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High Dairy Calcium Intake in Pubertal Girls: Relation to Weight Gain and Bone Mineral Status

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As the deficient calcium intake, especially during period of rapid skeletal growth, may exaggerate osteoporosis, we aimed to study impact of calcium-rich diets mainly from dairy products in healthy adolescent females on Bone Mineral Density (BMD) and Bone Mineral Content (BMC) and to check for any relation to body weight gain. In a community-based controlled study, 73 healthy females were enrolled whose mean age 11.5 years and sexual maturation almost at Tanner II. Over a period of 24 months, one group (milk group, n = 44) was supplemented with dairy food products to a daily allowance of 1600 mg calcium and the second group (control group, n = 29) was given the usual diets (daily calcium content 800 mg). Body Mass Index (BMI), body fat and lean, BMD and BMC using dual-energy x-ray absorptiometry as well as some serological markers of bone-calcium metabolism were measured at start of study then 6 monthly. Milk group showed statistically significant increases in BMD and BMC in the last 3 estimates compared to control (p = 0.04, 0.02, 0.03 for BMD and 0.04, 0.01, 0.02 for BMC, respectively). However, neither anthropometric measures nor serological parameters showed any marked difference in the studied groups whether at start or in any subsequent estimate. Teenage girls whose dietary calcium intake at or above recommended daily allowance had an increased rate of bone mineralization especially when started during pubertal growth spurt without any significant increase in body fat or weight gain.

Key words: Bone mineral, calcium intake, dairy

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INTRODUCTION

Osteoporosis is a major health problem accounting annually for more than 200,000 fractures occurring in UK mostly (85%) in women (Kanis and Pitt, 1992). The maximum bone mass that protects against pathologic fractures is attained during the adolescence; it is dependant upon endogenous factors like genetics and hormonal profile as well as exogenous factors like diet and physical activity (Ott, 1991). Ensuring a healthy bone growth in teenage girls by supra-physiological doses of dairy calcium had been proven in different ethnics to be the cornerstone for prevention of osteoporosis and pathologic fractures that usually occur in older women (Johnston *et al*., 1992; Chan *et al*., 1995; Merrilees *et al*., 2000).

The reluctance of teenagers to drink milk or to eat other dairy foods for fear of excess weight gain, obesity and destruction of self-image (Lappe *et al*., 2004) or due to its unpleasant taste, may constitute the major limiting factor for calcium supplementation in this critical age group. The objective of our current study is to define the impact of calcium supplementation from dairy food products on Bone Mineral Density (BMD) and Bone Mineral Content (BMC) among Egyptian adolescent girls. Also we aimed to shed any light on the relationship between dairy calcium supplements and body fat- net weight gain aiming to minimize milk phobia or avoidance and to put forwards a plan for modulation of exogenous factors that would determine the maximum bone mass.

MATERIALS AND METHODS

Subjects and Study design: Our subjects were pubertal girls aged 10-14 years; all were at the same sexual maturation of Tanner stage II. They were healthy volunteers recruited from different Egyptian schools in Eastern Province, Saudi Arabia in the period from October, 2002 to December, 2004 and they represented most of the social classes; manual and non-manual. Seventy three teenagers were enrolled; they had no history suggestive of medical illness and were not using any drug known to affect calcium or bone metabolism e.g., antacids, calcium, food supplement, anti-epileptic or contraceptive pills. None were following a specific dietary regimen or had a recent fracture. A written informed consent was obtained from all volunteers and their parents after full explanation of the plan and objectives of study.

In a controlled trial over a period of almost 24 months with regular evaluations every six months; subjects were allocated randomly to either of two groups; milk group (n = 44) was advised to take three servings daily; average 600 mL of dairy food products (full-cream, half-cream or skimmed according to individual's preference) during the whole period of study; calcium content of the three milk forms is virtually the same; about 750 mg day^{-1} to be completed to 1600 mg day^{-1} from other sources). The second group is used as a control (n = 29); its subjects were allowed to maintain on their regular diet (average daily calcium intake = 800 mg). The supplemented amounts were estimated according to the recommended daily dietary calcium allowances; 800 mg for children more than 10 years; 1200 mg for pubertal children; and finally 800 mg for adults with an extra-amount of 400 mg for pregnant or lactating women. These amounts are needed to ensure a daily absorption of 150 mg (Lichton, 1989).

Random assessment of the daily dietary calcium and caloric intake was done by asking the guardian or individual himself about the food of previous day to ensure a perfect compliance. Caloric intake and physical activity were standardized to meet an average daily caloric allowance of 2000-2500 depending upon the individual's physical activity avoiding the regular competitive sports (Riddoch *et al*., 1991).

Anthropometric measurements: Weight was measured to the nearest 100 g with a set of upright balance scales wearing light clothes but without shoes. Triceps skin fold thickness and mid-arm circumference were measured as indices of body fat and lean, all measurements were made early morning by the same observer at each time point; at start of study and then 6 monthly.

Bone minerals, body fat and lean content: Total Bone Mineral Density (BMD; g cm^{-2}), Bone Mineral Content (BMC; g cm^{-1}), fat mass and lean mass were measured by dual energy X ray absorptiometry using a densitometer (Hologic, Waltham, MA, USA). This method has a precision error (coefficient of variation) of 0.9-1.0% for BMD in children, being calibrated daily using phantoms according to the manufacturer advice (Katzman *et al*., 1991). BMD and BMC were measured at the mid-shaft radius on ulna. At a position one-third proximal to ulnar process, three cut scans were made and the mean value was estimated.

Biochemistry: Non-fasting blood and urine samples were taken in morning of each time visit. Bone formation was assessed by radiometric assays of serum osteocalcin (ELSA-OSTEO, Cis Bio International, Gif-sur-Yvette, France) and serum bone-specific alkaline phosphatase (Tandem-R OSTASE, Hybritech Europe, Liège, Belgium). Bone resorption was assessed by cross linkage assays

(ELISA) done in urine for N-telopeptides of type I collagen (Osteomark, Ostex International, Seattle, WA, USA) and free deoxypyridinoline (Pyrilinks-D, Metra Biosystems, Mountain View, CA, USA). The results were expressed as ratio to urinary creatinine. Serum parathyroid hormone PTH (Nichols Institute, San Juan Capistrano, CA, USA) was measured by radioimmunoassay.

Statistical analysis: Data were analyzed using the Statistical Package of Social Science (SPSS, 1999). Depending upon normality of parameter, mean±SD and t-test were used to check for differences between two groups but Mann-Whitney U test was used for non-parametric differences. Unpaired t-test was used for difference between two groups in one time measure, but paired t-test for difference in two measures of a given parameter within each group. For the non-parametric serologic markers, Wilcoxon test was used to analyze changes over time within each group and Friedman test was a repeated measure analysis for the five estimates of BMD and BMC.

RESULTS

Anthropometric measurements of the studied groups are shown in Table 1; the mean ages 11.2±0.6 years and 11.8±0.4 years for milk and control groups, respectively. At the start of study both groups were perfectly matched. Both groups showed similar increments in weight, BMI, body fat and body lean, although the milk group showed tendency towards gain in body lean and reduction in body fat (yet statistically insignificant, $p>0.05$) without any clinical or statistical impact on the net body weight.

The serial estimates of BMD and BMC of the studied groups over 24 months are shown in Table 2 and plotted in Fig. 1A and B, respectively, these two parameters were properly matched at start of study assuring the random group assignment. Significant increments in the last three estimates were noticed among milk groups compared to the control ($p = 0.04, 0.02, 0.03$ for BMD and 0.04, 0.01, 0.02 for BMC, respectively). Repeated measure analysis of these parameters showed statistically significant increments in milk group compared to control ($p = 0.03$ and 0.02 for BMD and BMC, respectively) (Table 2).

Table 1: Anthropometric measurements of the studied groups at start of study and after 24 months

	Milk group		Control group	
	Before	After	Before	After
1. Weight (kg)	42.5±8.9	43.2±9.2	44.1±9.4	44.6±8.3
2. Body Mass Index (BMI) (kg m^{-2})	19.3±2.9	19.5±3.2	19.9±3.3	19.7±4.1
3. Body fat (kg)	10.9±3.9	10.8±4.2	11.2±4.1	11.5±3.5
4. Body lean (kg)	31.4±6.3	32.6±7.2	32 .1±6.6	32.2±6.9

values expressed are mean±SD

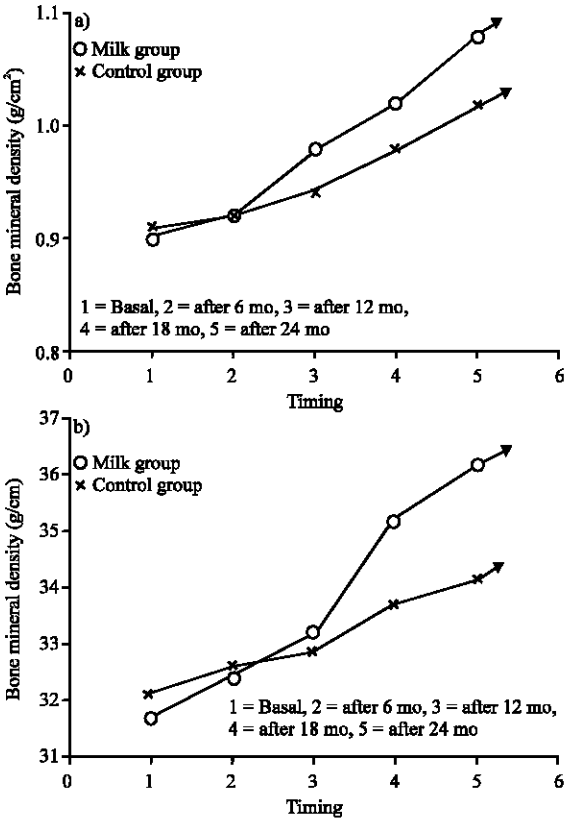

Fig. 1: Serial measures of bone mineral (g cm^{-2}) (a) and bonemineral content (g cm^{-1}) (b) in the studied groups throughout the study period

Table 3 showed the serological markers of bone metabolism in the studied groups at the start and end of study; they were almost similar indicating that calcium supplementation at or above the recommended allowance had no differential effect on bone turnover.

Table 2: The basal and follow-up values of bone mineral density (gm cm^{-2}) and bone mineral content (gm cm^{-1})

	Bone mineral density					Bone mineral content				
Test time	Basal	6 Mo.	12 Mo.	18 Mo.	24 Mo.	Basal	6 Mo.	12 Mo.	18 Mo.	24 Mo.
Milk group	0.90	0.92	0.98	1.02	1.08	31.7	32.4	33.2	35.2	36.2
Control group	0.91	0.92	0.94	0.98	1.02	32.1	32.6	32.9	33.7	34.2
p^1	0.22	0.12	0.04*	0.02*	0.03*	0.42	0.20	0.04*	0.01*	0.02*
p^2			0.03*					0.02*		

values expressed are means. Analysis of difference between the studied groups in each parameter at each time sitting (unpaired t-test, p^1) and repeated measure analysis (Friedman test, p^2). *p is statistically significant if less than 0.05

Table 3: Serological parameters of the studied groups at start and end of study

	Milk group		Control group		
	Basal	End	Basal	End	p^2
1. Alkaline Phosphate ($\mu g\ L^{-1}$)	80.1	58.2	82.3	60.8	0.97
p^1	0.002*		0.003*		
2. Osteocalcin ($ng\ mL^{-1}$)	127	91	134	90	0.11
p^1	0.004*		0.001*		
3. Telopeptide ($nmol\ mL^{-1}$)	350	285	360	290	0.98
p^1	0.02*		0.01*		
4. Deoxyprid-inolin ($nmol\ L^{-1}$)	17.1	14.3	16.9	15.8	0.35
p^1	0.03*		0.04*		
5. PTH ($pg\ mL^{-1}$)	22.4	18.8	24.8	20.4	0.27
p^1	0.003*		0.005*		

p^1 is the significance level between the basal and end parameter within the same group (Wilcoxon matched pairs signed ranks test). However, p^2 is the significance level between the studied groups after 24 months (unmatched pairs, Mann-Whitney-U test). *p is statistically significant if less than 0.05

DISCUSSION

Osteoporosis is a major health problem accounting for about 40% of pathologic fractures in women. Adolescence is the critical time for bone mineral deposition during which maximum peak bone mass is attained. Skeletal maturity is deemed to be the most important protective factor against pathologic fractures in later life (Kanis and Pitt, 1992; Ott, 1991).

In the current study, we tried to test the hypothesis that increased milk intake should increase bone mineral deposition which if maintained during the period of pubertal growth spurt would achieve the maximum peak bone mass. Up to the best of our knowledge, this hypothesis is firstly evaluated in the Egyptian teenage girls. Many clinical studies had been conducted in different ethnics evaluating this issue to control the growing dilemma of osteoporosis. In a study of 45 pairs of Indian identical twins over a period of 3 years; one twin received high calcium diet (1612 mg day^{-1}) and controlled by the other twin who received only 908 mg day^{-1}, they found increased calcium intake led to increased rate of gain in BMD and hence peak bone density could be attained before puberty (Johnston *et al*., 1992). In pubertal American girls, increased calcium intake at or above the recommended daily allowance was associated with increased rate of bone mineralization (Chan *et al*., 1995). In a randomized controlled trial done on 91 teenage New Zealand girls, it has been proven that high calcium supplement showed a consistent increase in BMD when assessed in different bony sites, but the increase in BMC was noticed only in some sites with no effect on vertebral height or width (Merrilees *et al*., 2000). It should be stressed that most of clinical researches evaluated the effect of dietary calcium of dairy source because of the big range of calcium variations in dairy foods compared to non-dairy calcium sources in human diet e.g., vegetables, fruits and fish and meat (Teegarden *et al*., 1999; Zemel *et al*., 2000).

In the present study, we have found significant increments in BMD and BMC of milk group, that received supra-physiologic doses of calcium supplement, in the measures at 12, 18 and 24 months compared to the control group that received the physiological calcium allowances. The overall impact of calcium supplement was unraveled by repeated measure analysis of Friedman test that showed statistically significant increments in BMD and BMC of milk group compared to the controls ($p = 0.03$ and 0.02, respectively). However, none of the serological markers for bone formation and bone resorption showed any significant difference between the two groups whether at the start or end study, indicating that the differential calcium supplementation had no selective effect on bone turnover although the significant suppression was a generalized rule in both groups.

Studying the impact of excessive dairy intake on body fat, lean and hence the rate of weight gain was the other facet of our study objective because obesity is also a growing problem nowadays not only in the civilized communities but also in the developing countries. Moreover, milk phobia or avoidance for fear of obesity was found to be a common complaint among teenagers of our culture (questionnaire in our study sheet, data were not shown). In the current study, both milk and control groups showed similar increments in weight, BMI, body fat and lean although milk group showed a predilection towards gain in body lean and reduction in body fat without any clinical or statistical impact on the net body weight. These results are closely similar to those reported in literatures especially when caloric intake is controlled (Chan *et al*., 1995; Merrilees *et al*., 2000; Phillips *et al*., 2003; Lappe *et al*., 2004).

We can conclude that calcium-rich diet specially from dairy food products should be encouraged in females during late childhood and adolescence to attain the maximum bone mass before puberty thus aiding in solution of the growing problem of female osteoporosis. No fear of obesity should be entertained with the high dairy intake if the caloric intake and exercise activity were standardized to be within the normal range.

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