reflect lifetime calcium intake. In agreement with our study, Lee *et al*, 1993 found that previous calcium intake was significantly associated with total body bone mineral content (TBBMC), whereas the cross-sectional association between current calcium intake and TBBMC was not established.

Vitamin D is the only vitamin the human body makes itself in any significant amounts. It has a significant role in tightly regulating serum calcium levels enhancing bone mineralization in children and adolescents (Peters and Martini, 2010).

Vitamin D intake level among our sample is far below the recommended level (Less than 25% of RDA, Table 3). Hypovitaminosis D is a major public health problem across all life stages in the Middle East. Predisposing factors for low vitamin D levels in children and adolescents, are limited sun exposure especially in girls due to clothing style, decreased outdoor activity, and decreased calcium content of diets, obesity, and lack of government regulation for vitamin D fortification of food (Fuleihan, 2009).

A strong highly positive association is observed between vitamin D intake and bone mineral variables indicating their deficient state of vitamin D. This is previously proved by meta-analysis of placebo controlled randomized controlled trials of vitamin D supplementation. It was found that vitamin D supplementation in deficient children and adolescents results in clinically important improvements in bone mineral density of the lumbar spine and total body bone mineral content. On the other hand, vitamin D supplements are unlikely to be beneficial in children and adolescents with normal vitamin D levels (Winzenberg *et al*., 2011).

Vitamin K intake appeared to be sufficient in our sample and it showed positive correlation with BMC and BMD in both sexes. Limited clinical research suggests that adequate vitamin K intake is associated with decreased bone turnover and decreased urinary calcium excretion (Institute of Medicine, 2001).

Because phosphorus and magnesium are abundantly distributed in the food supply coupled with good intestinal absorption, dietary phosphorus and magnesium deficiency is extremely rare (Institute of Medicine, 1997). In our study phosphorus and magnesium intake is more than 100% of RDA and is positively correlated with bone mineral variables.

Bone mass status of our sample of obese children and adolescents was influenced by both dietary and non-dietary variables. Sex-specific analysis revealed that, BMI in males and Tanner stage in females were the most important predictors of bone mineral density. Calcium and vitamin D intake were the consistent dietary significant predictors of bone measures among all nutrients in males and females. The inhibitory effect of PRAL was evident on BMC in females.

Predominance of non-dietary variables in this age period was observed in other previous studies (Boot *et al*., 1997; Chan *et al*., 2008). However, the positive effect of increased BMI must be taken cautiously; as body weight may contribute to fracture risk by placing extra burden on bones during falls (Peters and Martini, 2010). Good nutrition, especially protein, calcium and vitamin D, is important for justifying maximum bone mass accumulation during childhood and adolescence (IOF, 2007). The role of calcium and vitamin D is emphasized in our study as in previous studies (Cashman, 2007; Institute of Medicine 1997; Viljakainen *et al*., 2006).

Protein intake in our participants was accompanied by low calcium intake and high PRAL which partly suppressed its anabolic effect on bone health. This is in agreement with other studies (Thorpe *et al*., 2008; Vatanparast *et al* 2007).

A Limitation of this study is the cross-sectional design; it is carried out at one time point, providing some indication of the relationship between bone mass status and dietary variables and can't establish causal relationships. Another point to be mentioned is the lack of information about physical activity of the study population, since the interplay between physical activity and dietary calcium in bone health is potentially important (Boot *et al*., 1997).

In conclusion, detecting single-nutrient effects on bone health is difficult as the effects may be small, and the nutrient intakes themselves are often highly correlated. An appropriate intake of calcium accompanied by sources of alkali reducing PRAL is necessary for the anabolic effect of protein. Vitamin D supplementation is recommended in this age group since its synthesis in the skin or its intake from the diet is highly deficient. Consuming a well-balanced diet composed of a variety of foods, including calcium-rich foods, fruits and vegetables, grains and meat or beans on a daily basis is the best way to ensure an adequate intake of all these important bone-building nutrients. Obesity and overweight presented positive effect on bone mass of children and adolescents; however, follow up studies will be necessary to evaluate the influence of such characteristic on bone resistance in adulthood and at older age.

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