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ARTICLE

High dietary calcium intake and low adiposity: findings from a longitudinal study in Brazilian adolescents

Ingestão alta de cálcio na dieta e adiposidade baixa: achados de um estudo longitudinal em adolescentes brasileiros

Alta ingesta de calcio en la dieta y baja adiposidad: hallazgos de un estudio longitudinal en adolescentes brasileños

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Abstract

Epidemiological studies have supported the hypothesis that dietary calcium intake is protective for adiposity. This study aimed to estimate the association of dietary calcium with adiposity indicators during adolescence. This is a cohort study with high school adolescents (n = 962) from selected schools of the Metropolitan Region of Rio de Janeiro, Brazil, which were followed from 2010 to 2012. Calcium intake was assessed by a validated self-reported food frequency questionnaire. Cross-sectional and longitudinal analyses of dietary calcium intake were performed regarding body mass index (BMI), waist circumference (WC), body fat percentage (%BF), fat mass (FM), fat-free mass (FFM), fat mass index (FMI), and fat-free mass index (FFMI). The analysis of variance was used for cross-sectional analysis with baseline data and linear mixed models applied to assess changes across the follow-up. At baseline, BMI, %BF, fat mass, and FMI (p for trend < 0.05) had lower means at the highest quintile of calcium intake whereas FFM and FFMI had higher means (p for trend < 0.05), especially for boys. During follow-up, boys had decreased FMI at the 4th and 5th quintiles of calcium intake (p < 0.05); among girls, only WC was significantly lower at the 4th quintile than in the 1st. These results support the hypothesis that low calcium intake increases adiposity among adolescents.

Adolescent; Body Fat Distribution; Calcium; Obesity; Longitudinal Studies

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Introduction

Though several studies reported that dairy and dietary calcium could decrease the risk of obesity in children and adolescents [1,2,3,4,5], other prospective studies show that this association is still inconclusive [6,7]. Systematic reviews which suggested that dietary calcium or dairy intake is protective against excess weight in children, adolescents, and adults also had inconsistent results [8,9].

Dietary calcium was first associated with obesity by McCarron [10] and later by Zemel. [11]. According to Zemel's hypothesis, low calcium bioavailability increases the parathormone (PTH) and 1,25-Dihydroxyvitamin D3 synthesis as calcium-regulating mechanisms, leading to intracellular calcium concentration, which stimulates lipogenesis and suppresses lipolysis in the adipose tissue [11,12]. A meta-analysis of clinical control trials [13] showed that calcium intake around 1,000-1,400mg/day seems to enhance fat oxidation and is inversely correlated with PTH. Calcium intake of around 600-700mg/day is believed to boost the process of adipogenesis [14].

Brazilian nationwide surveys have shown that overweight and obesity increased in the country, reaching 20% of adolescents [15]. Meanwhile, the first *Brazilian National Dietary Survey* (INA) conducted in Brazil (2008-2009) [16] showed that, among Brazilian adolescents, the per capita dairy intake was lower than 100g/day and the average calcium intake was around 500mg/day [17]. Souza et al. [18] found similar results in their analysis of a 2013 nationally representative school-based study with adolescents. Data from the 2017-2018 INA also showed that low calcium intake persists among Brazilian adolescents, estimating that 98.1% of them had inadequate intake of this micronutrient [19]. This low intake and the increased overweight and obesity provides an opportunity to examine the relationship between calcium intake and adiposity. We thus sought to assess the association between dietary calcium intake and adiposity indicators among a cohort of Brazilian adolescents.

Material and methods

Study population

The *Adolescent Nutritional Assessment Longitudinal Study* (ELANA) was carried out to assess changes in anthropometric and body composition measures in a cohort of adolescents in high school followed from 2010 to 2012 in the Metropolitan Region of Rio de Janeiro, Brazil [20].

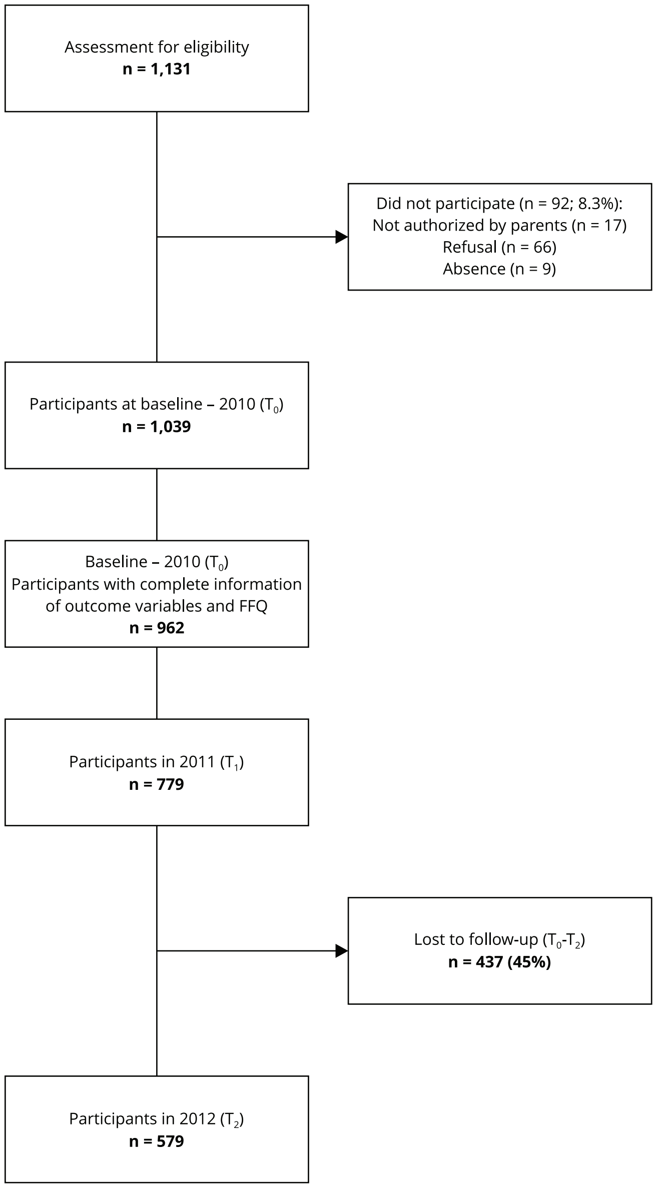
The ELANA sample size was originally estimated at 1,200 adolescents, which would allow estimating the variation of one unit of body mass index (BMI) based on a 5% alpha error, 80% test power, mean BMI of 21.9kg/m^2, standard deviation (SD) of 3.0kg/m^2, and up to 20% lost to follow-up. The sample design was stratified by gender and type of school (public or private) with 300 individuals in each stratum. The repetition of the outcomes over time was considered in the sample size estimation. The sample size in each quintile of calcium intake could detect differences from 0.63 to 1.5 units between the outcomes, with statistical power ranging from 71 to 99% and a 5% alpha error.

All high school students (n = 1,131) of the two public and four private schools selected by convenience for the study were invited to participate in ELANA. Students with physical disabilities which prevented nutritional assessment, students on a diet, and pregnant female students were not eligible for the study. Moreover, 92 students (8.3%) could not be examined for lack of parental consent or refusal to participate in the study or for being absent from school. The ELANA cohort thus included 1,039 high school adolescents at baseline (2010, T_0). Our study included 962 students (aged 13-19 years) after excluding 77 (7%) individuals with incomplete information on food consumption or anthropometric/body composition measurements. In total, 779 adolescents were followed until 2011 (T_1) and 579 were followed until 2012 (T_2). Figure 1 shows the flowchart of the participants and the measurements taken at baseline and at follow-up. Further details on ELANA sample design and participant selection are available elsewhere [20].

The ELANA was approved by the Ethics Research Committee of the Institute of Social Medicine of the State University of Rio de Janeiro (IMS/UERJ, n. 0020.0.259.000-09). A written informed consent for the survey was provided by the adolescents and by their parents or guardians.

Figure 1

Flowchart of the study participants at baseline and at follow-ups.



FFQ: *Food Frequency Questionnaire*.

Anthropometric and body composition measurements

Anthropometric measures included body weight (kg), height (cm), and waist circumference (cm), assessed by well-trained staff and in accordance with standard protocols [21] in 2010, 2011, and 2012.

Body weight was measured using a portable digital scale (Kratos, Cotia, Brazil) with 0.1kg of variation while the participant wore light clothes and no shoes. A stadiometer (Alturaexata, Belo Horizonte, Brazil) with a variation of 0.1cm was used to measure height twice, accepting a maximum variation of 0.5cm between the two measures. The average of both measurements was used in the analysis. BMI (kg/m^2) was estimated. Excess weight was defined as BMI Z-score > +1 according to gender and age distributions [22]. Waist circumference (WC) was assessed at the narrowest circumference of the trunk with an inelastic tape (Seca, Cotia, Brazil) with 0.1 cm accuracy. The WC was measured twice, allowing a maximum variation of 1.0cm between the two measurements, whose average was used in the analysis. Considering the absence of an international reference for WC distribution for adolescents, abdominal obesity was defined as values above the 90th percentile of WC distribution, specified by gender.

Body composition was measured at baseline and at the first year of follow-up (2011) using a bioelectrical impedance device (BIA RJL System, 101Q, Clinton Township, United States) with tetrapolar electrodes. Subjects were in the supine position and the electrodes were placed on the dorsal surface of their right foot and hand. A 50-kHz frequency electrical current was employed to measure resistance and reactance. Resistance values were used to estimate fat-free mass (FFM) using the equation for adolescents validated by Houtkooper et al. [23]. Fat mass (FM) was calculated as the difference between weight and fat-free mass. Body fat percentage (%BF) was obtained from: [(fat mass/total weight) * 100]. Excess body adiposity was defined as %BF ≥ 25% for boys and ≥ 30% for girls [24]. Since BMI is a global adiposity index, the body fat fraction was estimated using the fat mass index [FMI = fat mass/height^2 (kg/m^2)] whereas the body fat-free fraction was estimated with the fat-free mass index [FFMI = fat-free mass/height^2 (kg/m^2)].

Dietary intake assessment

Food intake was assessed using a qualitative self-administered *Food Frequency Questionnaire* (FFQ) referring to consumption in the last three months before the interview. The FFQ included 72 items and eight options for consumption frequency, ranging from "less than once a month or never" to "four or more times a day". This is a reduced version of an FFQ validated for adolescents from Rio de Janeiro [25]. To reduce the original FFQ, items with 90% of energy intake were selected and items consumed by less than 5% of the original group evaluated were excluded. Furthermore, several items were grouped into a broad category; different types of bread were simply listed as "bread". The three food records which assessed the validation of the original FFQ were used to assess the relative validity of the short FFQ, showing similar results: deattenuated and adjusted Pearson's correlation coefficients ranged from 0.16 to 0.42 for the reduced questionnaire (unpublished data) and from 0.17 to 0.47 for the complete version [25].

For the analysis, calibrated FFQ energy and nutrient intake were estimated as described by Araujo et al. [25]. In short, the database used for validating the original FFQ was used for the calibration process and, for each nutrient, linear regression models adjusted for age and gender were run, with the nutrient intake estimated from the mean of three food records as the dependent variable and from the FFQ as the independent variable. The intercept (α) and the slope (β) regression coefficients were computed to calibrate the energy and nutrient intakes estimated by the FFQ using the following equation:

$$\textit{FFQ calibrated energy or nutrient intake} = \alpha + \beta * FFQ_{\textit{reported energy or nutrient intake}}$$ [25]

The reported food frequency consumption was transformed into daily frequency and multiplied by the standard serving size associated with each item. The daily intake of energy, protein, carbohydrates, vitamins, and minerals – including calcium – was estimated using the *Brazilian Food Composition Table* (TBCA) [26].

Finally, dietary calcium intake was categorized into quintiles and the following cutoffs were estimated: 1st quintile (Q1): < 492.0mg/day; 2nd quintile (Q2): 492.0-567.9mg/day; 3rd quintile (Q3): 568.0-670.9mg/day; 4th quintile (Q4): 671.0-838.0mg/day; and 5th quintile (Q5): > 838.0mg/day.

Other covariates

Age (as a continuous variable), gender, and type of school (public and private) were obtained by a structured self-administered questionnaire, applied in class under supervision of the nutritionists responsible for ELANA. Physical activity was measured using the short version of the *International*

Physical Activity Questionnaire (IPAQ) validated for Brazilian adolescents [27]. Subjects were asked about their frequency and duration (in minutes) of walking, practice of specific moderate and vigorous activities, and sedentary habits (e.g., sitting) in the week before the interview. Physical activity was then categorized as: "very active" (vigorous: ≥ 5 days/week and ≥ 30 minutes per time and/or ≥ 3 days/week and ≥ 20 minutes per time + moderate and/or walking: ≥ 5 days/week and ≥ 30 minutes per time); "active" (vigorous: ≥ 3 days/week and ≥ 20 minutes per time and/or moderate or walking: ≥ 5 days/week and ≥ 30 minutes per time); "irregularly active A" (vigorous + moderate + walking: 5 days/week or 150 minutes/week); "irregularly active B" (those who did not match the frequency or duration of physical activity recommendations); or "sedentary" (those who do not practice physical activity for at least 10 minutes continuously per week) [28]. In this study, physical activity categories were divided into dichotomous categories: "active/highly active" (active + very active) and "sedentary/less active" ("sedentary" + "irregularly active A" + "irregularly active B").

Daily energy (Kcal/day) and vitamin D (mcg/day) intake and daily consumption frequency of fruits, vegetables, sugar-sweetened beverages, fast foods, and breakfast cereals were considered as potential confounders since they are usually associated with healthy or unhealthy dietary patterns and weight gain. All these variables were included in the longitudinal analysis to adjust the regression models.

Statistical analyses

Means and SD of continuous variables and proportions of categorical variables were estimated across quintiles of dietary calcium intake. For cross-sectional analysis, means of BMI, WC, FM, FFM, %BF, FMI, and FFMI were compared by analysis of variance (ANOVA) according to dietary calcium quintiles; Tukey's Post Hoc test compared the 1st quintile with the 2nd to the 5th quintiles. The Tukey's test compares the difference between each pair of means with appropriate adjustment for the multiple testing. The ANOVA F-test was applied to test for linear trend (increasing or decreasing) of means on each dependent variable across calcium intake quintiles.

For longitudinal analysis, linear mixed-effects models (LMM) were applied to assess the effect of dietary calcium intake on each adiposity measure (dependent variables) from baseline to the end of follow-up. Models were run for the whole sample and stratified by gender. The age (in years) of the adolescents at each point of follow-up was considered as the random effects in the LMM to guarantee that changes could be attributable to time instead of age. For each outcome variable, we constructed separated models including the random effects of both the intercepts and slopes. The correlation matrix was unstructured since this type generates full models, which are the best estimates of the regression parameters considering unbalanced and incomplete data (missing outcome variables), prevalent in longitudinal studies [29]. Changes in time of the outcome variables across quintiles of dietary calcium intake were assessed by including the interaction term between age and dietary calcium (age * dietary calcium intake) on LMM. A significant interaction of age * dietary calcium intake indicates a differential growth rate for each adiposity measure over time.

The multicollinearity of confounder variables in regression models was assessed by the variance inflation factor (VIF). The VIF values for each confounder variable ranged from 1.13 to 3.14 and were lower (except for energy with VIF = 3.14) than the cutoff point of 2.5, which indicates considerable collinearity [30].

The Cochran-Mantel-Haenszel test was applied to assess differences between the proportions of cases at baseline and new cases at the end of follow-up regarding excess weight, abdominal obesity, and excess body fat in the calcium intake quintiles.

Statistical analyses were conducted using SPSS software (https://www.ibm.com/) and statistical significance was set at p-value > 0.05.

Results

Table 1 shows the characteristics of the study population at baseline according to quintiles of dietary calcium. At baseline, 53% of participants were women, 50% were from private schools, and 71% were active/highly active. The Q5 of calcium intake included more boys (32%) than girls (9%) and, regarding type of school, 24% of students in Q5 were from public schools whereas 16% were from private schools. Adolescents in Q5 were also classified according to categories of physical activity, in which most were active/highly active (23%) rather than sedentary/low active (15%). The mean age was 15.7 (SD = 0.9) years. Overall, the mean calcium intake was 700 (SD = 310) mg/day. Means of calcium, vitamin D, energy intake, consumption frequency of breakfast cereals, fast foods, fruits/vegetables, and sugar-sweetened beverages were higher in Q5 than in Q1 (Table 1).

Table 2 shows significant differences between most of the baseline anthropometric and body composition variables means at highest quintiles of calcium intake and at the first quintile. For the total sample, means of BMI (p for trend = 0.003), FM (p for trend < 0.01), %BF (p for trend < 0.01), and FMI (p for trend < 0.01) were lower at Q4 and Q5 than in Q1. Differences between means of FFM (p for trend < 0.01) and FFMI (p for trend = 0.001) from Q3 to Q5 were not significant compared to

Table 1

Baseline characteristics for total sample in quintiles (Q) * of calcium intake of adolescents from the Metropolitan Region of Rio de Janeiro, Brazil, 2010.

Characteristics	Total sample	Dietary calcium intake (mg/day)					p-value **
		Q1	Q2	Q3	Q4	Q5	
	[n = 962]	[n = 192]	[n = 193]	[n = 192]	[n = 193]	[n = 192]	
Gender [%]							0.001
Boys	47	14	14	18	22	32	-
Girls	53	25	26	22	18	9	-
Type of school [%]							0.001
Public	50	17	17	20	22	24	-
Private	50	23	23	20	18	16	-
Physical activity [%]							0.001
Sedentary/less active	29	28	20	18	19	15	-
Active/highly active	71	17	20	18	21	23	-
Age (years) [mean (SD)]	15.7 (0.9)	15.6 (0.9)	15.6 (0.8)	15.7 (0.9)	15.8 (0.9)	16.0 (1.0)	0.001
Total calcium (mg/day) [mean (SD)]	700 (310)	450 (37)	525 (21)	617 (30)	738 (47)	1174 (391)	< 0.01
Vitamin D (mcg/day) [mean (SD)]	4.5 (3.0)	3.0 (0.5)	3.4 (0.6)	3.8 (0.9)	4.5 (1.4)	8.0 (5.0)	< 0.01
Total energy intake (Kcal/day) [mean (SD)]	2,672 (899)	1,959 (230)	2,212 (247)	2,515 (328)	2,805 (434)	3,879 (1200)	< 0.01
Frequency of breakfast cereals intake (times/day) [mean (SD)]	0.3 (0.7)	0.1 (0.2)	0.2 (0.4)	0.3 (0.6)	0.4 (0.7)	0.7 (1.2)	< 0.01
Frequency of fast-foods intake (times/day) [mean (SD)]	0.9 (1.0)	0.4 (0.4)	0.6 (0.5)	0.9 (0.7)	1.0 (0.9)	1.9 (1.4)	< 0.01
Frequency of fruits/vegetables intake (times/day) [mean (SD)]	4.2 (6.0)	1.3 (1.2)	1.7 (1.4)	2.1 (1.5)	2.5 (1.5)	3.0 (1.5)	< 0.01
Frequency of sugar-sweetened beverages intake (times/day) [mean (SD)]	3.3 (3.0)	1.6 (1.5)	2.5 (2.3)	3.2 (2.5)	3.8 (3.0)	5.4 (3.6)	< 0.01

SD: standard deviation.

* Q1: < 492.0g/day; Q2: 492.0-567.9mg/day; Q3: 568.0-670.9mg/day; Q4: 671.0-838.0mg/day; and Q5: > 838.0mg/day;

** p-value of the chi-squared test and ANOVA F-test.

Table 2

Baseline means (and standard deviation – SD) of anthropometric and body composition variables according to quintiles (Q) * of dietary calcium intake among adolescents from the Metropolitan Region of Rio de Janeiro, Brazil.

Anthropometric variables	Dietary calcium intake (mg/day)					p-value **
	Q1 [mean (SD)]	Q2 [mean (SD)]	Q3 [mean (SD)]	Q4 [mean (SD)]	Q5 [mean (SD)]	
Total sample						
Body mass index (kg/m2)	23.0 (3.9)	21.5 (3.7) ***	22.1 (4.5)	21.7 (3.9) ***	21.5 (3.8) ***	0.003
Waist circumference (cm)	72.8 (8.9)	69.7 (7.8) ***	71.8 (9.8)	71.2 (8.7)	71.9 (7.9)	0.89
Fat mass (kg)	19.9 (6.8)	15.2 (7.5)	15.5 (8.8)	13.7 (7.7) ***	12.2 (7.2) ***	< 0.01
Fat-free mass (kg)	45.6 (9.8)	43.5 (9.2)	46.3 (10.2)	46.5 (9.5)	50.0 (9.3)	< 0.01
% body fat	26.7 (7.2)	25.0 (8.1)	24.2 (8.6) ***	22.1 (8.1) ***	19.1 (8.1)) ***	< 0.01
Fat mass index (kg/m2)	6.2 (2.5)	5.7 (2.8)	5.6 (3.1)	5.0 (2.8) ***	4.3 (2.5) ***	< 0.01
Fat-free mass index (kg/m2)	16.7 (2.5)	16.0 (2.4)	16.5 (2.6)	16.7 (2.5)	17.3 (2.3)	0.001
Boys						
Body mass index (kg/m2)	23.7 (3.9)	21.9 (4.2)	22.1 (4.5)	22.0 (4.1)	21.4 (4.1) ***	0.003
Waist circumference (cm)	77.8 (8.6)	73.8 (8.4)	74.2 (9.2)	74.2 (8.9)	73.0 (7.8) ***	0.001
Fat mass (kg)	15.2 (7.2)	13.0 (8.2)	12.8 (8.6)	12.2 (8.6)	10.9 (7.0) ***	0.01
Fat-free mass (kg)	55.2 (8.6)	52.4 (8.4)	53.8 (9.0)	52.6 (7.8)	52.9 (8.4)	0.14
% body fat	21.2 (7.2)	18.8 (8.7)	18.1 (7.2)	17.3 (7.5) ***	16.4 (6.9) ***	< 0.01
Fat mass index (kg/m2)	5.0 (2.3)	4.4 (2.7)	4.2 (2.7)	4.1 (2.9)	3.6 (2.3) ***	0.01
Fat-free mass index (kg/m2)	18.5 (2.3)	17.7 (2.5)	17.9 (2.3)	17.8 (2.5)	17.8 (2.2)	0.12
Girls						
Body mass index (kg/m2)	22.6 (3.8)	21.3 (3.5)	22.1 (4.5)	21.3 (3.7)	21.8 (3.7)	0.22
Waist circumference (cm)	70.3 (8.0)	67.8 (6.7)	69.8 (9.8)	67.9 (7.2)	68.9 (7.4)	0.31
Fat mass (kg)	17.8 (6.5)	16.3 (7.0)	17.5 (8.4)	15.4 (6.2)	16.0 (6.6)	0.08
Fat-free mass (kg)	41.1 (6.7)	39.3 (5.9)	40.6 (6.8)	39.8 (6.0)	41.3 (5.6)	0.66
% body fat	29.3 (5.3)	27.9 (5.8)	28.9 (6.6)	27.2 (5.1)	27.1 (5.8)	0.01
Fat mass index (kg/m2)	6.8 (2.3)	6.3 (2.6)	6.6 (3.0)	6.0 (2.2)	6.1 (2.3)	0.06
Fat-free mass index (kg/m2)	15.8 (2.1)	15.2 (2.0)	15.5 (2.2)	15.5 (1.9)	15.7 (1.8)	0.85

* Q1: < 492.0g/day; Q2: 492.0-567.9mg/day; Q3: 568.0-670.9mg/day; Q4: 671.0-838.0mg/day; and Q5: > 838.0mg/day;

** F-test for linearity trend for means;

*** p-value < 0.05 of Tukey test for multiple comparisons of means in relation to the Q1.

differences with Q1. Boys had lower means of BMI (p for trend = 0.003), WC (p for trend = 0.001), FM (p for trend = 0.01), and FMI (p for trend = 0.01) in Q5 than in Q1; they also had lower %BF means (p for trend < 0.01) in both Q4 and Q5. Among girls, means of adiposity indicators from Q2 to Q5 were equal compared to Q1, except for %BF means, which decreased across the quintiles of calcium intake (p for trend = 0.01) (Table 2).

Table 3 shows the adjusted anthropometric and body composition variable means (and SD) at the end of follow-up according to the quintiles of calcium intake at baseline, estimated from linear mixed models for longitudinal analysis. Changes in the means over time are estimated with the interaction term between age * dietary calcium intake. For the total sample and for boys, those at Q4 (p for interaction = 0.03) and Q5 (p for interaction = 0.02) quintiles had lower FMI means at the end of follow-up than at baseline. Girls at Q4 of calcium intake had the lowest WC (p for the interaction = 0.03) compared to those at Q1.

At baseline, the proportions of excess weight were lower at Q5 than at Q1 for the total sample (p = 0.02), for boys (p = 0.02), and for girls (p = 0.06). Accordingly, the proportion of abdominal obesity was lower at Q5 than Q1 for the total sample (p = 0.06) and for boys (p = 0.07). The proportion of

Table 3

Estimated means * and standard deviations (SD) of anthropometric and body composition variables at the end of follow-up according to quintiles (Q) ** of dietary calcium intake at baseline among adolescents from the Metropolitan Region of Rio de Janeiro, Brazil, 2010-2012.

Anthropometric variables	Dietary calcium intake (mg/day)				
	Q1 [mean (SD)]	Q2 [mean (SD)]	Q3 [mean (SD)]	Q4 [mean (SD)]	Q5 [mean (SD)]
Total sample					
Body mass index (kg/m2)	23.1 (0.3)	21.9 (0.3)	22.5 (0.3)	22.1 (0.3)	22.6 (0.4)
Waist circumference (cm)	74.0 (0.7)	71.3 (0.7)	72.6 (0.6)	71.7 (0.6)	72.8 (0.9)
Fat mass (kg)	16.3 (0.6)	14.6 (0.6)	15.5 (0.6)	14.1 (0.6)	15.0 (0.8)
Fat-free mass (kg)	48.5 (0.6)	46.4 (0.6)	47.8 (0.5)	46.9 (0.5)	48.6 (0.7)
% body fat	24.6 (0.6)	22.9 (0.5)	23.3 (0.5)	22.5 (0.5)	22.8 (0.7)
Fat mass index (kg/m2)	11.5 (0.5)	10.5 (0.5)	10.6 (0.5)	8.9 (0.5) ***	9.7 (0.7) ***
Fat-free mass index (kg/m2)	17.2 (0.2)	16.6 (0.2)	16.8 (0.2)	16.8 (0.2)	17.8 (0.2)
Boys					
Body mass index (kg/m2)	23.1 (0.6)	21.7 (0.6)	22.3 (0.5)	22.4 (0.4)	22.7 (0.5)
Waist circumference (cm)	77.1 (1.2)	73.4 (1.2)	74.8 (1.0)	74.8 (0.9)	75.5 (1.0)
Fat mass (kg)	14.5 (1.1)	12.6 (1.0)	12.4 (0.9)	12.4 (0.8)	12.8 (0.8)
Fat-free mass (kg)	54.8 (1.1)	52.5 (1.4)	54.3 (1.0)	53.7 (0.8)	55.3 (0.9)
% body fat	20.1 (1.0)	17.7 (1.0)	17.3 (0.8)	17.3 (0.7)	17.9 (0.7)
Fat mass index (kg/m2)	10.3 (1.0)	8.6 (0.1)	8.3 (0.8)	7.4 (0.7) ***	8.4 (0.7) ***
Fat-free mass index (kg/m2)	18.3 (0.3)	17.6 (0.3)	18.0 (0.2)	18.0 (0.2)	18.5 (0.2)
Girls					
Body mass index (kg/m2)	23.4 (0.4)	22.1 (0.4)	22.6 (0.4)	21.5 (0.4)	21.9 (1.0)
Waist circumference (cm)	71.7 (0.9)	69.2 (0.7)	70.1 (0.8)	67.8 (0.9) ***	69.1 (2.1)
Fat mass (kg)	18.8 (0.7)	17.0 (0.6)	17.8 (0.7)	15.7 (0.8)	15.4 (1.8)
Fat-free mass (kg)	42.1 (0.6)	40.0 (0.5)	41.0 (0.5)	39.5 (0.6)	41.9 (1.4)
% body fat	29.8 (0.6)	28.3 (0.5)	28.9 (0.5)	27.1 (0.6)	26.7 (1.5)
Fat mass index (kg/m2)	13.1 (0.7)	12.4 (0.6)	12.6 (0.7)	9.7 (0.8)	10.0 (1.6)
Fat-free mass index (kg/m2)	16.2 (0.2)	15.5 (0.2)	15.7 (0.2)	15.4 (0.2)	15.7 (0.5)

* Estimated means (SD) obtained from linear mixed models (LMM) adjusted for gender, age, type of school, physical activity, frequency of fruits/vegetables intake, frequency of fast-food intake, frequency of breakfast cereals intake, frequency of sugar-sweetened beverages intake, and vitamin D and total energy intake;

** Q1: < 492.0g/day; Q2: 492.0-567.9mg/day; Q3: 568.0-670.9mg/day; Q4: 671.0-838.0mg/day; and Q5: > 838.0mg/day;

*** Significant p-value (< 0.05) for changes in time (interaction term of calcium intake * age) compared to the Q1.

excess body fat was lower at Q5 for the total sample ($p < 0.01$), boys ($p = 0.002$), and girls ($p = 0.003$). No association was observed between calcium intake and the incidence of excess weight, abdominal adiposity, and excess body fat at the end of the follow-up (Table 4).

Subjects lost to follow-up (n = 437) differ from those who completed the study regarding gender and type of school; however, since 76% (n = 779) of the cohort completed the first follow-up, contributing with at least two observations during the period, they were included in the longitudinal analyses with the LMM. Moreover, losses were more frequent in public schools (59.5%), likely because these students often have higher mobility between schools than students from private ones. No significant differences were observed between subjects that completed the study and those lost to follow-up regarding BMI ($p = 0.31$), WC ($p = 0.07$), and %BF ($p = 0.19$) at baseline (data not shown).

Table 4

Baseline and new cases at the end of follow-up (%) of excess weight, abdominal obesity, and excess body fat according to quintiles (Q) * of dietary calcium intake among adolescents from the Metropolitan Region of Rio de Janeiro, Brazil, 2010-2012.

	Baseline			Follow-up **		
	Total [n = 993]	Boys [n = 466]	Girls [n = 527]	Total [n = 419]	Boys [n = 169]	Girls [n = 250]
Excess weight	26.2	28.5	24.1	8.4	11.2	6.4
Q1	36.3	46.8	31.4	12.3	20.0	9.8
Q2	23.0	26.6	21.3	5.6	13.3	2.6
Q3	24.3	23.3	25.0	6.1	6.5	5.9
Q4	23.9	27.9	19.6	10.3	12.2	8.7
Q5	24.0	25.3	20.0	8.4	8.5	6.7
p-value ***	0.02	0.02	0.06	0.44	0.51	0.47
Abdominal obesity	9.7	9.7	9.7	3.1	1.3	4.4
Q1	14.2	16.1	13.3	4.7	2.9	5.5
Q2	6.5	7.8	5.9	2.4	NS	3.4
Q3	12.9	10.5	14.7	4.1	2.7	5.0
Q4	7.5	11.5	3.1	1.8	NS	3.4
Q5	7.5	6.0	12.0	2.4	1.5	5.3
p-value ***	0.06	0.07	0.34	0.34	0.67	0.83
Excess body fat	29.7	17.1	40.8	No new cases	No new cases	No new cases
Q1	44.4	31.7	50.4	No new cases	No new cases	No new cases
Q2	34.5	23.8	39.6	No new cases	No new cases	No new cases
Q3	31.8	14.1	45.1	No new cases	No new cases	No new cases
Q4	23.1	17.6	29.0	No new cases	No new cases	No new cases
Q5	15.2	9.5	32.0	No new cases	No new cases	No new cases
p-value ***	< 0.0001	0.002	0.003	No new cases	No new cases	No new cases

NS: no cases were identified.

* Q1: < 492.0g/day; Q2: 492.0-567.9mg/day; Q3: 568.0-670.9mg/day; Q4: 671.0-838.0mg/day; and Q5: > 838.0mg/day;

** Excluded cases at baseline;

*** p-value of the Cochran-Mantel-Haenszel test.

Discussion

In this study, at baseline, adolescents at Q4 and Q5 quintiles had lower means of body adiposity indicators (BMI, FM, %BF, and FMI) and higher means of lean mass indicators (FFM and FFMI) than those at the lowest quintile, with differences according to gender. Longitudinally, calcium intake was inversely associated with changes over time for FMI in boys at Q4 and Q5 and for WC in girls at Q4. Q5 had a lower proportion of excess weight and excess body fat than Q1 at baseline, but not at the end of the follow-up.

These results corroborate similar international [1,2,3] and national studies [4,5] that reported an inverse association between calcium intake and adiposity in adolescents. A systematic review of longitudinal studies (n = 10) [8] and a review conducted by Spence et al. [9] including clinical trials (n = 11) and cross-sectional (n = 23) and longitudinal studies (n = 13) also found that calcium and/or dairy helps prevent obesity. A meta-analysis including 33 studies, out of which nine were conducted with children and adolescents, found an inverse correlation between calcium intake and body weight gain among these age groups [31], indicating that calcium intake could benefit obesity prevention more than obesity treatment.

The association between calcium intake and weight gain is likely inconsistent because of the variety of studies, which have large cultural differences in calcium/dairy consumption, different lengths of follow-up, limited outcome variables (usually based on BMI), and different methods for diet assessment and statistical analysis procedures.

Furthermore, a threshold of dietary calcium intake around 600-700mg/day [14] may start the biological mechanisms that regulate calcium levels, thus increasing intracellular calcium concentrations in the adipogenesis process into adiposity cells [11,12]. Individuals whose usual calcium intake exceeds this threshold could be on calcium homeostasis with no increases in adipogenesis rate in fat tissues. This condition could partly explain the controversial results in epidemiological studies since the definition of low or high calcium intake varies according to its original population and may be above or below this biological threshold.

The average calcium intake in Brazilian adolescents is low. The 2017-2018 INA estimated a 457mg intake [19], similarly to the threshold of Q1 estimated in our study (450mg/day) but below the threshold associated with the onset of adipogenesis into adiposity cells [14]. On the other hand, the mean calcium intake in Q5 was 1,174mg/day, close to the estimated average requirement (EAR) of calcium (1,100mg) for 9-18 year old children and adolescents [32]. Even if half of the adolescents in the highest quintile do not reach the recommended intake, the Q5 cutoff limit (838.0mg/day) is higher than the threshold associated with the increased risk of overweight [14].

The findings of this cross-sectional analysis support Zemel's hypothesis [11] considering that adolescents at Q5, especially boys, had lower means for most adiposity indicators and higher means of lean mass indicators. The longitudinal analysis found that high calcium intake was inversely associated with changes over time (calcium intake * age) for FMI in boys at Q4 and Q5 and for WC in girls at Q4. At baseline, boys were more likely to be classified at Q5 (32%) than girls (9%), which could explain the gender differences related to the association between calcium intake and adiposity indicators. Moreover, waist circumference alone is also a good indicator for visceral adipose depots [33,34] in children and adolescents. Waist circumference values increased over time could be more sensitive to detect abdominal fat changes, especially in girls, as shown in this study [35].

Our findings are similar to those of other longitudinal studies. The *Avon Longitudinal Study of Parents and Children* – ALSPAC (n = 2,455) found that children with high calcium intake (1,140mg/day) at 10 years old had a lower risk of excessive body weight and FM at 13 years old (36) than children with low calcium intake (564mg/day). Our results are also similar to those of the IDEFICS study [37], a large European cohort of children and adolescents (n = 6,696) which found an inverse association at baseline of total calcium intake with all the adiposity indices in boys and with the sum of skinfolds and FMI among girls. The authors of the cohort study also found that the prevalence of overweight/obesity decreased across the tertiles of calcium intake for both sexes. Prospectively, at baseline, boys at the highest tertile of calcium intake had significantly lower increase in BMI, WC, and FMI z-scores whereas girls had lower increase in WC z-score only. Moreover, boys had lower risk of overweight/obesity at the highest tertile of calcium intake than in the first [37].

Similarly to our findings for boys, Barr [38] observed that girls with calcium intake below the median (773mg/day) had higher baseline body weight, total FM, percentage body fat, and percentage visceral fat than those above the median, with no changes after two years of follow-up. However, in a randomized controlled trial [39], girls aged 13-14 years with usual calcium intake ≤ 600mg/day were supplemented with ≥ 1,200mg/day of calcium from low-fat milk and yogurt for 12 months, gaining amount of body fat similar to the control group (≤ 600mg/day). This shows no association between calcium or dairy and the decrease of body fat or weight gain in girls. These results lead us to ask whether the supplementation of calcium and dairy can counteract a physiological stimulus to weight and fat gain, as commonly observed during adolescence. To our understanding, they are not.

Dairy products are the major calcium source in a diet and rich in other nutritional components such as conjugated linoleic acid (CLA) and vitamin D, both involved in the lipolysis process [40]. Moreover, other components such as lactose, cholecalciferol (vitamin D3), and casein phosphopeptides enhance their bioavailability [41,42]. This effect's attribution to dietary calcium per se or to other components in dairy products, or even both, is still undefined. In fact, two meta-analyses [43,44] suggested that dairy consumption is inversely and longitudinally associated with the risk of childhood and adolescence overweight/obesity.

This study has limitations. The period of follow-up may have been too short to capture small changes on anthropometric and body composition measures as those observed in the study. We did not consider the sexual maturation stage, but adolescents included in this study had a mean age of 15.7 years, indicating that most of them may have completed all five stages of pubertal development [45];

therefore, changes in body weight and composition may not result from the hormonal transformations of puberty. Moreover, linear mixed models included age as the random effect which seems to minimize the absence of sexual maturation stage information. The mixed linear models can generate better estimators of changes over time by minimizing the effect of common problems related to longitudinal designs, including the drop-outs during follow-up, unbalanced data, and unequal spacing between time intervals [29]. Finally, the losses to follow-up did not bias our results because they did not differ regarding the main exposures and outcomes.

This study also has strengths. Firstly, we analyzed indicators of body fat besides BMI to assess changes in weight and fat gain during follow-up. Secondly, we applied a validated FFQ specially designed for adolescents including the representative main food sources of calcium in the Brazilian diet. Finally, we calibrated dietary data to improve the energy and nutrient estimates and minimize information bias usually associated with the FFQs.

Conclusions

This study's results corroborate Zemel's hypothesis about the effect of dietary calcium on adiposity in adolescents, especially among boys. To our knowledge, this is the first prospective study in Brazil to analyze the association between calcium intake and adiposity measures in adolescents. This study supports the hypothesis that dietary calcium intake is inversely associated with changes in adiposity among adolescents in a low/middle-income country with a high prevalence of calcium intake inadequacy. Further studies should consider that a threshold of low calcium intake could enhance adipogenesis.

Contributors

A. B. V. Moraes contributed to data collection, analysis, and interpretation and the drafting, writing, and revision of the manuscript. G. V. Veiga, R. Sichieri and R. A. Pereira contributed to the study conception and design, data interpretation, and critical revision of the manuscript. V. B. Azeredo revised the manuscript. All authors approved the final version of the manuscript.

Additional informations

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Resumo

Estudos epidemiológicos têm sustentado a hipótese que a ingestão de cálcio na dieta pode proteger contra a adiposidade. O estudo teve como objetivo estimar a associação entre ingestão de cálcio e indicadores de adiposidade durante a adolescência. O estudo de coorte analisou adolescentes do Ensino Médio (n = 962) de escolas selecionadas na Região Metropolitana do Rio de Janeiro, Brasil acompanhados entre 2010 e 2012. A ingestão de cálcio foi avaliada com um questionário validado de autorrelato de frequência alimentar. Foram realizadas análises transversais e longitudinais de ingestão de cálcio em relação ao índice de massa corporal (IMC), circunferência da cintura (CC), percentual de gordura corporal (%GC), massa gorda (MG), massa magra (MM), índice de massa gorda (IMG) e índice de massa magra (IMM). A análise transversal usou ANOVA com dados da linha de base, e modelos mistos lineares foram aplicados para avaliar as mudanças ao longo do seguimento. Na linha de base, foram observados valores médios mais baixos para IMC, %GC, MG e IMG (p para tendência < 0,05) no quintil mais alto de ingestão de cálcio, em que foram estimados valores médios mais altos de massa magra e índice de massa magra (p para tendência < 0,05), principalmente em meninos. Durante o seguimento, os meninos mostraram uma redução no IMG no quarto e quinto quintis de ingestão de cálcio comparado com o primeiro quintil (p < 0,05), enquanto nas meninas, apenas a CC foi significativamente mais baixa no quarto quintil de ingestão de cálcio comparado com o primeiro quintil. Os resultados corroboram a hipótese do papel da baixa ingestão de cálcio no aumento da adiposidade em adolescentes.

Adolescente; Distribuição da Gordura Corporal; Cálcio; Obesidade; Estudos Longitudinais

Resumen

Los estudios epidemiológicos han apoyado la hipótesis de que la ingesta de calcio en la dieta puede ser protectora de la adiposidad. El objetivo del estudio fue estimar la asociación del calcio dietético con los indicadores de adiposidad durante la adolescencia. Se trata de un estudio de cohorte con adolescentes de secundaria (n = 962) de escuelas seleccionadas del Área Metropolitana de Río de Janeiro, Brasil, que fueron seguidas desde 2010 hasta 2012. La ingesta de calcio se evaluó mediante un cuestionario validado de frecuencia de alimentos autoinformado. Se realizaron análisis transversales y longitudinales de la ingesta de calcio en la dieta en relación con el índice de masa corporal (IMC), la circunferencia de la cintura (CC), el porcentaje de grasa corporal (%GC), la masa grasa (MG), la masa libre de grasa (MLG), el índice de masa grasa (IMG) y el índice de masa libre de grasa (IMLG). Se utilizó el ANOVA para el análisis transversal, utilizando los datos de referencia y se aplicaron modelos lineales mixtos para evaluar los cambios a lo largo del seguimiento. En la línea de base, se observaron medias más bajas de IMC, %GC, MG e IMLG (p para tendencia < 0,05) en el quintil más alto de ingesta de calcio, para el que se estimaron medias más altas de MLG y IMLG (p para tendencia < 0,05), especialmente para los chicos. Durante el seguimiento, los chicos presentaron una reducción del IMLG en el 4º y 5º quintiles de ingesta de calcio (p < 0,05), mientras que entre las chicas, sólo la CC fue significativamente menor en el 4º quintil de ingesta de calcio en comparación con el 1º. Estos resultados apoyan la hipótesis de que la baja ingesta de calcio puede tener un papel en el aumento de la adiposidad entre los adolescentes.

Adolescente; Distribución de la Grasa Corporal; Calcio; Obesidad; Estudios Longitudinales

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