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Influence of calcium and vitamin D intakes on body composition in children and adolescents

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Abstract:	Identifying nutritional strategies to maintain a healthy body weight and reduce the comorbidities associated with obesity is extremely important. We aimed to investigate whether calcium and vitamin D intakes are associated with body composition measurements in a population of children and adolescents. A cross-sectional study was conducted involving 1060 children and adolescents (65.8% females; 34.2% males) aged 9 to 19. Fat mass, percentage of fat mass, and fat-free mass were measured using a body composition analyzer (TANITA BC-418MA®). The mean dietary calcium and vitamin intakes were 829.66 ±328.34 mg/day and 200.78±400.91 IU/day. Linear regression analysis revealed a lack of significant association between daily calcium and vitamin D intakes and body composition measurements after adjusting the model for age, sex, maturation status, and energy intake. Dietary calcium and vitamin D intakes do not appear to be associated with higher adiposity measurements in children and adolescents.

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3 1 **Influence of calcium and vitamin D intakes on body composition in children and**
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5 2 **adolescents**

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9 4 **Abstract**

10 5 Identifying nutritional strategies to maintain a healthy body weight and reduce the
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12 6 comorbidities associated with obesity is extremely important. We aimed to investigate
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14 7 whether calcium and vitamin D intakes are associated with body composition
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16 8 measurements in a population of children and adolescents. A cross-sectional study was
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30 15 maturation status, and energy intake. Dietary calcium and vitamin D intakes do not
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32 16 appear to be associated with higher adiposity measurements in children and adolescents.
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39 18 **Key words:** dietary calcium; vitamin D; fat mass; body composition; adolescents.
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1 Introduction

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7 Obesity in children and adolescents continues to be a global public health problem
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9 (Ogden, Carroll, Kit, & Flegal, 2012). The related disease risks include cardiovascular
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11 disease, type 2 diabetes, hypertension and some cancers (Freedman, Mei, Srinivasan,
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13 Berenson, & Dietz, 2007). Diet is the major modifiable factor associated with child and
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15 adolescent obesity (Fock & Khoo, 2013). Therefore, identifying nutritional strategies to
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17 maintain a healthy body weight and reduce the comorbidities associated with obesity is
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19 extremely important. As nurses are involved in implementing preventive programs,
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21 their role in improving dietary habits in children and adolescents is particularly relevant
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23 and could help reduce the impact of childhood obesity.
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29 Recently, it was recognized that calcium intake is important in regulating adiposity and
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31 the risk of obesity (Zemel, Shi, Greer, Dirienzo, & Zemel, 2000). The possible
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33 mechanisms of this association have not yet been fully elucidated, but it has been
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35 proposed that increased dietary calcium suppresses adipocyte intracellular Ca^{2+} ,
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37 regulating energy metabolism and stimulating lipolysis (Zemel, 2003). Likewise, an
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39 important function of dietary vitamin D has been established, whereby it modulates
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41 anti-inflammatory and pro-apoptotic effects in adipocytes (Sergeev & Song, 2014;
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43 Vanlint, 2013).
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48 The possible beneficial effect of dairy products and calcium intake on weight
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50 management have been investigated, yielding inconsistent results (Booth, Huggins,
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52 Wattanapenpaiboon, & Nowson, 2015; Dicker, Belnic, Goldsmith, & Kaluski, 2008;
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54 Eilat-Adar et al., 2007; Lee, Cho, Lee, Kim, & Cho, 2014; Macdonald, New, Campbell,
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1 & Reid, 2003; Rautiainen et al., 2016; Rosell, Håkansson, & Wolk, 2006; Varena,
2 Binelli, Casari, Zucchi, & Sinigaglia, 2007). Some previous studies have reported an
3 inverse association between calcium intake and weight, but this finding has not been
4 consistent across all studies (Eilat-Adar et al., 2007; Louie, Flood, Hector, Rangan, &
5 Gill, 2011; Macdonald et al., 2003; Phillips et al., 2003; Schwingshackl et al., 2016). In
6 addition, most studies have focused on adults, so there is limited data on the relationship
7 between Ca intake and anthropometric measurements in children and adolescents
8 (Berkey, Rockett, Willett, & Colditz, 2005; García-Lorda et al., 2007; Keast, Hill
9 Gallant, Albertson, Gugger, & Holschuh, 2015; Lin et al., 2012; Moore, Singer,
10 Qureshi, & Bradlee, 2008; Moreira, Padez, Mourão, & Rosado, 2005; Nezami, Segovia-
11 Siapco, Beeson, & Sabaté, 2016; Phillips et al., 2003; Spence, Cifelli, & Miller, 2011).
12 Similarly, the results of studies exploring the potential effect of vitamin D intake on
13 body weight are not conclusive (Kamycheva, Joakimsen, & Jorde, 2003; Keast et al.,
14 2015).

15 Additionally, attention has been focused on the effect of dietary calcium and vitamin D
16 on anthropometric indicators of obesity, principally body mass index (BMI) (Berkey et
17 al., 2005; Keast et al., 2015). Although BMI is a measure of obesity, its relationship
18 with body composition is controversial since BMI cannot distinguish fat from lean mass
19 (Wells & Fewtrell, 2006).

20 In this context, the purpose of this study was to investigate the association of calcium
21 and vitamin D intakes with body composition measurements in a population of children
22 and adolescents.

24 **Methods**

25 *Design and sample*

1 A cross-sectional study was conducted involving 1060 ethnically homogeneous children
2 and adolescents (65.8% females and 34.2% males) aged 9 to 19, recruited from public
3 education centers in XX, XX. A member of the research team visited the study subjects
4 in their academic centers and explained the objectives and characteristics of the study.
5 Subject inclusion criteria included: good health (not undergoing medical treatment
6 related to managing body weight), and an age of between 9 and 19. Subjects with major
7 acute or chronic conditions who had made significant lifestyle changes in the preceding
8 months that could have affected body weight were excluded. Written informed consent
9 was obtained from all participants or parents and the study was approved by the local
10 ethics committee at the University of XXX and conducted in accordance with the
11 Declaration of Helsinki.

12 *Body composition measurements*

13 Body weight (kg), fat mass (kg), percentage of fat mass (%), and fat-free mass (kg)
14 were measured twice (without shoes and in light clothes) to the nearest 0.1 kg using a
15 body composition analyzer (TANITA BC-418MA[®]). A Harpenden stadiometer (Holtain
16 602VR[®]) was used to take height measurements. Each participant was asked to stand
17 erect with their back, buttocks and heels in continuous contact with the vertical height
18 rod of the stadiometer and their head orientated in the Frankfurt plane. The horizontal
19 headpiece was then placed on top of the head of the participant to measure their height.
20 Height was measured twice without shoes to the nearest 0.5 cm. The average of the two
21 values for each measurement were used in the analysis. The measurements were taken
22 in the morning after a 12-hour fast and 24 hours abstention from exercise. The
23 corresponding intra-observer technical error (reliability) of the measurements was
24 0.95%. The same trained research assistant performed all the measurements. The BMI
25 of the study subjects was categorized into BMI-categories using the age-sex specific

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3 1 cut-offs from Cole et al. (Cole & Lobstein, 2012).
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6 2 *Dietary intake assessment*
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9 3 Daily energy, calcium, and vitamin D intakes were assessed using a 72-hour diet recall
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11 4 interview considering intakes on Thursday, Friday and Saturday to capture weekly
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13 5 variations across weekdays and the weekend. In a face-to-face interview with trained
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15 6 investigators, individuals were asked to recall all food consumed in the preceding
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17 7 72 hours, including nutritional supplements and beverages. Standard household
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19 8 measures and pictorial food models were employed during the interviews to define
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21 9 amounts when requested. The completed food records were analyzed using a
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23 10 computerized nutrient analysis program (Nutriber 1.1.5).
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28 12 *Maturation status assessment*
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31 13 Maturation status (self-reported) was assessed using the Tanner classification (five
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33 14 stages: I–V) (Tanner & Whitehouse, 1976). Each participant entered an isolated room.
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35 15 Using a set of images exemplifying the various stages of sexual maturation, they
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37 16 categorized the development of their own genitalia (for boys), breasts (for girls),
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39 17 armpits (for boys), and pubic hair (for both genders). The reproducibility of our data
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41 18 was up to 85%.
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45 20 *Statistical analysis*
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48 21 The Kolmogorov-Smirnov statistic was applied to verify data distribution normality.
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50 22 We applied logarithmic transformations as needed to ensure normal distribution of the
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52 23 following variables: daily energy intake, calcium intake and vitamin D intake. **The data**
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54 24 **was expressed as the mean ± standard deviation for normal distribution and median**
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56 25 **(interquartile range) for non-normal distribution. The independent two-sample t-test or**
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1 Mann-Whitney U-test was used to compare variables with normal or non-normal
2 distribution, respectively, between groups. The outcome variables were body weight
3 (kg), fat mass (kg), percentage of fat mass (%), fat-free mass (kg), and BMI (kg/m²).
4 Linear regression analyses were conducted to determine the association between
5 calcium and vitamin D intakes and body composition variables. In the overall
6 population, regression analyses were adjusted by the following confounding factors:
7 age, sex, maturation status, and total energy (kcal). In the stratified analysis by sex,
8 linear regressions were adjusted by age, maturation status, and total energy (kcal).
9 Maturation status was treated as a categorical variable with five levels (stages 1-5). The
10 results are reported as percentage change (β) with 95 % confidence intervals (95 % CI).
11 SPSS Statistics version 21.0 (SPSS, Chicago, IL, USA) was used for all the analyses. P-
12 values < 0.05 were considered to be statistically significant.

14 Results

15 The characteristics of the participants were summarized separately for males and
16 females (Table 1). The study subjects included in this study comprised 697 girls and
17 363 boys with a mean age of 16.45±3.74 years. The average BMI was 21.74±3.9 kg/m²
18 for the girls and 22.24±4.10 for the boys and a significant difference was not observed.
19 The prevalence of overweight/obesity was 17.7% in the girls and 22.9% in the boys, and
20 62.9% of the subjects were of normal weight. Significant differences were observed
21 between males and females with respect to height, weight, fat mass, and fat-free mass (p
22 < 0.001). The reported energy intake was higher in the males (2109.40 ±623.10) than
23 the females (1943.60 ±600.52) (p < 0.001). The mean dietary calcium and vitamin
24 intake were 829.66 ±328.34 mg/day and 200.78±400.9 IU/day in the overall population.
25 The Recommended Dietary Allowances (RDA) according to the 2010 Institute of

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3 1 Medicine Dietary Reference Intake committee, of 1300 mg/day for calcium and 600
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5 2 IU/day for vitamin D intake for children aged 9-18, were not reached by 92.8% and
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7 3 90.8% of the subjects, respectively (*Dietary Reference Intakes for Calcium and Vitamin*
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9 4 *D*, 2011).

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13 6 Linear regression analysis revealed a lack of significant association between daily
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15 7 calcium intake and body composition measurements after adjusting the model for age,
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17 8 sex, maturation status, and energy intake (Table 2). Similarly, when vitamin D intake
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19 9 was analyzed, there was no significant association with any of the adiposity indices
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21 10 after adjusting for confounders (Table 3).

11 12 **Discussion**

13 The purpose of this study was to investigate the relationships between dietary calcium
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15 14 and vitamin D intakes and body composition by assessing fat mass (kg), percentage of
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17 15 fat mass (%), and fat-free mass (kg) in a population of 1060 children and adolescents.

16 We found a lack of association between dietary calcium and vitamin D intakes and
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18 17 adiposity measurements in both females and males, as well as in the entire sample after
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20 18 adjusting for known confounders. In agreement with our findings, data from a
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22 19 community-based survey of 3044 children reported that calcium does not show
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24 20 significant associations with BMI (Moreira et al., 2005). Furthermore, dairy food
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26 21 consumption was not associated with higher body weight or adiposity in a study based
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28 22 on data from the National Health and Nutrition Examination Survey (NHANES 2005-
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30 23 2008) in children aged 8-18 (Keast et al., 2015). Similarly, Lin et al. found no
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32 24 significant association between dairy intake and lower adolescent BMI (Lin et al.,
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34 25 2012), and Nezami et al. concluded that dairy consumption was not linked to obesity in

1 a population of adolescents (Nezami et al., 2016). Interestingly, our findings are
2 consistent with most of the longitudinal studies reviewed by Loui et al. (Loui et al.,
3 2011).

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5 In contrast, in recent years, some studies have shown an inverse association between
6 body weight and dietary calcium, supporting the hypothesis that calcium intake exerts a
7 protective effect against weight gain and promotes weight loss in children and
8 adolescents (García-Lorda et al., 2007; Spence et al., 2011). Possible causes of
9 inconsistencies in the data reported between the studies could be due to differences in
10 sample characteristics (sex distribution, ethnicity, or the prevalence of
11 overweight/obesity), dietary assessments (24-hour dietary recalls or food frequency
12 questionnaires), and analysis methods (adjusting versus not adjusting dietary calcium
13 for energy intake). Indeed, one longitudinal study conducted in adolescents concluded
14 that the effect of dietary calcium seems to be explained mainly by energy intake, since
15 the relationship was attenuated when adjusted for energy (Berkey et al., 2005).
16 Furthermore, the significant relationship between dietary calcium and body weight has
17 been described in obese, but not in lean subjects (Phillips et al., 2003). Since our
18 population has lower rates of overweight and obesity (20.3%), this could have affected
19 the power to identify significant results. Additionally, it must be highlighted that mean
20 calcium and vitamin D intakes were remarkably low in our sample, since only 7.2% and
21 9.2% of subjects met the RDA for calcium and vitamin D, respectively (*Dietary
22 Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride,*
23 1997). It may therefore be possible that very low calcium and vitamin D intakes do not
24 exert important effects on adiposity levels. Moreover, the effects might depend on the
25 dietary source of calcium, as it has been demonstrated that source of the dietary calcium

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3 1 may exert a significant effect on fat loss during energy restriction (Zemel & Miller,
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5 2 2004). Finally, it should be noted that since body weight and adiposity are highly
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7 3 multifactorial variables, it is improbable that dietary calcium and vitamin D are the only
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9 4 factors underlying lower BMI and fat mass.
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13 6 The fact that adiposity was assessed using fat mass, fat-free mass, and percentage of fat
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15 7 mass, in addition to traditional indicators such as BMI, is a major strength of this work
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17 8 since most studies have only focused on how calcium and vitamin D intakes affect
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19 9 anthropometric measurements of obesity. Secondly, our data is from a large, well-
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21 10 characterized sample of 1060 Spanish children and adolescents that is representative of
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23 11 our population. On the other hand, this study is limited by its cross-sectional design and
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25 12 a causal relationship cannot be proven. Furthermore, it also has certain limitations
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27 13 inherent to the use of 72-hour recall since this is prone to under reporting and relies on
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29 14 memory (Shim, Oh, & Kim, 2014). Nevertheless, in order to minimize any recall bias,
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31 15 face-to-face interviews were conducted by a well-trained interviewer and the subjects
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33 16 also received training prior to participating in the study. To improve the accuracy of the
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35 17 food descriptions, standard household measures and pictorial food models were used. In
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37 18 addition, we estimated the misreporting level in our study cohort using the Goldberg
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39 19 cut-off method (Black, 2000), showing that the potential level of underreporting in our
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41 20 population is not relevant. Finally, although our models were adjusted for important
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43 21 covariables, including sex, age, maturation status, and energy intake, some unmeasured
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45 22 covariables such as physical activity may account for a proportion of the variation in
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47 23 obesity measurements.
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3 1 In conclusion, our analysis reveals that dietary calcium and vitamin D intakes do not
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5 2 appear to be associated with higher measures of adiposity in children and adolescents.
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7 3 Further prospective studies are warranted to clarify the current understanding of the
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9 4 effects of dietary calcium and vitamin D intakes on adiposity during childhood and
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11 5 adolescence.
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For Peer Review

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1 Table 1. Descriptive characteristics of the study population.

	Females	Males	Total
N (%)	697(65.8)	363(34.2)	1060
Age	16.91(3.53)*	15.57(3.96)	16.45(3.74)
Height (m)	1.60(0.09)*	1.64(0.15)	1.61(0.11)
Weight (kg)	56.38(12.04)*	61.46(17.88)	58.12(14.51)
BMI (kg/m²)	21.74(3.9)	22.24(4.10)	21.91(3.97)
Fat mass (%)	24.28(7.84)*	17.21(8.35)	21.86(8.69)
Fat mass (kg)	14.63(7.94)*	10.85(6.87)	13.33(7.79)
Fat-free mass (kg)	41.76(6.02)*	50.63(14.13)	44.79(10.48)
Daily energy intake (kcal/day)	1874.13[802.26] *	2050.28[823.32]	1948.57[819.50]
Calcium intake (mg/day)	781.41[425.6]	808.66[414.9]	788.66[421.00]
Vitamin D intake (µg/day)	1.50[2.77] *	1.93[3.12]	1.60[2.82]

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3 Data are expressed as the mean ± standard deviation for normal distribution and median [interquartile
4 range] for non-normal distribution.

5 * p < 0.05 between females and males.

6 BMI: body mass index.
7

1 Table 2. Association between dietary calcium intake (mg/day) and body composition variables.

	Dietary calcium intake					
	Females*		Males*		Total**	
	B (95% CI)	p-value	B (95% CI)	p-value	B (95% CI)	p-value
Weight (kg)	0.0001 (-0.003, 0.003)	0.924	-0.003 (-0.007, 0.002)	0.242	-0.001 (-0.003, 0.001)	0.484
BMI (kg/m²)	0.0001 (-0.001, 0.001)	0.766	-0.001 (-0.002, 0.001)	0.297	-0.0002 (-0.001, 0.001)	0.657
Fat mass (%)	0.0003 (-0.002, 0.002)	0.719	-0.0002 (-0.003, 0.002)	0.834	0.00009 (-0.001, 0.002)	0.910
Fat mass (kg)	0.0004 (-0.001, 0.002)	0.653	-0.001 (-0.003, 0.001)	0.507	-0.00006 (-0.002, 0.001)	0.936
Fat-free mass (kg)	-0.001 (-0.00005, 0.002)	0.287	-0.001 (-0.004, 0.001)	0.313	-0.00007 (-0.001, 0.001)	0.915

2
3 * Adjusted by age, maturation status and total energy intake.

4 **Adjusted by sex, age, maturation status and total energy intake.

5 B (95% CI): standardized regression coefficient (95% confidence interval).

1 Table 3. Association between dietary vitamin D intake ($\mu\text{g}/\text{day}$) and body composition variables.

	Dietary vitamin D intake					
	Females		Males		Total	
	B (95% CI)	p-value	B (95% CI)	p- value	B (95% CI)	p-value
Weight (kg)	-0.008 (-0.151, 0.134)	0.909	-0.251 (-0.725, 0.223)	0.298	-0.030 (-0.177, 0.117)	0.690
BMI (kg/m^2)	-0.005 (-0.055, 0.045)	0.844	-0.048 (-0.189, 0.092)	0.499	-0.011 (-0.058, 0.036)	0.649
Fat mass (%)	-0.033 (-0.135, 0.070)	0.532	-0.065 (-0.354, 0.224)	0.659	-0.042 (-0.140, 0.056)	0.398
Fat mass (kg)	-0.033 (-0.136, 0.070)	0.531	-0.130 (-0.379, 0.118)	0.304	-0.049 (0.142, 0.044)	0.298
Fat-free mass (kg)	0.015 (-0.042, 0.072)	0.612	-0.123 (-0.416, 0.171)	0.412	0.009 (-0.071, 0.090)	0.818

2
3 * Adjusted by age, maturation status and total energy intake.

4 **Adjusted by sex, age, maturation status and total energy intake.

5 B (95% CI): standardized regression coefficient (95% confidence interval).