HIGH DAIRY CALCIUM INTAKE IN PUBERTAL GIRLS: RELATION TO BODY WEIGHT GAIN AND BONE MINERAL STATUS

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ABSTRACT

Background: Lack of calcium intake may exaggerate osteoporosis which is most evident during period of rapid skeletal growth.

Objective: To study effect of high calcium intake mainly from dairy products in healthy adolescents females on their body weight, bone mineral density (BMD) and bone mineral content (BMC).

Subjects and methods: In a community-based, randomized controlled study, 73 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.

Results: 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 the anthropometric measures nor the serological parameters showed any marked difference in the 2 studied groups whether at start or in any subsequent estimate.
Conclusion: 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, Diary).

INTRODUCTION
Osteoporosis is a major health problem accounting annually for > 200,000 fractures occurring in UK mostly affecting women (85%) (1). The maximum bone mass which is the most important protector against fracture occurring in later life is mainly attained during adolescence; it is dependent upon endogenous factors including genetic and hormonal profile as well as exogenous factors like the diet and physical activity (2).

Teenage girls are usually reluctant to drink milk or to eat other dairy product foods for fear of weight gain and obesity and hence destruction of self-image. Some adolescents dislike milk and its products because of to its unpleasant taste (questionnaire in our present study, data not included). Milk avoidance in this critical period was deemed by many authors to be the basis of most pathological fractures occurring in later life. Therefore ensuring a healthy bone growth in teenagers is the cornerstone for prevention of osteoporosis and pathologic fractures occurring in old women (3-5).

The main objective of this work is to define the relationship of calcium supplementation (dairy food products) on body fat and weight gain and to suggest a plan for modulation of exogenous factors determining BMD and BMC.

SUBJECTS AND METHODS
Subjects and Study design: Our subjects included pubertal Egyptian girls who were healthy volunteers aged 10-14 years and enrolled from governmental and Egyptian schools in the Eastern Province, Saudi Arabia representing most of the social classes (manual and non-manual). Seventy three teenagers were enrolled having no history of any medical illness or using any drug known to affect calcium or bone metabolism (e.g. antacids, calcium, food supplement, anti-epileptic). None were following a specific dietary regimen or had a recent
fracture. A written informed consent was obtained from all volunteers and their parents following explanation of the study procedures. This randomized controlled trial was conducted over a period of 24 months starting at February 2002 with 6 monthly evaluations and terminated towards end of April 2004. Subjects were classified into a milk group (n = 44) advised to take daily 3 servings (average 600 ml) of dairy food products (full, half-cream or skimmed according to the subject's preference; calcium content of the 3 forms of milk is virtually the same; about 750 mg/day, to be completed to daily calcium intake of 1600 mg from other food sources) and a control group (n = 29) who were allowed to maintain on their habitual diet (average daily calcium intake = 800 mg).

The recommended daily dietary calcium allowances were estimated to be 800 mg for children < 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 calcium absorption of 150 mg (6). Random assessment of the daily dietary intake (calcium and caloric content) was done by asking the guardian or individual himself about the food of previous day to ensure perfect compliance. Caloric intake and physical activity were standardized to meet an average daily caloric intake of 2000-2500 depending upon the physical activity avoiding the regular competitive sports (7).

Anthropometric measurement: 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 (TST) and mid-arm circumference (MAC) were measured as indices of body fat and lean, all measurements were made in the morning, by the same observer at each time point (at the beginning and at 6 month intervals).

Bone minerals, body fat and lean content: Total bone mineral density (BMD; gm/cm²), bone mineral content (BMC; gm/cm), 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 accord-
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ing to the manufacturer advice (8). BMD and BMC were measured at the midshaft radius on ulna; it is located by measuring the distance between the midpoint of the ulnar styloid process and the proximal edge of olecranon and marking a position that was one-third proximal to ulnar process. Three cut scans were made at that position and the mean values were estimated.

Biochemistry: Non-fasting blood and urine samples were taken in the 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 a ratio to urinary creatinine concentration. Serum parathyroid hormone (Nichols Institute, San Juan Capistrano, CA, USA) was measured by radioimmunoassay.

Statistical analysis: Data were analyzed using SPSS for windows version 10.0 (9). Mean ± SD were used to express central values and dispersion unless otherwise indicated. T-test and Mann-Whitney U test were used for the comparison between two groups (depending upon the normality of parameter being checked by Kolmogrov Smirnov test). Unpaired t-test was used for difference between 2 groups in one time measure, but paired t-test was used in one group for difference in two time-measures. For non-parametric variables (serological markers), Wilcoxon matched pairs signed ranks test was used to analyze changes over time within each group. Repeated measure analysis (Friedman test) was done for the 5 estimates of BMD and BMC (basal and 6 monthly follow-up values).

RESULTS

Anthropometric measurements of the two studied groups at the start (before) as well as end of study (after) were shown in table (1); at start of study both groups were perfectly matched while at end of study although both groups showed similar in-
crements in weight, BMI, body fat and body lean, yet milk group showed tendency towards gain in body lean and reduction in body fat (statistically insignificant; \( P > 0.05 \)) without any clinical or statistical difference between the 2 groups regarding the net body weight.

Serial estimates of BMD and BMC showed significant increments with oral calcium supplements whether at or above the recommended daily allowances (P2 of Freidman test were 0.03 and 0.02 respectively). However, the increments in milk group were significantly higher than the control group at 12, 18 and 24 month estimates (P1 for unpaired t-test were 0.04, 0.02, 0.03 for BMD and 0.04, 0.01, 0.02 for BMC respectively); table: 2 and figure: 1.

Serological parameters for bone formation (osteocalcin and serum bone-specific alkaline phosphatase) and urinary markers for bone resorption (N-telopeptides of type I collagen and free deoxypyridinoline) as well as serum parathyroid hormone were significantly suppressed at the end of study in the 2 studied groups (P1 for matched pairs for all of these parameters in each group was < 0.05) with no statistically significant difference between both groups; neither at the start nor at the end of study (table: 3).

Table (1): Anthropometric measurement for the 2 studied group at start of study and after 24 moths, values expressed are mean ± SD.

<table>
<thead>
<tr>
<th></th>
<th>Milk group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>1. Weight (kg)</td>
<td>42.5 ± 8.9</td>
<td>43.2 ± 9.2</td>
</tr>
<tr>
<td>2. Body mass index</td>
<td>19.3 ± 2.9</td>
<td>19.5 ± 3.2</td>
</tr>
<tr>
<td>(BMI) in kg/m²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Body fat (kg)</td>
<td>10.9 ± 3.9</td>
<td>10.8 ± 4.2</td>
</tr>
<tr>
<td>4. Body lean (kg)</td>
<td>31.4 ± 6.3</td>
<td>32.6 ± 7.2</td>
</tr>
</tbody>
</table>
Table (2): The basal and follow-up values of BMD (gm/cm²) and BMC (gm/cm): values expressed are means.

<table>
<thead>
<tr>
<th>Test Time</th>
<th>Bone mineral density (BMD)</th>
<th>Bone mineral content (BMC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Milk group</td>
<td>0.90</td>
<td>0.92</td>
</tr>
<tr>
<td>Control group</td>
<td>0.91</td>
<td>0.92</td>
</tr>
<tr>
<td><em>p</em></td>
<td>0.22</td>
<td>0.12</td>
</tr>
<tr>
<td><em>p</em></td>
<td>0.03*</td>
<td>0.02*</td>
</tr>
</tbody>
</table>

Analysis of difference between the two groups in each parameter at each time sitting (unpaired t-test, *p*¹) and repeated measure analysis (Friedman test, *p*²).

Table (3): Serological parameters of the two studied groups at start and end of study, (Wilcoxon matched pairs signed ranks test).

<table>
<thead>
<tr>
<th>1. Alk. Phosph. (in ug/l)</th>
<th>Milk group</th>
<th>Control group</th>
<th><em>p</em>²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>80.1</td>
<td>82.3</td>
<td>0.97</td>
</tr>
<tr>
<td>End</td>
<td>58.2</td>
<td>60.8</td>
<td></td>
</tr>
<tr>
<td>2. Osteocalcin (ng/ml)</td>
<td>127</td>
<td>134</td>
<td>0.11</td>
</tr>
<tr>
<td>Basal</td>
<td>91</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>End</td>
<td>134</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>3. Telopeptide (nmol/ml)</td>
<td>350</td>
<td>360</td>
<td>0.98</td>
</tr>
<tr>
<td>Basal</td>
<td>285</td>
<td>290</td>
<td></td>
</tr>
<tr>
<td>End</td>
<td>360</td>
<td>290</td>
<td></td>
</tr>
<tr>
<td>4. Deoxyxypidinol (nmol/l)</td>
<td>17.1</td>
<td>16.9</td>
<td>0.35</td>
</tr>
<tr>
<td>Basal</td>
<td>14.3</td>
<td>15.8</td>
<td></td>
</tr>
<tr>
<td>End</td>
<td>16.9</td>
<td>15.8</td>
<td></td>
</tr>
<tr>
<td>5. PTH (pg/ml)</td>
<td>22.4</td>
<td>24.8</td>
<td>0.27</td>
</tr>
<tr>
<td>Basal</td>
<td>18.8</td>
<td>20.4</td>
<td></td>
</tr>
<tr>
<td>End</td>
<td>24.8</td>
<td>20.4</td>
<td></td>
</tr>
</tbody>
</table>

*P*¹ is the significance level between the basal and end parameter within the same group (matched pairs). However, *P*² is the significance level between the 2 studied groups after 24 months (unmatched pairs).
Figure (1): Serial measures of BMD (gm/cm²) and BMC (gm/cm) in the 2 studied groups throughout the study period.

1=Basil, 2=after 6 mo, 3=after 12 mo.
4=after 18 mo, 5=after 24 mo
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 agent against these pathologic fractures in late: life (1&2).

In the present study, we tested the hypothesis that increased milk intake should increase the 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. On the other hand, many clinical studies (randomized controlled and retrospective) were conducted in different ethnics evaluating this issue to control the growing dilemma of osteoporosis.

In a study including 45 pairs of Indian identical twins over a period of 3 years; one twin received high calcium diet (1612 mg/day) and controlled by the other twin (whose daily calcium intake was 908 mg), the authors concluded that increased calcium intake would increase the rate of gain in BMD and hence the peak bone density could be attained before puberty reducing the risk of fractures (3). Increased dietary calcium intake at or above the recommended daily allowance (primarily provided from the dairy products) in American pubertal girls, was found to be associated with increased rate of bone mineralization (4). In a randomized controlled trial in 91 teenage New Zealand girls, it has been proven that the high calcium supplement (mainly from dairy products) showed a consistent increase in BMD assessed in different bony sites, but the increase in BMC was noticed only in some sites (not consistent) with no effect on the vertebral height or width (5). 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 food compared to non-dairy calcium source in human diet e.g. vegetables, fruits and fish and meat (10 & 11).

In the present study, we have found that BMD and BMC of milk group showed significant increment in the last 3 estimates (at 12, 18 and 24 months) compared to the control
group, with the significance level P1 was 0.04, 0.02, 0.03 for BMD and 0.04, 0.01, 0.02 for BMC respectively. The overall impact of calcium supplement is unraveled by repeated measure analysis showing that BMD and BMC to be statistically significant increased in milk compared to control group (P² = 0.03 and 0.02 respectively). However, none of the serological markers for bone formation and resorption showed any significant difference between the 2 studied groups whether at the start or end of study, indicating that calcium supplementation had no effect on bone turnover.

Studying the impact of excessive dairy products intake on the body fat, lean and hence the rate of weight gain is a principal goal in our study because obesity is also a growing problem nowadays in civilized communities. Moreover, phobia from obesity was noticed by the way to be the main cause for milk products avoidance in the teenage girls (questionnaire in our study sheet, data not included).

In the present study, both milk and control groups showed similar increments in weight, BMI, body fat and body lean, although the milk group showed a predilection towards gain in body lean and reduction in body fat (yet statistically insignificant, P > 0.05) without any clinical or statistical difference between the 2 studied group regarding the net body weight.

These results are very similar to those reported in literatures where no evidence was found for the effect of dairy food consumption on the body fat % or BMI z-score during adolescence (4, 5 & 12). Moreover it was proved that increased dietary calcium (primarily provided from dairy food) did not cause excessive weight gain, if caloric intake was controlled (13).

However increased dietary calcium associated with body weight reduction was firstly noticed at 1988 and 1989 in rats (14 & 15), then proved 10 years later in human (10, 11 & 16); but these authors recommended further studies specifically designed to address this issue. Moreover, the perfect impact of dairy calcium supplementation on weight reduction is particularly evident during periods of weight control program, so some authors recommended that the US trend to remove dairy products from weight control program should be changed and instead the use of

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low-fat dairy food products in weight reduction regimens should be encouraged (17).

In the light of our results as well as the above recommendations we can conclude that calcium-rich diet especially from dairy food products should be encouraged in females during late childhood and adolescence to attain the maximum bone mass before puberty thus solving the growing problem of female osteoporosis. Meanwhile no fear of obesity should be entertained from much dairy intake if caloric intake and exercise activity are standardized to be within the average range.

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