ORIGINAL ARTICLE

Dietary calcium intake and risk of obesity in school girls aged 8-10 years

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Background: Some studies have demonstrated the role of calcium in reducing body mass index (BMI) or fat mass. Though, BMI does not provide very valid information about changes in body fat mass, Fat Mass Index (FMI) relates body fat mass to height and allows comparing body fat mass of individuals at different heights. This study investigated the possible association between dietary calcium intake (CI) and other nutritional factors and weight status of girls aged 8-10 years. **Materials and Methods:** In this case-control study, 110 girls aged 8-10 with FMI at or above 7.2 kg/m^2 as cases and 307 girls with FMI less than 7.2 kg/m^2 as controls were recruited through multistage cluster random sampling. FMI at or above 7.2 kg/m^2 was considered as the cutoff point for obesity. Body fat mass was assessed by a stand on bio impedance analyzer. In order to assess CI, participants were asked to complete a validated food frequency questionnaire. **Results:** Mean and standard deviation of CI in the case group was significantly lower than the control group 649 ± 103 and 951 ± 152 mg/d, respectively ($P < 0.01$). After Adjustment for total energy intake, the percentage of energy from fat, carbohydrate and protein in quartiles of physical activity, inverse association between CI and obesity was significant and in the highest quartile of physical activity the association was weaker. By further adjustment for the effect of fruits and vegetable intake inverse association between CI and obesity became weaker but yet was significant. **Conclusion:** The inverse relationship between CI and FMI remained significant even after controlling for confounding factors. FMI may be more accurate, compared to BMI, in showing the association between CI and obesity.

Key words : Calcium, fat mass index, girls

INTRODUCTION

Obesity has become an epidemic issue all around the world among children.[1] Based on results from the National Health Examination Survey, 18.8% of children 6-11 years old in the United States were overweight in year 2003-2004.[2] Prevalence of obesity and overweight in school age children in Iran based on the Center for Disease Control and Prevention (CDC 2000) is estimated to be 4.4% and 9.8%, respectively[3] and the prevalence rate of obesity and overweight in school age children in Iran based on The World Health Organization (WHO 2007) was reported 14% and 6% respectively.[4]

Based on the CDC growth chart, overweight was defined as being at or above the 95th percentile and at risk for overweight was defined as being between the 85th and the 95th percentile of sex-specific BMI-for-age in a specified reference population.[5] Unlike the CDC chart, the terminology used in this study referrers to overweigh as obese and being at risk of overweight as overweigh, relative to body mass index (BMI).

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Several factors are associated with the increase in incidence of obesity in children. Among these factors low physical activity, high intake of foods, presence of obesity in parents, socio-economic and nutritional factors are important.[6-9] Several published studies on nutritional behaviors among children have reported that high intake of milk and other dairy products,[10] high intake of vegetables and other sources of fiber,[11] and low intake of sweetened beverages[8] are associated with lower levels of BMI and body fat mass.

Results of studies which exploring the association between calcium intake (CI), specifically from dairy sources, and BMI, body weight, or body fat are not conclusive. To determine the long-term effect of a dairy-rich diet in weight control of young children, one study conducted a randomized controlled trial and showed that a using a dairy rich diet could be

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a practical strategy for weight control in overweight children.[12] Another study concluded that CI was inversely associated with BMI, sum of skinfolds, and trunk skinfolds in children.[13] However, an interventional study on young women, showed that there were not any differences in weight change, BMI, fat mass and fat free mass (which was measured with dual energy X-ray absorptiometry [DXA]), among subjects with high calcium and normal CI over one year.[14] Another interventional study showed that longer intake of calcium was not associated with lower body fat.[15] Body fat was assessed by DXA. BMI is a crude but readily obtainable measure of excess body fat. Therefore, it is not an accurate measure of changes in body fat and body compounds.[16] It measures excess weight relative to height, and does not discriminate body fat from fat-free mass.[17]

Fat Mass Index (FMI), which is defined as body fat (kg), divided by the square of height (m^2), is an indicator of nutritional status[18] and takes into account fat mass content in addition to height and weight. With FMI, one is able to compare body fat mass of individuals with different heights. That may explain the inconsistency in the results of the studies.

In the present study, we used FMI as an indicator of obesity in children. By using FMI, we were able to compare body fat mass of individuals with different heights, and to compare the intake of daily calcium in obese and non-obese children.

MATERIAL AND METHODS

Subjects

In this case-control study, the study population was 8-10 years old girls from primary schools in the city of Isfahan, in Iran. Multistage cluster random sampling was conducted for sample selection. Ten schools were randomly selected from five education and training districts of the city, and 11 children were selected from each school as cases. In total, 110 children were randomly selected as cases. For each case, three controls were randomly selected from the same class and the same school.

As the reference standards for FMI based on a large subject population have not been clearly defined, on account of larger sample size of Nakao's study which led creating a reference norm for Japanese boys and girls, we applied the latter cutoff for our study.[19] We considered 90^{th} percentile of FMI value for 9-11 years girls as the cut off point for obesity. Subsequently, based on the Nakao's *et al*. reference norm, FMI at or above 7.2 kg/m^2 was considered as a criterion for selecting cases; and FMI less than 7.2 kg/m^2 was considered as a criterion for setting controls. Furthermore, we computed the 90^{th} percentile value of FMI for the 420 participants in our study. The value was 7.4, which was 0.2 more than Nakao *et al*. value for 90^{th} percentile. In total, 110 girls in the case group and 307 girls in the control group were recruited.

Anthropometric measurements

Body weight was measured without shoes and with minimum clothing using a digital scale (Tanita, Japan) with 0.1 kg accuracy. The scale was calibrated before each measurement session. Height was measured with 0.1 cm accuracy with stadiometer. BMI was calculated as weight in kilograms divided by height in meters squared. To measure fat mass, we used a bio impedance analyzer (BIA) (Tanita 418 MA, Tokyo, Japan). Compared to DXA, BIA has an acceptable accuracy and provides on average; 2-6% lower values for fat mass.[20] The device uses a multi frequency electrical level to estimate both extracellular and intracellular fluids. It has eight polar electrodes and it incorporates a digital scale for measuring body mass. BIA was used under constant conditions, before exercise, in a fasting situation. For all of the subjects, the same device was used. Fat mass (kg) were divided by height squared (m^2) to calculate FMI. FMI at or above 7.2, according to the 90^{th} percentile of reference norm of Nakao study in Japan[19] was considered as a criterion for categorizing obesity and FMI less than 7.2 was considered as criterion for not being obese.

Assessment of dietary intake

A 56-item food frequency questionnaire (FFQ) was used to evaluate the CI of children for 1 year ago. The questionnaire was filled out by interviewing mothers or caregivers and children. Validity and reliability of this questionnaire in children in Tehran had been examined previously.[21] Validity of FFQ was determined by comparison with five 24 h dietary recalls. After controlling for total energy intake, correlation coefficients between the two methods were 0.35 and 0.65 for validity and reliability, respectively.

The amount and frequency of food consumption during the previous year on a daily, weekly or monthly basis was assessed by this FFQ. This questionnaire categorized food items into the following subcategories: Group 1: Cereals including bread, rice, cookies, biscuits and pancakes; group 2: Grains; group 3: Milk, milk shake, yoghurt, cheese, ice-cream, yoghurt drink; group 4: Eggs, red meat, fish and poultry; group 5: Tomato, cucumber, lettuce, cabbage, spinach and green leaf vegetables; group 6: Oranges, apples, dates, dried fruits, raisins, sloe, blueberries; group 7: Nuts, cocoa chocolate, soy beans, seeds, and sesame paste. Quantities were estimated from photographs of portion sizes and from household measures.[22]

In addition to the FFQ, subjects completed 3 day food records including 1 weekend day and 2 weekdays to assess their total intake of energy and macronutrient. These records were filled out by mothers or caregiver and children. Explicit written instructions in addition to an explanatory oral session on how to complete records on beverages, food, supplements, and estimating portion sizes were provided to mothers. Expert nutritionists controlled all records for accuracy.[23]

Each food and beverage was coded according to the prescribed protocol and then analyzed for content of energy and CI and serving of daily dairy products, fruits and vegetables intake, based on U.S. Department of Agriculture (USDA) database and Iranian food composition table, by using NUTRITIONIST IV software.

In order to evaluate physical activity of the children, we utilized Baecke *et al*.[24] questionnaire. Information was collected on the level of habitual physical activity. The questionnaire consisted of components of physical activity, sports during leisure time, and physical activity during leisure time, excluding sports.[25] To compare physical activity scores between the two groups, Beacke scores were computed across quartiles.

Statistical analysis

Quantitative variables were expressed as mean ± Standard deviation (SD) and qualitative variables, as median and interquartile range. The Kolmogorov–Smirnov test was used to test the normality of the distribution of variables. Independent samples comparisons in terms of quantitative variables were done using two independent samples *t*-test and Mann-Whitney *U* test as appropriate. Multinominal logistic regression modeling was used for modeling the relationship between CI with FMI, adjusting for potential confounding variables, including: Energy intake (in kcal/d), percentage of energy from fat, carbohydrate and protein (percent/d), physical activity level (light, moderate, or severe), and consumption of fruits and vegetables (serving/d). We categorized physical activity scores based on quartiles. Quartiles cut points for physical activity were based on the distribution of scores among the control group. Cochran–Armitage test for trend also was conducted. All statistical analyses were done using SPSS software (version 16; SPSS Inc., Chicago IL). *P* value, 0.05 was considered significant.

RESULTS

The mean and standard deviation of FMI and BMI for the 417 children were 6 ± 2.1 kg/m^2 and 19.4 ± 3 kg/m^2, respectively.

The means and SDs age (y), anthropometric measurements and physical activity status for case and control groups are shown in Table 1. The mean of, BMI ($P < 0.05$), fat mass ($P < 0.01$), percent of fat mass ($P < 0.01$), waist circumference ($P < 0.01$) were significantly higher in cases, as compared to controls. The mean of fat free mass ($P < 0.01$) was significantly higher in controls, as compare to cases. Most of the subjects in the control group were in 4th quartile of physical activity score and the majority of the subjects in the case group were in the 3rd quartile. Cases had significantly higher scores of physical activity in 3th and 4th quartile ($P < 0.05$), and lower score in 1th quartile [Table 1].

Compared with the subjects in the control group, cases had more intake of total energy ($P < 0.01$) and more intake of fat (percent of total energy from fat) ($P < 0.01$) and low intake of protein (percent of total energy from protein) ($P < 0.05$). Children in case and control groups showed a significant difference in CI and dairy products, fruits and vegetables consumption. Compare to cases, controls consumed significantly more from mentioned food groups ($P < 0.01$) and there was no significant difference between two groups in intake of the meat and meat products ($P = 0.06$) [Table 2].

Multinominal-adjusted odds ratios and 95% CIs for obesity and its features for CI in quartiles of physical activity are shown in Table 3. In low quartiles of physical activity the association between CI and obesity is stronger. In model 1, we adjusted obesity for the effect of total energy intake, the percentage of energy from fat, carbohydrate and protein (OR and 95% CI in quartiles of physical activity respectively 0.69 (0.59-0.71), 0.74 (0.61-0.86), 0.83 (0.81-0.85), 0.87 (0.95-0.96). After adjustment, the inverse association between CI and obesity was significant and in the 1st quartile of physical activity the association was significantly stronger than 4th quartile. In model 2, we further made adjustments for the effect of fruit and vegetable intake, resulting that the OR became weaker (OR 95% CI in quartiles of physical activity respectively 0.85 (0.63-0.96), 0.86 (0.79-0.99),

Table 1: Characteristics of the subjects in case and control group

Variable	Case (*n*=110) mean (SD)	Control (*n*=307) mean (SD)	*P**
Age (year)	8.6±0.4	8.8±0.72	<0.07
BMI (kg/m^2)	22.2±2.7	18.5±2.6	<0.01
Fat mass (kg)	15.6±2.4	9.4±3.3	<0.01
Fat mass percent (p)	34.2±3.6	27±4.7	<0.01
Fat free mass (kg)	24.4±4.5	30.1±3.4	<0.01
Waist circumference (cm)	73.2±6.8	62±6.8	<0.01
Quartile of physical activity score**			
1 (%)	22.4	7.2	<0.05***
2 (%)	23.3	25.9	<0.05***
3 (%)	27.8	32.8	<0.05***
4 (%)	26.5	34.1	<0.05***

*= *P* values based on *t*-test or Mann-Whitney test; **=Cutoffs were: <2.15; 2.15 to <2.28; 2.28 to <2.56; and ≥ 2.56 for quartiles 1-4; respectively; ***= The result of Cochran-Armitage test for trend; {BMI=Body mass index}

Table 2: Dietary intake of participants in case and control group

Dietary intake	Case (*n*=110)	Control (*n*=307)	*P*[a]
Nutrients			
Total energy (kcal/d)	2354±291[b]	1887±298[c]	<0.01
Carbohydrate (% of total energy)	35±3.1[b]	36±3.6[b]	<0.08
Protein (% of total energy)	25±3.2[b]	28±3.4[b]	<0.05
Fat (% of total energy)	40±6.1[c]	36±5.2[c]	<0.01
Calcium (mg/d)	657±103[b]	981±152[b]	<0.01
Food groups (serving/d)			
Fruits[d]	1.3±0.3[b]	2.1±0.7[b]	<0.01
Vegetables[e]	0.7±0.2[b]	1.1±0.5[b]	<0.01
Dairy[f]	1±0.3[b]	2.5±0.4[b]	<0.01
Meat[g]	2.1±0.4[b]	2±0.7[b]	<0.06

[a]*P* values based on *t*-test or Mann-Whitney test; [b]mean±SD; [c]median±interquartile range; [d]Includes apples, oranges, bananas, peaches, grapes, strawberries, pears, watermelon, grapefruit, prunes, pomegranates, kiwi, persimmons, raisins, figs, coconuts, apricots, sweet lemon, and lemon; [e]Includes onions, cucumbers, lettuce, carrots, cauliflower, brussels sprouts, kale, cabbage, spinach, mixed vegetables, corn, green beans, green peas, peppers, beets, potatoes, tomatoes, broccoli, and celery; [f]Includes milk, yogurt, and cheese; [g]Includes beef, liver, chicken hearts and kidneys, hamburger, sausages, processed meats, meat in a sandwich, tuna fish, and other fish

Table 3: Multinominal-adjusted odds ratios (OR and 95% calcium intakes [CIs]) for obesity across CI

OR (95% CI)	Quartile of physical activity			
	1 (*n*=45)	2 (*n*=104)	3 (*n*=129)	4 (*n*=132)
*Model 1	0.69 (0.59-0.71)	0.74 (0.61-0.86)	0.83 (0.81-0.85)	0.87 (0.95-0.96)
**Model 2	0.85 (0.63-0.96)	0.86 (0.79-0.99)	0.89 (0.74-0.88)	0.99 (0.97-0.98)

*= Model 1 was adjusted for total energy, percentage of energy from fat, carbohydrate and protein; **= Model 2 was further adjusted for fruits and vegetables intake; {CI=Calcium intake}

0.89 (0.74-0.88), 0.99 (0.97-0.98). The inverse association between CI and obesity became weaker but significant.

DISCUSSION

The result of this study indicates that FMI is inversely associated with daily dietary CI and the inverse relationship between FMI and CI remained significant after adjusting for confounding factors.

Results from other studies are not conclusive in this regard. One study reported an inverse association between CI and body fat in 8-year-old children by conducting a 5-year longitudinal study.[25] In this study, body fat was measured with DXA and CI was assessed by food record. Another study reported an inverse association between BMI and body weight in children.[26] Yet another study reported inverse association between BMI, sum of skinfolds and trunk skinfolds and calcium consumption in children.[13] Other studies showed beneficial effects of dairy products in regulating body weight.[27-29] Zemel *et al*.[30] reported that isocaloric substitution of dairy products in obese adults reduced total body fat and decreased trunk fat. Contrary to our findings, some other studies found no relationship between CI and BMI, body weight and fat mass in children. An interventional study showed that children with rich calcium diet had an increase in BMI, weight and fat mass similar to the group with normal calcium diet.[31] Another study assessed CI in children through 24 h recall and measured body fat with DXA. They did not find any association between CI and BMI, body weight or body fat.[32] Some interventional studies, as well, did not find any association between CI and weight or fat mass change. One review study consists of nine randomized dairy product supplementation trials reported that seven of the studies did not find any association between CI and weight change. Two studies found greater weight gain in the group with dairy product intake.[29] One study did not find any association between higher intake of dairy product and change in body weight or fat mass over 1 year in young women.[33] Another study reported inverse association between calcium supplements and weight gain.[34] In contrast, one study found no association between calcium supplementation and body fat, but reported an inverse association between body fat and dietary CI.[35]

Our study was in agreement with studies that showed inverse association between dietary calcium and dairy products consumption, on the one hand, and body fat, BMI, and the incidence of obesity, on the other.[36-43]

The results on association between CI and obesity remain controversial. One reason could be that previous studies focused mostly on BMI or fat mass as an indicator of obesity. BMI is not a good yardstick for assessing obesity, because BMI does not discriminate body fat from fat-free mass.[16,17] In this study, by applying FMI we were, therefore, able to compare body fat mass of individuals with different heights, and further assess the association between CI and obesity.

One explanation for the effect of calcium on body fat is that by high CI calciotropic hormones (calcitriol and parathyroid hormones) are suppressed, and this ultimates a decrease in intracellular calcium concentration in adipose tissue and subsequently increases lipolysis and thermogenesis. As a consequence, a decrease in body fat deposition occurs.[44] Another mechanism which can describe calcium's anti-adiposity effect is that with high CI, high fecal fat loss and inhibiting fat absorption occurs, and it will contribute to a reduction in energy balance.[45]

Protein content of dairy products and their amino acids composition could play a role in reducing obesity. Compared to other macronutrients, protein is more effective in suppressing appetite. One component of dairy products that could have a role in modulating adiposity is whey protein. The rich concentration of branched-chain amino acids in whey protein (26% leucine, isoleucine, and valine)[46,47] could play a specific metabolic role (leucine specifically) in supporting protein synthesis[48] by repartitioning of dietary energy from adipose tissue to skeletal muscle.[44] This suggests that high amounts of calcium in dairy products in combination with whey protein can have synergistic effect to diminish fat mass.[44]

Another mechanism that expresses the anti-obesity role of dairy products is related to calcitriol which suppresses the expression of uncoupling protein2 (UCP2) via vitamin D nuclear receptor.[49] UCP2 has a role in inducing a mitochondrial proton leak and hence increasing thermogenesis. In addition, it induces apoptosis in adipocytes. High intake of calcium in mice, by suppressing 1,25-(OH) 2-D3 levels enhanced adipocytes apoptosis and UCP2 expression and inhibited the decline in thermogenesis.[50] So high calcium diet increased fat oxidation and decreased lipid accumulation.

There are some limitations in our study. We designed a case-control study to explore the association between CI and FMI. Further interventional studies will provide stronger evidence on this association. In this study, we utilized BIA (Tanita 418 MA) to assess fat mass. Even though the device is one of the most valid instruments after DXA to measure fat mass, measuring fat mass with DXA can provide stronger evidence. Furthermore, we did not have a reference norm in Iran to define a cutoff point for FMI. Even though we computed 95th percentile for percent of fat mass for our population, the sample size (410) was not large enough to conclude a cut off points for FMI. Therefore, we applied reference norms from Nakao study. To our knowledge this was the only study in this age group with large enough sample size that could be used to indicate a cutoff point for FMI. It is recommended that future studies assess the components of dairy products and related mechanisms of action which are responsible for this effect.

An advantage of this study was using FMI as a measure to assess obesity. Whereas BMI may not provide accurate information about changes in body fat mass, FMI relates body fat mass to height and let us compare the body fat mass of individuals with different heights.

In conclusion, we found evidence that indicates an inverse relation between CI and FMI after controlling for potential confounding factors. Further studies are required to corroborate our findings.

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