



## ORIGINAL ARTICLE

# Relationship between dietary calcium intake and adiposity in female adolescents<sup>☆</sup>



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### KEYWORDS

Adolescents;  
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### Abstract

**Background and objective:** The prevalence and magnitude of obesity in children and adolescents increase rapidly. Besides genetic and environmental factors, calcium intake has recently been identified as a dietary factor that is inversely related with body mass index and development of overweight and obesity.

The purpose of this study was to assess the correlation between dietary calcium intake and body mass index and fat distribution in female adolescents.

**Materials and methods:** This was a cross-sectional study where anthropometric variables (weight, height, body mass index, waist and hip circumference) were collected in 244 female adolescents to establish total body adiposity and fat distribution. A 24-h recall and a food frequency questionnaire were used to assess total calorie, calcium, and dairy products intake. **Results:** Calcium intake was inversely related to body mass index ( $p < .05$ ), waist circumference ( $p < .05$ ), hip circumference ( $p > .05$ ), and waist to hip ratio ( $p < .05$ ). Overweight (8.3%) and obese (0.7%) adolescents had a lower mean calcium intake than adolescents of normal weight ( $p = .06$ ).

**Conclusions:** Dietary calcium intake and, to a lesser extent, consumption of dairy products are inversely related to total and abdominal adiposity, and also to the prevalence of overweight in this group of adolescents.

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**PALABRAS CLAVE**

Adolescentes;  
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Sobrepeso

**Relación entre ingesta dietética de calcio y adiposidad corporal en adolescentes mujeres****Resumen**

*Antecedentes y objetivo:* La prevalencia y magnitud de la obesidad en niños y adolescentes se incrementa aceleradamente. Aparte de los factores genéticos y ambientales, se ha identificado recientemente la ingesta de calcio dietético como uno de los factores que se correlaciona inversamente con el índice de masa corporal y con el desarrollo de sobrepeso y obesidad.

El objetivo del presente estudio es determinar la correlación entre la ingesta dietética de calcio con el índice de masa corporal y la distribución de la grasa en adolescentes mujeres.

*Materiales y métodos:* Es un estudio de corte transversal en el cual se realizó la evaluación antropométrica (peso, talla, índice de masa corporal, circunferencia de cintura, circunferencia de cadera) en 244 adolescentes mujeres, para establecer la adiposidad corporal total y la distribución de la grasa. Se utilizó el recordatorio de 24 h y un cuestionario de frecuencia de consumo para determinar la ingesta calórica total, ingesta de calcio y consumo de lácteos.

*Resultados:* La ingesta de calcio se correlacionó inversamente con el índice de masa corporal ( $p < 0,05$ ), circunferencia de cintura ( $p < 0,05$ ), circunferencia de cadera ( $p > 0,05$ ) e índice cintura cadera ( $p < 0,05$ ).

Las adolescentes con sobrepeso (8,3%) y obesidad (0,7%) tuvieron una ingesta promedio de calcio inferior al de las adolescentes de peso normal ( $p = 0,06$ ).

*Conclusiones:* La ingesta dietética de calcio y en menor grado el consumo de productos lácteos se relaciona inversamente con la adiposidad total y abdominal así como con la prevalencia de sobrepeso en las adolescentes estudiadas.

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**Introduction**

Non-communicable chronic diseases represent a worldwide health problem.<sup>1</sup> They have a substantial impact on health and have serious adverse effects on the quality of life, favor early mortality, and cause high costs to society. Cardiovascular disease, cancer, chronic respiratory tract disease, and diabetes account for 60% of deaths worldwide. It is estimated that 41 million people will die from non-communicable chronic diseases in 2015.<sup>2</sup>

Obesity is considered by the World Health Organization to be the "epidemic of the 21st century", and its prevalence has increased and continues to increase in developed countries, as well as in countries with economies in transition, and has acquired epidemic proportions. Overweight and obesity account for approximately 2.6 million deaths annually. Considering its multifactorial etiology, genetic factors appear to have played only a secondary role in the increased prevalence of obesity, while environmental factors such as diet and physical inactivity emerge as the most significant determinants of increased adiposity in the past 30 years.<sup>2</sup>

The prevalence and magnitude of obesity are also rapidly increasing in children and adolescents.<sup>3</sup> Childhood obesity is the most common cause of insulin resistance in children and adolescents and is associated with dyslipidemia, type 2 diabetes mellitus, long-term vascular complications, and usually with increased morbidity and mortality in adulthood.<sup>3,4</sup>

Research has often focused on the identification of combinations of macronutrients able to regulate body weight, but the effects of micronutrients need to be further

explored. Some minerals are involved in energy metabolism and in insulin secretion and action, and may interfere with obesity control.<sup>5</sup>

Studies searching for epidemiological explanations of increased adiposity have identified dietary calcium intake as one of the factors inversely correlated to the body mass index (BMI). Thus, consistent evidence concerning the role of calcium and dairy product consumption in obesity prevention has started to appear in recent years. The first report by McCarron, two decades ago, of the finding of lower body weights in US people who reported higher calcium intake in the National Health Survey opened a line of basic, clinical, and molecular research aimed at first confirming, and then explaining the mechanisms by which a mineral apparently unrelated to the energy equation could contribute to weight loss.<sup>6</sup> Short-term studies have reported negative associations between calcium consumption and the BMI, percent fat, and waist circumference.<sup>7</sup> Experimental studies in rats to determine the effect of subchronic dietary calcium intake on body weight and lipid metabolism showed that the administration of calcium added to a high-fat diet decreased body weight and abdominal fat contents during treatment, from which a potential decrease in fat absorption and a potential increase in fatty tissue apoptosis was inferred.<sup>8-10</sup>

A reanalysis of several prospective studies designed to assess the effect of dairy products on the prevention of osteoporosis showed that groups treated with calcium or with a greater intake of dairy products consistently had less risk of overweight.<sup>11</sup> A greater relative risk of high body adiposity was found in people with the lowest calcium intake, and the risk gradually decreased as calcium intake increased; relative risk was 0.75 for the second quartile,

0.40 for the third quartile, and 0.16 for the fourth quartile of calcium intake in women. The intake of dairy products, dietary protein, and calcium was associated with more favorable body composition in obese males and females.<sup>12-14</sup>

The results of clinical studies of calcium intake found significant negative associations between calcium intake and body weight in all age groups, and the range of probability to be overweight (BMI >25) was 2.25 for females in the lower half of calcium intake.<sup>15-17</sup>

On the other hand, some studies have reported that increased consumption of dairy products with no energy restriction does not lead to a significant change in body weight or composition.<sup>18</sup>

Based on the foregoing and because of the inconsistency of the effects of dairy products on body weight and composition reported by various observational and experimental studies, a study was undertaken to assess whether the intake of calcium and dairy products in diet correlated to the degree of adiposity and development of overweight in a group of female adolescents.

## Objectives

To determine whether dietary calcium intake correlates to the BMI and body fat distribution in female adolescents.

To determine whether dairy product intake correlates to the BMI and body fat distribution in female adolescents.

## Materials and methods

This was a cross-sectional, observational, epidemiological study that enrolled 244 female adolescents aged 12–19 years, randomly selected from public and private schools from the list of schools of the Ministry of Education of Ecuador.

All participants voluntarily agreed to take part in the study, and written informed consent signed by their parents or tutors was obtained. Adolescents with any physical disability that made the taking of anthropometric measurements difficult and those who had a clinical history of endocrine or metabolic problems, or cardiovascular, respiratory or musculoskeletal disease were excluded from the study.

The study was approved by the bioethics committee of Universidad San Francisco in Quito.

The sample was estimated with Epi Info (version 6) software using the following parameters: 95% confidence interval, expected prevalence of overweight of 12%, and 7% precision.

## Definition of variables

**Anthropometrics.** The following anthropometric measurements were taken: body weight: using a SECA scale (Germany); height: using a stadiometer; waist circumference: measured halfway between the costal margin and the iliac crest; and hip circumference: measured at the widest part of the hip, at the level of the greater trochanters, using a non-stretchable measuring tape.

The BMI was calculated as: weight (kg)/height (m<sup>2</sup>).

**Overweight and obesity.** Overweight and obesity were defined based on the BMI. Overweight was defined as a BMI above the 85th percentile, and obesity as a BMI above the 95th percentile. The resulting values were compared to those provided in the tables of the National Center for Health Statistics (NCHS) according to age and sex.<sup>19</sup>

**Fat distribution.** The waist/hip ratio was used to determine body fat distribution.

**Intake of calories, macronutrients, calcium, and dairy products.** This was calculated using a 24 h recall instrument. To facilitate recall of the size of portions and to decrease recall bias, participants were shown the book with pictures of food portions of the American Dietetic Association. To determine differences between week days and weekends, three recalls per person were used in adolescents from state schools, and two recalls in those from private schools. The information thus collected was analyzed with Food Processor software (Nutrition Analysis ESHA), with which total calorie and macronutrient intake, percent macronutrients in total calories, and daily calcium intake were determined.

Based on the frequency distributions obtained for this group of adolescents, calcium consumption was divided into three groups: adequate intake: adolescents who consumed 1200 mg/day or more; low intake: those consuming less than 1200 mg/day; very low intake: those consuming 600 mg/day of calcium or less.

A consumption frequency questionnaire was used to assess the consumption of dairy products (milk, cheese, and yogurt). Adequate intake was defined as the consumption of one or more portions of dairy products daily, and low intake as the consumption of less than one portion daily.

**Data analysis.** Variables were analyzed using descriptive statistics and are given as mean  $\pm$  one standard deviation for continuous variables, and as percentages for nominal variables. The differences between groups were analyzed using a Student's *t* test for continuous variables and a  $\chi^2$ -square for categorical variables. To establish the association of the BMI, waist circumference, hip circumference, and waist/hip ratio with predictive variables, multiple regression analyses were performed. Values of  $p \leq 0.05$  were considered statistically significant. Excel 2013 software was used to record data, and SPSS version 22 for Windows was used for processing and statistical analysis.

## Results

Multiple regression analysis showed that calcium intake adjusted for calorie intake negatively correlated to the BMI ( $p < 0.05$ ), waist circumference ( $p < 0.05$ ), hip circumference ( $p > 0.05$ ), and waist/hip ratio ( $p < 0.05$ ) (Table 1).

As regards the relationship between nutritional state and calcium intake, adolescents with overweight were found to have a lower mean calcium intake than adolescents of normal weight ( $p = 0.06$ ); adolescents with overweight were also found to have a lower calorie intake as compared to adolescents of normal weight ( $p = 0.005$ ) (Table 2).

With regard to the association of calcium intake and body weight, overweight and obese adolescents were found to have a very low and low calcium intake, and no adolescent in these groups had an adequate calcium intake ( $p = 0.48$ ) (Table 3).

**Table 1** Multiple regression analysis between calcium intake and different anthropometric parameters.

Predictive variable	Anthropometric measurements	<i>r</i>	<i>p</i>
Calcium intake (mg)	Body mass index (BMI)	-0.21	0.001
	BMI percentile	-0.13	0.040
	Waist circumference	-0.17	0.007
	Hip circumference	-0.081	0.20
	Waist/hip ratio	-0.14	0.026

**Table 2** Calorie and calcium intake by nutritional status.

Nutritional status	Mean intake of				
	Calcium (mg)	SD	Calories	SD	No.
Low weight	453.00	338.28	1.992.1	789.8	14
Normal weight	507.84	367.98	1.736.7	742	206
Overweight	494.73	386.84	1336.5	483.1	24
Total	485.19	364.36	1.738.10	738.1	244
	<i>p</i> = 0.06		<i>p</i> = 0.005		

**Table 3** Relationship between calcium intake and body weight.

Calcium intake	Low weight	Normal	Overweight	Obesity
Very low	8 (57%)	97 (47.1%)	14 (63.6%)	2 (100%)
Low	5 (35.7%)	97 (47.1%)	8 (36.4%)	0 (0%)
Adequate	1 (7.1%)	12 (5.8%)	0 (0%)	0 (0%)
Total	14	206	22	2
	<i>p</i> = 0.48			

Finally, adolescents with a low intake of dairy products (milk, cheese, and yogurt) had on average greater values of BMI (20.09 vs 19.58) and waist circumference (71.3 vs 69.18) as compared to adolescents with adequate intake ( $p > 0.05$ ).

## Discussion

The results of this study show that the dietary intake of calcium and dairy products was inversely correlated to body adiposity and overweight and obesity in these female adolescents.

Other similar cross-sectional studies conducted on adolescents have also shown a negative correlation between calcium intake and anthropometric indicators (weight, the BMI, fat mass, and percent fat).<sup>20</sup> The pathophysiological mechanism of this association appears to be related to the oxidative capacity of adipose tissue, with high calcium intake inducing a decrease in intracellular calcium levels in adipose tissue, so promoting fat oxidation, rather than fat deposition, and, by contrast, low dietary calcium intake having the reverse effect.<sup>21</sup> Various studies in humans have similarly shown an inverse relationship between high calcium intake and the presence of metabolic disorders related to obesity such as high blood pressure, diabetes, and insulin resistance.<sup>22</sup>

In addition, some studies have reported that the consumption of calcium from dairy products promotes the loss of fat mass in premenopausal obese women.<sup>23</sup> Finally, animal models have provided a potential mechanism through which low calcium intake could influence body fat deposits.<sup>24</sup> Among these mechanisms, it has been suggested that a low calcium diet increases intracellular calcium concentrations by stimulating calciotropic hormones such as 1,25 dihydroxyvitamin D and parathormone, which may increase intra-adipocyte calcium. These elevated intra-adipocyte calcium concentrations may then increase the rate of lipogenesis and inhibit lipolysis, resulting in increased adiposity. Thus, an increased dietary calcium intake could be proposed to prevent the triggering of this cascade by maintaining low calciotropic hormone levels, therefore decreasing intracellular calcium and lipid contents in adipocytes.<sup>25</sup>

On the other hand, calcium has been seen to dose-dependently increase the fecal excretion of fatty acids. A person consuming 2500 kcal with one third of the energy coming from fat and also taking 2 g of elemental calcium daily, could be expected to excrete an additional 1% of energy from fat daily, and an energy loss of 3010 kcal/year in feces could be anticipated. This energy deficiency could cause a change in body weight of -0.4 kg/year. The effects of calcium on fat excretion are however insufficient to explain the greater difference in body weight shown by some

studies in animals and humans, particularly of calcium from dairy products. Studies conducted in transgenic mice have also shown that high calcium diets stimulate the expression of the uncoupling proteins in adipose tissue (UCP)2 and skeletal muscle (UCP)3.<sup>26,27</sup>

An additional mechanism by which dietary calcium intake may affect body adiposity is an effect on triacylglycerol absorption from the gastrointestinal tract. Calcium fortification has been shown to increase the percentage of saturated fat excreted in feces by 6–13% daily. A high calcium diet also significantly decreased total cholesterol by 6%, LDL cholesterol by 13%, and the apolipoprotein B level by 7% as compared to a low calcium diet. Dietary calcium intake may therefore be inversely related to the development of metabolic syndrome.<sup>28</sup> It would be interesting to assess whether greater calcium intake, mainly from diet, not only decreases the risk of developing overweight or obesity, but also whether the apparent lowering effect on lipid and glucose levels is able to reduce long-term cardiovascular risk.

Our study mainly found that dietary calcium intake is the factor with the greatest correlation to total and abdominal adiposity, and also to the presence of overweight and obesity, while the intake of dairy products also showed a negative correlation, although not a significant one. This agrees with most studies, which found a negative correlation between the dietary intake of calcium, especially from dairy products, and the development of overweight and obesity.<sup>29</sup>

The explanation for the lack of a significant correlation in our study between dairy product intake and adiposity level, in contrast to the significant association found for dietary calcium, may be that no differentiation was made of the type of dairy products preferentially consumed, i.e. if they were whole milk or skimmed milk products. Most probably, in agreement with our dietary habits, a majority consumed whole milk dairy products with high fat contents, which would explain the poor correlation between the consumption of dairy products and body adiposity, while total calcium intake was negatively and significantly associated with total adiposity, and especially with clinically important abdominal adiposity, which is closely related to glucose metabolism, hypertriglyceridemia, low HDL cholesterol, increased blood pressure, metabolic syndrome, type 2 diabetes, and cardiovascular disease.<sup>30</sup> An alternative explanation for the non-significant association between dairy product intake and the development of overweight may be the small sample size of the study.

In any case, regardless of the mechanism involved, most studies support the conclusion that dietary calcium may play a role in body fat regulation and the hypothesis that increased dietary calcium intake may decrease future weight gain and reduce the risk of developing cardiometabolic disorders. However, only small clinical studies specifically designed for exploring the effects of dietary calcium on body weight, total adiposity, and lipid and glucose levels have been conducted. Prospective clinical studies should be conducted to determine whether body weight may be modified by calcium supplementation or by increasing low-fat dairy products in diet, and whether these measures may also decrease the risk of developing those metabolic changes related to visceral fat accumulation, such as diabetes or cardiovascular disease.

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## Conflicts of interest

The authors state that they have no conflicts of interest related to the conduct of this research study.

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## References

- Schmidt MI, Duncan BB, Azevedo e Silva G, Menezes AM, Monteiro CA, Barreto SM. Doenças crônicas não transmissíveis no Brasil: carga e desafios atuais. *The Lancet Saúde do Brasil*. Maio: Saúde no Brasil 4; 2011. p. 61–74.
- World Health Organization. 2008–2013 action plan for the global strategy for the prevention and control of noncommunicable diseases. Geneva: WHO; 2008. Available from: <http://www.who.int/nmh/publications/9789241597418/en/>
- Cadenas-Sánchez C, Artero EG, Concha F, Leyton B, Kain J. Anthropometric characteristic and physical fitness level in relation to body weight status in Chilean preschool children. *Nutr Hosp*. 2015;32:346–53.
- Kamijo K, Khan NA, Pontifex MB, Scudder MR, Drollette ES, Raine LB, et al. The relation of adiposity to cognitive control and scholastic achievement in preadolescent children. *Obesity*. 2012;20:2406–11.
- Cominetti C, Marreiro DDN, Cozzolino SMF. Minerais e obesidade in Cozzolino SMF. Biodisponibilidade de nutrientes. 3.ª ed Barueri, SP: Manole; 2009. p. 811–41.
- McCarron DA. Dietary calcium as an antihypertensive agent. *Nutr Rev*. 1984;42:223–5.
- Onakpoya IJ, Perry R, Zhang J, Ernst E. Efficacy of calcium supplementation for management of overweight and obesity: systematic review of randomized clinical trials. *Nutr Rev*. 2011;69:335–43.
- Yanovski JA, Parikh SJ, Yanoff LB, Denkinger BI, Calis KA, Reynolds JC, et al. Effects of calcium supplementation on body weight and adiposity in overweight and obese adults. *Ann Intern Med*. 2009;150:821–9.
- Barrientos-Alvarado C, Becerra-Reséndiz V, Flores-Gómez A, Beltrán-García X. El calcio minimiza la absorción de grasas en ratas. *Rev Hosp Jua Mex*. 2013;80:219–23.
- Sergeev IN1, Song Q. High vitamin D and calcium intakes reduce diet-induced obesity in mice by increasing adipose tissue apoptosis. *Mol Nutr Food Res*. 2014;58:1342–8.
- Wenersberg MH, Smedman A, Turpeinen AM, Retterstol K, Tengblad S, Lipre E, et al. Dairy products and metabolic effects in overweight men and women: results from a 6-mo intervention study. *Am J Clin Nutr*. 2009;90:8.
- Reid IR, Ames R, Mason B, Bolland MJ, Bacon CJ, Reid HE, et al. Effects of calcium supplementation on lipids, blood pressure, and body composition in healthy older men: a randomized controlled trial. *Am J Clin Nutr*. 2010;91:131–9.

13. Bortolotti M, Rudelle S, Schneiter P, Vidal H, Loizon E, Tappy L. Dairy calcium supplementation in overweight or obese persons: its effect on markers of fat metabolism. *Am J Nutr.* 2008;88:877–85.
14. Murphy KJ, Crichton GE, Dyer KA, Coates AM, Pettman TL, Milte C, et al. Dairy foods and dairy protein consumption is inversely related to markers of adiposity in obese men and women. *Nutrients.* 2013;5:4665–84.
15. Bueno MB, Cesar CLG, Martini LA, Fisberg RM. Dietary calcium intake and overweight: an epidemiologic view. *Nutrition.* 2008;24:1110–5.
16. Harvey-Berino J, Casey B, Lauber R, Starinski A. The impact of calcium and dairy product consumption on weight loss. *Obes Res.* 2005;13:1720–6.
17. Esteves EA, Rodrigues CAA, Paulino ÉJ. Ingestão dietética de cálcio e adiposidade em mulheres adultas. *Rev Nutr.* 2010;23:543–52.
18. Abargouei AS1, Janghorbani M, Salehi-Marzjarani M, Esmailzadeh A. Effect of dairy consumption on weight and body composition in adults: a systematic review and meta-analysis of randomized controlled clinical trials. *Int J Obes (Lond).* 2012;36:1485–93.
19. Kuczmarski RJ, Ogden CL, Guo SS, Grummer-Strawn LM, Flegal KM, Mei Z, et al. CDC growth charts for the United States: methods and development. *Vital Health Stat.* 2000;11:2002.
20. Vargas-Hernández G, Romero-Velarde E, Vásquez-Garibay EM, Vizmanos-Lamotte B, Troyo-Sanromán R. Calcium intake and adiposity in adolescents aged 12–16 years in Guadalajara, Mexico. *Arch Latinoam Nutr.* 2013;63(June):157–63.
21. Zemel MB, Thompson W, Milstead A, Morris K, Campbell P. Calcium and dairy acceleration of weight and fat loss during energy restriction in obese adults. *Obes Res.* 2004;12:582–90.
22. Reid IR, Ames R, Mason B, Bolland, Mark J, Bacon CJ, Reid HE, et al. Effects of calcium supplementation on lipids, blood pressure, and body composition in healthy older men: a randomized controlled trial. *Am J Clin Nutr.* 2010;91:131–9.
23. Josse AR, Atkinson SA, Tarnopolsky MA, Phillips SM. Increased consumption of dairy foods and protein during diet- and exercise-induced weight loss promotes fat mass loss and lean mass gain in overweight and obese premenopausal women. *J Nutr.* 2011;141:1626–34.
24. Sun X, Zemel MB. Calcium and dairy products inhibit weight and fat regain during ad libitum consumption following energy restriction in Ap2-agouti transgenic mice. *J Nutr.* 2004;134:3054–60.
25. Parikh S, Yanovski J. Calcium intake and adiposity. *Am J Clin Nutr.* 2003;77:281–7.
26. Zemel MB. Mechanisms of dairy modulation of adiposity. *J Nutr.* 2003;133:2525–65.
27. Villarroel P, Villalobos E, Reyes M, Cifuentes M. Calcium, obesity, and the role of the calcium-sensing receptor. *Nutr Rev.* 2014;72(October):627–37.
28. Reid IR, Mason B, Horne A, Ames R, Clearwater J, Bava U, et al. Effects of calcium supplementation on serum lipid concentrations in normal older women: a randomized controlled trial. *Am J Med.* 2002;112:343–7.
29. Keast DR, Gallant KM, Albertson AM, Gugger CK, Holschuh NM. Associations between yogurt, dairy, calcium, and vitamin d intake and obesity among U.S. children aged 8–18 years: NHANES, 2005–2008. *Nutrients.* 2015;7:1577–93.
30. Coniglio R. Relación entre la obesidad central y los componentes del síndrome metabólico. *Acta Bioquim Clin Latinoam.* 2014:191–201.