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Dairy products and total calcium intake at 13 years of age and its association with obesity at 21 years of age

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Abstract

Background/objectives Dairy products and specifically calcium have been suggested to play a role in obesity development but more longitudinal evidence is still needed. The objective of this study was to assess the association between dairy products and total calcium intake at age 13 and body mass index at age 21.

Subjects/methods This longitudinal study included 2159 individuals from the Epidemiological Health Investigation of Teenagers cohort (EPITeen), Porto, Portugal, evaluated at ages 13 and 21. Assessment consisted of anthropometrics measurements and structured questionnaires namely a semi-quantitative food frequency questionnaire to appraise food consumption in the past 12 months. Linear regression models were run in 941 individuals with complete information of confounders: gender, follow-up period, parents' education, physical activity, energy, and total calcium intake.

Results Negative association was found on total calcium intake at age 13 with BMI at age 21 (model 0: $\beta = -0.059$ (95% CI: -0.113, -0.004) and model 1: -0.057 (95% CI: -0.113, -0.002)), however, no statistically significant association was found when adjusting for energy intake (model 2: $\beta = -0.031$ (95% CI: -0.110, 0.047)). There were no associations between milk, yogurt, and cheese consumption at age 13 and BMI at age 21 when adjusting for confounders.

Conclusions This study did not support an independent effect of dairy products or total calcium intake in adolescence on later early adulthood adiposity.

Introduction

Worldwide obesity is one of the most prevalent evitable disease in the world, increasing to more than double in three decades [1]. The prevalence of overweight and obesity in children and adolescents is increasing at worrying rates, both in developed and developing countries [2, 3]. Since these entities are preponderant risk factors for cardiovascular disease, metabolic syndrome, and type 2 diabetes mellitus [4, 5] efforts should be made to reduce this trend. Among the multiple modifiable risk factors, the role of calcium in the obesity pathophysiology needs to be clarified

[2, 6] and its importance in fat accumulation in children and adolescents remains to be definitely established [7–9].

One of the most cited mechanisms is the impact of intracellular calcium in lipid metabolism inside the adipocytes [10]. In fact, the decrease in intracellular calcium induced by the decrease of parathyroid hormone (PTH) and $1,25(OH)_2D_3$ [11] promotes a decrease in lipogenesis and an increase in lipolysis and fat oxidation [7]. Furthermore, $1,25(OH)_2D_3$ also has a role in the size of fat storage inside adipocytes [12] and in their differentiation and proliferation [13]. Other plausible mechanisms were also proposed for the possible beneficial effect of calcium in adiposity. The decrease of fat absorption enhanced by calcium binding to bile acids and by forming insoluble fatty acid soaps [14] leads to an increase of fecal fat excretion which eventually will diminish the energy intake. Another explanation is that nutrients from dairy products can also be related to body fat regulation. For instance, conjugated linoleic acid and proteins influence fat accumulation and regulation of appetite, respectively [7].

Some review studies support the hypothesis that dairy consumption is inversely associated with the development

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of childhood obesity [5, 7, 8, 15, 16]. Observational studies have been showing results of a negative relationship between dairy consumption and body mass index (BMI) [17–22], body fat mass [23, 24], abdominal circumference [21, 25–27] and skin folds [21, 26] both cross-sectionally [21, 25–27] and longitudinally [17–20, 22–24], however none of these studies evaluated calcium intake effect. Additionally meta-analysis of observational studies [15, 16] and randomized controlled trials [16, 28, 29], in children and adolescents, showed discrepant results. Although beneficial influence of calcium intake in long-time obesity is supported mostly by cross-sectional studies there is the need to confirm this effect with more longitudinal and experimental studies.

The aim of this study was to evaluate the association of total calcium intake and dairy consumption at 13 years of age and BMI at 21 years of age.

Subjects and methods

Participants

The participants in this study are part of the Epidemiological Health Investigation of Teenagers cohort (EPITeen), Porto, Portugal [30], which started in the academic year 2003/2004 and was carried out in public and private schools in Porto, Portugal. These individuals were born in 1990 and were evaluated at ages 13, 17, and 21. In this study, only measurements from ages 13 and 21 were used because dietary assessment was not conducted at age 17.

At the beginning of the cohort, 2159 of 2942 eligible adolescents (73.4%) agreed to participate in the study. For the data analysis, 986 participants with complete information for weight, height and milk, yogurt, and cheese intake, in both moments of evaluation, were included.

Therefore, outliers were excluded ($n = 45$) when meeting the following criteria: missing answer to 57 or more items of food; total energy intake more than 3 times the interquartile range over the third quartile or under the first quartile; intake of fruit or vegetables more than more than 1.5 times the interquartile range over the third quartile or under the first quartile. After exclusions, sample ended with 941 participants for statistical analysis.

Data collection

Data collection was assessed by questionnaire including sociodemographic variables (age, gender, parents' education, aggregate monthly income, and social class), medical history (diagnosis of diabetes, asthma, and eating disorders), and behavioural aspects (smoking status, alcohol drinking, physical activity, and food intake including

calcium supplementation). Dietary assessment was performed by using a semi-quantitative food frequency questionnaire (FFQ) relative to consumption of 86 food items in the previous 12 months, with frequency varying from never to 6 or more per day. The questionnaire was validated to adult population [31] and adapted for adolescents [32]. Total calcium intake was evaluated multiplying the frequency by the correspondent pre-determined food mean portion sizes and using the software "Food Processor Plus" version 7 (ESHA Research, Salem, OR, USA) adapted for Portuguese foods, as previously described [31].

Physical activity at age 13 was defined as extracurricular physical activities with at least 20 min of duration and at age 21 it was adapted to adult life pattern, i.e., physical exercise at free time from most of the time sitting to most of the time active. Anthropometry was performed by trained health professionals and applying standardized procedures. Weight and height were measured with the participants wearing light clothing and no shoes. Weight was measured in kilograms with decimal approximation through digital scale (Tanita TBF-300, Tanita Corporation of America, Inc., Illinois, USA) and height was measured in meters with decimal approximation through portable stadiometer (seca 213, seca gmbh & co., Hamburg, Deutschland).

At age 13, participants were classified according to the Centers for Disease Control and Prevention, USA, with underweight/normal-weight if BMI was below the 85th percentile, overweight if BMI was at or above the 85th percentile, and as obese if the BMI was at or above the 95th percentile [33]. At age 21, WHO classification for adults [34] was used: underweight, $<18.5 \, kg/m^2$; normal-weight, ≥ 18.5 and $<25 \, kg/m^2$; overweight, ≥ 25 and $<30 \, kg/m^2$; obese, $\geq 30 \, kg/m^2$.

Statistical analysis

All statistical procedures were performed in the software "Statistical Package for the Social Sciences" (SPSS) v23.0 (IBM Corporation, New York, NY, USA). The exposures categorical variables that were frequency of milk consumption (sum of skim, semi-skimmed, and full fat milks), yogurt, cheese, and dairy products consumption (total sum of milks, yogurt, and cheese) were converted to continuous variables by multiplying the frequency of each dairy product by the specified mean portion size (244 g for a cup of milk, 125 g for one yogurt and 30 g for a slice of cheese). Exposures were categorized into three groups of frequency of consumption or tertiles for compare purposes and also converted to continuous variables for estimation of association with BMI. The obtained values were taken per 100 g of eaten food and in the case of calcium per 100 mg ingested.

Table 1 Participants' characteristics at 13 and 21 years of age by gender

	13 years old			21 years old		
	Girls ($n = 501$) n (%)	Boys ($n = 440$)	p-value[2]	Girls ($n = 501$) n (%)	Boys ($n = 440$)	p-value[2]
BMI (kg/m^2)						
Underweight/Normal weight	390 (77.8)	327 (74.3)		407 (81.2)	311 (70.7)	
Overweight	73 (14.6)	69 (15.7)		67 (13.4)	107 (24.3)	
Obese	38 (7.6)	44 (10.0)	0.343	27 (5.4)	22 (5.0)	<0.001
Parents education maximum level						
1–4th grade	44 (9)	44 (10.3)		–	–	
5–6th grade	52 (10.7)	32 (7.5)		–	–	
7–9th grade	85 (17.5)	69 (16.1)		–	–	
10–11th grade	72 (14.8)	46 (10.7)		–	–	
12th grade	82 (16.8)	90 (21)		–	–	
College	152 (31.2)	147 (34.3)	0.116	–	–	
Extracurricular physical activity[1]						
Never	114 (23.1)	42 (9.8)		–	–	
Less than once a week	62 (12.6)	28 (6.5)		–	–	
Once a week	69 (14)	42 (9.8)		–	–	
2 to 3 times a week	177 (35.8)	142 (33.1)		–	–	
4 to 6 times a week	33 (6.7)	76 (17.7)		–	–	
Almost all days of the week	39 (7.9)	99 (23.1)	<0.001	–	–	
Free time physical activity						
Most time sitting	–	–		149 (29.7)	150 (34.2)	
Most time standing and/or walking (not running)	–	–		262 (52.3)	189 (43.1)	
Most time very active	–	–		90 (18.0)	100 (22.8)	0.016
Intake per day	Mean (SD)		p value[3]	Mean (SD)		p value[3]
Energy (MJ/kcal)	10.1 (3.0) / 2421.8 (708.7)	10.3 (2.8) / 2455.3 (683.7)	0.462	8.6 (2.4) / 2046.6 (576.5)	10.1 (3.3) / 2515.3 (777.4)	<0.001
Milk (g)	393.5 (290.1)	419.5 (281.7)	0.164	292.9 (221.8)	292.8 (223.8)	0.999
Yogurt (g)	104.1 (95.6)	101.5 (95.3)	0.674	101.9 (86.8)	96.1 (94.1)	0.325
Cheese (g)	16.1 (20.8)	15 (17.8)	0.361	15.2 (15.0)	18.5 (19.0)	0.004
Dairy products (g)	513.7 (314.4)	535.9 (311.7)	0.277	410 (234.4)	407.4 (253.8)	0.87
Calcium (mg)	1129.7 (447.3)	1144.9 (446.6)	0.604	966.9 (350.6)	1106.6 (489.2)	<0.001

[1]Extracurricular physical activities with at least 20 min of duration

[2]Tested with chi-square test

[3]Tested with t-test

Underweight and normal-weight BMI categories at age 21 were combined for comparison with categories at age 13. Parents' years of education, corresponding to the maximum complete years of both parents at age 13, was grouped according to usually Portuguese study cycles.

Data were described according to gender and groups were compared using chi-square test for categorical variables, t-test and ANOVA for continuous variables. Significance level was settled to ≤0.05. Linear regression models were performed to estimate the association between the various baseline exposures and BMI at 21 years of age. Concerning the possible existence of different confounders three models were defined. Model 0 was crude model; Model 1 was adjusted for gender, follow-up period, parents maximum education level, and extracurricular physical activities with at least 20 min of duration at age 13; Model 2 was model 1 plus adjustment for free time physical activity and energy intake at age 21; Model 3 was model 2 plus

Table 2 Mean of body mass index at 21 years of age according to groups of dairy products and calcium intakes by gender

Intake groups at 13 years of age	n (%)	BMI at 21 years of age (kg/m²) Mean (SD)[1]		
		Total (n = 941)	Girls (n = 501)	Boys (n = 440)
Milk				
1 cup or less per day	527 (56.0)	23.0 (4.0)	22.6 (3.9)	23.5 (4.0)
2–3 cups per day	342 (36.3)	22.6 (3.3)	22.1 (3.3)	23.2 (3.2)
More than 3 cups per day	72 (7.7)	23.8 (4.4)	24.5 (5.2)	23.0 (3.1)
p-value		0.05	0.002	0.572
Yogurt				
4 units or less per week	477 (50.7)	23.0 (3.8)	22.5 (3.8)	23.5 (3.7)
5–6 units per week or 1 per day	330 (35.1)	23.2 (4.1)	22.9 (4.3)	23.7 (3.8)
2 units or more per day	134 (14.2)	22.2 (3.0)	22.0 (3.1)	22.3 (3.0)
p-value		0.041	0.383	0.039
Cheese				
4 slices or less per week	426 (45.3)	22.9 (3.8)	22.7 (3.9)	23.2 (3.7)
5–6 slices per week or 1 per day	233 (24.8)	22.8 (4.1)	22.1 (4.4)	23.7 (3.7)
2 slices or more per day	282 (30.0)	23.1 (3.5)	22.8 (3.5)	23.4 (3.5)
p-value		0.819	0.301	0.548
Gender-specific tertiles[2]				
Dairy products				
1		23.2 (4.2)	22.8 (4.2)	23.7 (4.3)
2		22.8 (3.5)	22.3 (3.6)	23.3 (3.3)
3		22.8 (3.6)	22.6 (3.9)	23.1 (3.3)
p-value		0.277	0.578	0.291
Calcium				
1		23.4 (4.2)	23.0 (4.3)	23.7 (4.1)
2		22.7 (3.7)	22.1 (3.6)	23.5 (3.6)
3		22.7 (3.5)	22.6 (3.8)	22.9 (3.3)
p-value		0.056	0.072	0.099

[1] Tested with ANOVA test

[2] Girls: Tertile 1 < 319.0 g; Tertile 2 ≥ 319.0 g and <665.6 g; Tertile 3 ≥ 665.6 g. Boys: Tertile 1 < 321.1 g; Tertile 2 ≥ 321.1 g and <676.4 g; Tertile 3 ≥ 676.4 g

[3] Girls: Tertile 1 < 889.7 mg; Tertile 2 ≥ 899.7 and <1283.6 mg; Tertile 3 ≥ 1283.6 mg Boys: Tertile 1 < 923.7 mg; Tertile 2 ≥ 923.7 mg and <1280.4 mg; Tertile 3 ≥ 1280.4 mg

adjustment for total calcium intake at age 21. Possible confounders such as alcohol intake, smoking, self-reported diabetes, asthma and eating disorders, calcium supplements, monthly household income, and social class were tested and did not influence the final models.

Ethical considerations

Conditions for appropriate confidentiality and data protection were guaranteed in all procedures. Both the participants and their parents have been informed about the purpose and study design through oral and written information and written informed consents were also obtained from both parts. The development of EpiTeen cohort had ethical approval from the Ethics Committee for Health of the Hospital de São João/Faculty of Medicine of Porto University.

Results

Descriptive characteristics of the sample at 13 and 21 years of age, by gender, are shown in Table 1. At age 13, 7.6% of girls and 10.0% of boys were obese while at age 21 these prevalences were 5.4 and 5.0%, respectively. Over 30% of individuals have at least one parent with higher education and more than 50% with at least 10 years of education. Boys have significantly higher physical activity than girls in both moments. At age 13, no significant differences were observed between boys and girls regarding the intake of energy or calcium and consumption of dairy products. At age 21, boys consumed significantly more energy, cheese and calcium than girls.

Table 2 shows the BMI mean at age 21 of the sample by groups of milk, yogurt, cheese, dairy products, and total calcium intake at age 13. Considering the total sample and regarding categories of milk, yogurt, cheese, total dairy products, and total calcium intake, significant differences of BMI mean values were observed only in yogurt. Significant differences were observed in the BMI mean values according to category of milk in girls ($p = 0.002$) and according to category of yogurt in boys ($p = 0.039$). The higher mean values of BMI were found in the highest category of milk and the lower were found in the highest category of yogurt.

Associations from linear regression between milk, yogurt, cheese, and dairy products (per 100 g) and calcium (per 100 mg) intake at age 13 and BMI at age 21 are shown in Table 3. Negative associations were found for all exposures, however, statistical significant associations were only found for total calcium intake in crude model and in model 1 (model 0: $\beta = -0.059$ (95% CI: −0.113, −0.004); model 1: $\beta = -0.057$ (95% CI: −0.113, −0.002)). After further adjustment for physical activity, energy and total calcium intake at age 21 the effect did not remained statistically significant. The energy intake at age 21 was the variable that showed more influence in the final model. The analysis was also performed according to gender and similar results were found.

Table 3 Association (β) between dairy products and calcium intake at 13 years of age and body mass index at 21 years of age

Intake per day at 13 years of age[1]	BMI at 21 years of age (kg/m^2)			
	β (95% CI)[2]			
	Model 0	Model 1	Model 2	Model 3
Milk	−0.015 (−0.099, 0.070)	−0.021 (−0.107, 0.066)	−0.005 (−0.091, 0.081)	−0.018 (−0.106, 0.069)
Yogurt	−0.247 (−0.501, 0.008)	−0.247 (−0.504, 0.011)	−0.208 (−0.462, 0.046)	−0.226 (−0.481, 0.029)
Cheese	−0.638 (−1.889, 0.613)	−0.392 (−1.658, 0.875)	−0.228 (−1.480, 1.024)	−0.291 (−1.544, 0.962)
Dairy products	−0.043 (−0.119, 0.033)	−0.044 (−0.122, 0.033)	−0.033 (−0.109, 0.043)	−0.044 (−0.122, 0.033)
Calcium	−0.059 (−0.113, −0.004)	−0.057 (−0.113, −0.002)	−0.037 (−0.092, 0.019)	−0.045 (−0.101, 0.011)

[1]Milk, yogurt, cheese and dairy products are taken per 100 g and calcium is taken per 100 mg

[2]Model 0: crude model; Model 1: adjusted for follow-up period, parents maximum education level and physical activity at age 13; Model 2: model 1 plus adjustment for free time physical activity and energy intake at age 21; Model 3: model 2 plus adjustment for total calcium intake at age 21

Mean difference of total calcium intake according to changes in BMI categories between age 13 and 21, by gender, was also analysed (data not shown) and no statistical significant differences were found between categories.

Discussion

The main result of this study was the lack of association between the dairy products or total calcium intake at age 13 and later BMI at age 21. Although a tendency for negative association between total calcium intake and adiposity was observed, the association did not remained statistically significant after adjustment for energy intake. These results are consistent with Berkey et al. [35] which results showed that milk intake in individuals aged between 9 and 14 years old was negatively and significantly associated with BMI 3 years later. However, this effect was lost when adjusted for energy intake. Other longitudinal studies conducted in adolescents showed no association between the consumption of milk [20, 36, 37] or dairy products [38] and BMI with adjustment for energy intake [34, 36] and for calories from fat [20]. Also a meta-analysis of randomized controlled trials in adults [39] concluded that the consumption of dairy products is not associated with weight change or fat mass when the intervention is not energy restricted or when the time of intervention is long.

The decrease in the amount of daily ingested calcium between ages 13 and 21 found in the present study was consistent with the pronounced decline in milk consumption considering that this is an important source of calcium in the diet practiced in Western countries. This reduction in milk consumption was also observed in a study conducted in the Framingham cohort [20] revealing the replacement of milk for soft drinks through adolescence thereby increasing energy intake. Thus, the amount of energy consumed seems to have a fundamental role in BMI and, if it is reduced, it can enhance the possible benefit of dairy consumption,

especially low-fat dairy. Besides energy, other dairy components could have an effect on adiposity. Longitudinal studies in English [22] and North American adolescents [40] showed that milk consumption in individuals between ages 10 and 15 was negatively associated with BMI, both with adjustment for energy intake [22] or unadjusted [40]. Other cross-sectional studies [27, 41, 42] and a meta-analysis [16] also showed negative association but some attention must be given especially on cross-sectional studies since reverse causality could be present.

The inconsistency between studies could also be explained by several factors such as the different methodologies used. For example, to estimate body fat of the individuals, the standard method would be to measure objectively fat percentage or fat mass [43], but using BMI, despite being also suitable [43], it is more inaccurate because it corresponds to the total body mass/m^2. However, from studies conducted in adolescents using body fat as outcome, only one cross-sectional study [44] and one longitudinal study [20] showed negative association. Moreover, cross-sectional [28, 40], longitudinal [38, 45] and randomized controlled trials studies [46, 47] failed to establish any relationship between milk or dairy consumption and fat percentage or fat mass. Another methodological difference was the dietary data collection method in which researchers used either the semi-quantitative FFQ or the 3-day dietary record. Although data from diaries is more accurate, the FFQ is one of the most used alternatives in large population studies and it was considered a valid method for the purpose of group comparisons [31]. The questionnaire used in the present study showed to be valid for nutritional assessment in the Portuguese adult population [31] and in pregnant women [48]. To minimize the relative imprecision, a larger sample size is possibly needed to achieve the adequate statistical power. We also decided to exclude extreme outliers based on energy intake and on fruit and vegetables intake, since these food items could reflect any misreporting beyond the energy intake, namely

for food items that could be sources of calcium intake. The regression models with and without outliers were performed and the estimates were similar. The use of this FFQ allows the estimation of ingested calcium from a large number of different foods and at same time the estimation of energy intake, used as a main possible confounder.

Yet, other strengths and limitations of this study deserve discussion. The longitudinal study design reduces the effect of reverse causality. Additionally, the availability of a considerable number of possible confounders used in the final models was an advantage. However, we could not exclude the possibility of residual confounders by other unmeasured variables.

Some limitations are also present in this study. There was no evaluation for pubertal growth stage, seric concentrations of $1,25(OH)_2D_3$ and PTH, and calcium retention [49], that are important factors in calcium metabolism and utilization during adolescence [7]. The fact that only two follow-up evaluations being considered in the current study may difficult the analysis of a tracking effect. The possible confounding by BMI at age 13 was not taken into account in this study since it could be considered an intermediate step in the causal chain. The possible beneficial effect of calcium can be attenuated by the theory that most adolescents stays in the same BMI category in the future adulthood [50]. The fact that more than 60% of adolescents in this study at age 13 above the 85th percentile were considered overweight or obese at age 21 supports that theory (data not shown). Another factor that may also be involved is the increasing globalization of nourishment which leads to that food exposures are increasingly similar among individuals, which results in the difficulty in finding associations with relevant magnitude.

In this longitudinal study, no statistically significant relation was found between dairy products or total calcium intake at age 13 and BMI at age 21. The results of this study, associated with other studies developed on this topic, suggest no effect or modest beneficial effect of calcium, not independent of energy intake. More longitudinal and interventional studies in adolescents assessing total calcium intake and adjusting for energy intake are needed to elucidate this possible effect.

Compliance with ethical standards

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