



## Original article

# Association of calcium and dairy product consumption with childhood obesity and the presence of a Brain Derived Neurotropic Factor-Antisense (BDNF-AS) polymorphism



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## SUMMARY

**Background & aims:** Calcium and dairy products have multiple health benefits. The objective of this work was to evaluate the association between calcium/dairy intake, blood pressure, the BDNF-AS rs925946 polymorphism and nutritional status in a group of schoolchildren.

**Methods:** As part of the GENYAL study to childhood obesity prevention, 221 children belonging to different areas of the Community of Madrid were enrolled. Anthropometric and dietary data were collected, and children were genotyped according to the rs925946 polymorphism. Adjusted logistic and linear models were used to describe the data.

**Results:** A significantly lower consumption of calcium in overweight versus normal weight children was observed ( $811.0 \pm 174.1$ ;  $859.0 \pm 195.9$ ;  $954.0 \pm 223.1$  mg; for obesity, overweight and normal weight, respectively,  $p = 0.010$ ). Moreover, an inverse association between blood pressures and calcium intake was detected ( $\beta = -0.006$  ( $-0.011, -3e^{-4}$ )),  $p = 0.040$ . The number of dairy servings/day showed a protective effect against overweight (OR = 0.48 (0.29, 0.75),  $p = 0.001$ ). Finally, common homozygous children (GG) showed an inverse association between the calcium intake and the BMI ( $\beta = -0.003$  ( $-0.006, -0.001$ ),  $p = 0.004$ ), which was not observed in children carrying the T allele ( $\beta = -1.3e^{-4}$  ( $-0.0022, 0.0024$ ),  $p = 0.93$ ).

**Conclusion:** Calcium and dairy were strongly associated with the nutritional status and blood pressure. The identification of differential effects of calcium/dairy consumption on the nutritional status according to genetics may contribute to the personalization of future nutritional advice.

**Trial registration:** This study is registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) NCT03419520.

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**Abbreviations:** WHO, World Health Organization; BMI, Body Mass Index; BDNF, Brain Derived Neurotropic Factor; BDNF-AS, Brain Derived Neurotropic Factor-Antisense; SNP, Single Nucleotide Polymorphism; RDIs, Recommended Daily Intakes; FM, Fat Mass; IOTF, International Obesity Task Force.

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## 1. Introduction

The prevalence of childhood overweight and obesity has raised over the last decades and it is still one of the main health objectives established by the 2013 World Health Assembly [1].

According to last data from the World Health Organization (WHO), overweight children and adolescents between 5 and 19 years old rose from 4% in 1975 to 18% in 2016, while obesity rates increased from 1% to 14% [2]. In Spain, like other high-income countries, Body Mass Index (BMI) seems to be stabilized; however, the overweight prevalence is still very high [1,3]. Specifically, according to the ALADINO, a study carried out by the Spanish

Agency for Consumer Affairs, Food Safety and Nutrition, the prevalence of childhood overweight in Spain is 23.2% (22.4% boys, 23.9% girls), while 18.1% is the prevalence of obesity (20.4% boys, 15.8% girls) [4]. These numbers make Spain one of the European countries with the highest obesity rates [5].

In this way, it has been observed that the dietary habits of Spanish children have worsened during the last decades, approaching the dietary patterns of Westernized countries, by abusing of poor quality foods with high caloric value and moving away from the Mediterranean diet [6]. Several studies have shown that the recommended food intakes are not achieved, which might lead to bad consequences on children's health, affecting their growth, mental health and their short and long-term wellbeing [7].

Calcium is an essential mineral at all stages of life, as it contributes to bone health. At the same time, together with the dairy products consumption, it has been associated with other beneficial functions for health, such as prevention of excess body weight or abdominal fat, inflammation, blood pressure, cardiovascular disease or type 2 diabetes [8–11]. For this reason, its appropriate consumption may have a clear impact on health status as well as on disease prevention.

Although calcium requirements will depend on the child's physiological and developmental states [12], there are not many studies that evaluate these mineral intakes in Spanish children, although the latest data reflect that it is insufficient [13,14].

On the other hand, it has been lately suggested that genetics may modulate responses to dietetic factors, which may contribute to more personalized recommendations with respect to nutrient requirements and to reduce the risk and prevalence of nutritional diseases [15,16]. The *Brain Derived Neurotrophic Factor (BDNF)* gene has been widely studied because of its high expression in the brain and its effects on synapses [17]. At the same time, it has been associated with eating disorders and obesity [18], acting as the main regulator of energy homeostasis [19]. Besides, its expression in other tissues has also been studied, such as in bones where it has been reported to participate in the development of cartilage [20], in ossification and in bone remodeling [21]. Moreover, the *BDNF antisense RNA (BDNF-AS)* gene, despite sharing 7 exons-introns with *BDNF* [22], has been less studied. However, it has been proposed as a possible regulator of the *BDNF* expression as it might directly inhibit the transcription and/or regulation of the neurotrophin *BDNF* by the superposition of its antisense RNA transcripts to the transcribed RNA of the *BDNF* gene [23]. The genetic variant rs925946 of the *BDNF-AS* gene is a single nucleotide polymorphism (SNP) associated with a greater weight gain and a fast growth during early childhood [24], as well as with the metabolic syndrome and with a low dietary calcium intake during adolescence [25].

Based on these data, the objective of the present study was to evaluate the possible association between calcium/dairy products intake and body composition and blood pressure parameters in a group of schoolchildren from Madrid. At the same time, it was also evaluated whether that association was regulated by the rs925946 polymorphism of the *BDNF-AS* gene. This work forms part of *GENYAL study to childhood obesity prevention*.

## 2. Material and methods

### 2.1. Study population

This work forms part of *GENYAL study to childhood obesity prevention*. The main objective of this study is to design and validate a predictive model that identifies those children who would benefit most from actions aimed at reducing the risk of obesity and its complications, considering both environmental and genetic

factors. Given the large number of endpoints and associations analyzed, and the absence of initial guesses for the variability for many of them, it was not possible a rigorous and univocal estimation of the sample size. We therefore decided to use the largest sample possible given the available resources. It is expected that the data in this initial phase will provide estimates for the variability that will allow sample size calculations for later phases of the study.

From this first phase, 221 schoolchildren (116 girls and 105 boys) in 1st and 2nd grades of primary school were included. The Ministry of Education of this Community was responsible for the selection of 6 different public schools among the Community of Madrid by considering the number of students in each center and the socioeconomic status of the different districts, so that the selection was representative of the average household income defined by the Spanish National Statistics Institute [26]. Thus, the selected schools were placed in 5 different districts of Madrid. Every School Board showed their interest and consent to participate in the study.

The duration of the project is planned to last for 5 years (2017–2021) with annual data collection, including anthropometric and nutrigenetic assessment, blood pressure measurements, and questionnaires about physical activity, dietary and socio-sanitary aspects. The initial data collection was carried out during March 2017 and the results of the assessment are analyzed here.

### 2.2. Ethical statement

This research was approved by the Research Ethics Committee of the IMDEA Food Foundation (PI:IM024) and has been registered as a clinical study in *ClinicalTrials.gov* (NCT03419520).

All families gave their written informed consent to participate. The study protocol follows the guidelines laid down in the Declaration of Helsinki.

### 2.3. Assessment of calcium and dairy products' intake

In order to calculate the schoolchildren's calcium and dairy intakes, their families had to fill out a 48-h food record of nonconsecutive days (a working day and a weekend day) as recommended by the European Food Safety Authority (EFSA). To facilitate this task, families were given some guidelines to convert homemade measurements (spoons, cups, etc.) into grams of food if they could not weigh the food.

Thereafter, children's dietary records were analyzed using the *DIAL* software version 3.5.0.3 (Alce Ingeniería, Madrid, Spain) [27]. This program uses validated dietary guidelines for the Spanish population, so that the children's adherence to the calcium Recommended Daily Intakes (RDIs) estimated in 800 mg/day [28] was calculated. Dairy portions considered by the program included: milk, yogurt, cheese, commercial milk shakes and other preparations containing milk such as flans or ice creams [29].

### 2.4. Anthropometric and blood pressure measurements

Children were assessed at their schools early in the morning by trained nutritionists following standard techniques and the international WHO guidelines specific for this population [30]. Measurements were taken twice in a row, considering the average as the final result.

Height was determined using a Leicester height rod with a millimetric accuracy (Biological Medical Technology SL, Barcelona, Spain). Body weight and fat mass (FM) percentage were assessed using a Body Composition Monitor (BF511- OMRON HEALTHCARE Co., Ltd, Kyoto, Japan). BMI was calculated as body weight (kg)/

height (m)<sup>2</sup> and classified in accordance with the International Obesity Task Force (IOTF) percentiles [31].

To perform blood pressure measurement, an automatic digital monitor was used (OMRON M3-Intellisense, The Netherlands) with a cuff suitable for children. It was measured with the children relaxed and sat up straight, with their back against the chair, their feet flat on the floor and their legs or ankles uncrossed. It was controlled that their arm was resting on a firm surface with the cubital fossa at the level of the heart and the palm of the hand upwards. The measurements were taken before the recess to ensure a sufficient resting period prior to testing (at least 30 min). The blood pressure was evaluated twice, with a brief break between readings. The average of both was considered as the final data. Results were classified according to the percentiles established by the Spanish Association of Pediatrics [32].

### 2.5. Genotyping of the variant rs925946 of the BDNF gene

DNA was obtained from saliva samples collected the same day of the anthropometric evaluation. For this purpose, it was used a sterile swab free of human RNAses, DNAses and DNA (300263DNA-Hisopos Deltalab polystyrene and polyester). Children had to have their mouth clean and avoid eating or drinking 30 min prior to collection. Three samples were taken per children. As samples were collected, they were directly conserved in refrigeration until all the children were evaluated. Immediately after, they were frozen at  $-80^{\circ}\text{C}$  until their processing. Genomic DNA was extracted from the buccal swabs using the INVISORB® SPIN TISSUE MINI KIT (Strattec), according to the manufacturer's instructions. Samples were lysed in the presence of proteinase K and a specific lysis buffer. The lysate was then purified and finally it was eluted in a free EDTA solution. For genotyping, the DNA samples were loaded in TaqMan® OpenArray® Real-Time PCR plates (Life Technologies Inc., Carlsbad, CA) already configured with the specific selected SNPs with specific waves for each allele marked with a different fluorophore to determine de genotype. This process was made using the OpenArray® AccuFill™ System (Life Technologies Inc., Carlsbad, CA). Once it was charged, a PCR was made and the chips were read in the QuantStudio® 12K Flex Real-Time PCR Instrument (Life Technologies Inc., Carlsbad, CA). The results were analyzed using the TaqMan® Genotyper software (Life Technologies Inc., Carlsbad, CA), which assigns automatically the genotype to each sample according to the amount of detected signal for each fluorophore. Data analysis was made by TaqMan Genotyper Software v1.3 (autocaller confidence level > 90%).

### 2.6. Statistical analysis

The software R version 3.4 ([www.r-project.org](http://www.r-project.org)) was used for statistical analyses. Qualitative data were presented as percentages and absolute frequencies while quantitative data were expressed as mean, median, standard deviation, minimum, maximum and interquartile range.

Moreover, the associations between the anthropometric and dietary variables, as well as the interaction with the rs925946 polymorphism, were established by logistic and linear models adjusted for sex and age. Multiple comparisons were adjusted by Bonferroni and by the total 20 dietary variables included in the study. The association between calcium intake and blood pressure was also adjusted for BMI values. Finally, a dominant model was used for the interaction analysis between rs925946 and calcium intake versus BMI.

All statistical tests were considered bilateral and  $p < 0.05$  as significant results.

## 3. Results

Among the total of 221 schoolchildren whose parents accepted to participate in the study, 52.50% were girls ( $n = 116$ ) and 47.50% boys ( $n = 105$ ). Participants were between 6 and 9 years old, although the 83% were presented 6–7 years of age. According to the percentile classification of the IOTF, 69.60% were normal weight, 25.40% presented a ponderal overload (16.30% overweight, 9.10% obese) and finally, 5.00% were underweight. No significant differences were found between sexes ( $p = 0.773$ ). With respect to the FM percentage obtained by bioelectrical impedance, 26.10% of the participants presented high results according to their sex and age.

With regard to the blood pressure assessment, 14.90% of the schoolchildren presented higher values than the recommended for their sex and age, so that they were categorized as normal-high blood pressure and hypertensive children.

Among the 221 schoolchildren enrolled in the study, a total of 201 fulfilled the nutritional questionnaires, 93 girls and 108 boys. Calcium daily average intakes, percentage of adherence to the RDIs and number of dairy portions are shown in Table 1. No significant differences were found in the analysis stratified by sex.

Table 2 shows the mean values of calcium intake, percentage of adherence to the RDIs and the number of dairy portions according to the IOTF nutritional status' categories. There it can be observed how the calcium and dairy portions gradually decrease as the classification rises from normal weight to obesity.

An inverse significant association was observed between the schoolchildren's BMI and the mg of calcium intake/day ( $\beta = -0.002$  ( $-0.003, -0.0003$ )  $p = 0.019$ ) (data adjusted for sex and age) (Fig. 1). This same association was identified with the adherence to the calcium RDIs ( $\beta = -0.015$  ( $-0.028, -0.0030$ )  $p = 0.019$ ) (data adjusted for age and sex). When adjusting these by the 20 nutritional variables the significant association was lost ( $p = 0.402$  in both cases).

When analyzing the association between the schoolchildren's calcium intake (mg) and the FM percentage analyzed by bioelectrical impedance, no significant association was found, but a trend towards significance was observed ( $\beta = -0.004$  ( $-0.009, 0.001$ ),  $p = 0.060$ ).

Moreover, when the sample of schoolchildren was divided into normal weight and ponderal overload (overweight + obesity), the number of dairy servings per day showed a protective effect against overload (OR = 0.479 (0.293, 0.753),  $p = 0.0014$ ) (data adjusted for sex and age) (Fig. 2). This effect remained significant after adjusting for the 20 nutritional variables analyzed in the study and maintained a trend towards significance after multiple comparison analysis that included a total of 50 variables from different categories included in the study (nutritional, anthropometric, socio-sanitary, etc.) ( $p = 0.056$ ).

Consumption of magnesium, phosphorus, the phosphorus/calcium ratio and D vitamin per day, according to the nutritional status was evaluated and no significant differences were found according to nutritional status (Supplementary Tables 1 and 2).

Regarding the schoolchildren's blood pressure, significant differences were also observed in the regression analyses adjusted for sex, age and BMI. Precisely, systolic (SBP) and diastolic (DBP) blood pressures were inversely associated with the calcium daily intake ( $\beta = -0.006$  ( $-0.011, -3e^{-4}$ ),  $p = 0.040$ ; and  $\beta = -0.005$  ( $-0.009, -0.001$ ),  $p = 0.010$ , respectively) and the percentage of adherence to the calcium RDIs ( $\beta = -0.045$  ( $-0.087, -0.003$ ),  $p = 0.040$ ;  $\gamma\beta = -0.039$  ( $-0.070, -0.008$ ),  $p = 0.010$ ; respectively). The stratified diagnosis by sex and age of the blood pressure was inversely associated with the percentage of adherence to the calcium RDIs (OR = 0.981 (0.964, 0.997),  $p = 0.020$ ).

**Table 1**  
Consumption of calcium and dairy portions per day. Differences between sexes.

	Total (n = 201)	Boys (n = 108)	Girls (n = 93)	p
Calcium (mg)	922.02 ± 220.22	932.96 ± 235.22	909.32 ± 201.93	0.444
Calcium RDI (%)	115.25 ± 27.53	116.62 ± 29.40	113.66 ± 25.24	0.444
Dairy portions/day	2.61 ± 0.80	2.63 ± 0.84	2.59 ± 0.76	0.851

RDI = Recommended Daily Intake.

**Table 2**  
Consumption of calcium and dairy portions per day, according to the IOTF nutritional status' classification.

	Underweight (n = 11)	Normal weight (n = 143)	Overweight (n = 31)	Obesity (n = 16)	p
Calcium (mg)	835.00 ± 217.67	954.00 ± 223.11	859.00 ± 195.91	811.00 ± 174.13	0.010
Calcium RDI (%)	104.37 ± 27.21	119.32 ± 27.89	107.48 ± 24.49	101.42 ± 21.77	0.010
Dairy portions/day	2.30 ± 0.70	2.74 ± 0.80	2.30 ± 0.76	2.26 ± 0.66	0.007

RDI = Recommended Daily Intake.

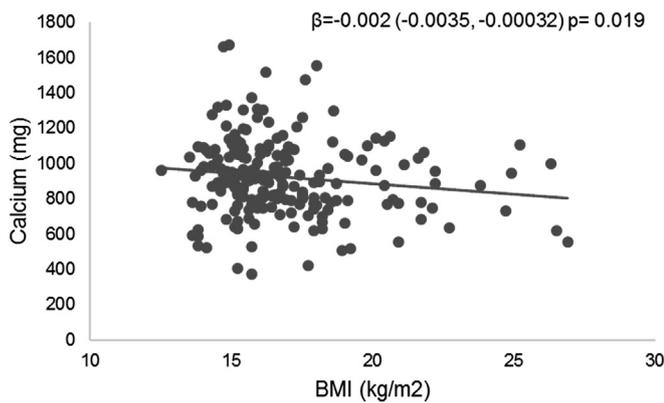
The distribution by genotypes according to the genetic variant rs925946 showed that 55.91% of the participants were GG common homozygous (n = 123), 36.82% heterozygous (n = 81), and 7.27% TT homozygous (n = 16). No significant differences were observed regarding the BMI between both genotypes (BMI (GG) = 16.97 ± 0.47 kg/m<sup>2</sup>, and (GT + TT) = 17.00 ± 0.540 kg/m<sup>2</sup>).

An interaction very close to the significance (p = 0.054) was observed between the genetic variant and the BMI. Indeed, GG common homozygous reduced on average 0.34 kg/m<sup>2</sup> the BMI for each 100 mg of calcium (β = -0.003 (-0.006, -0.001), p = 0.004).

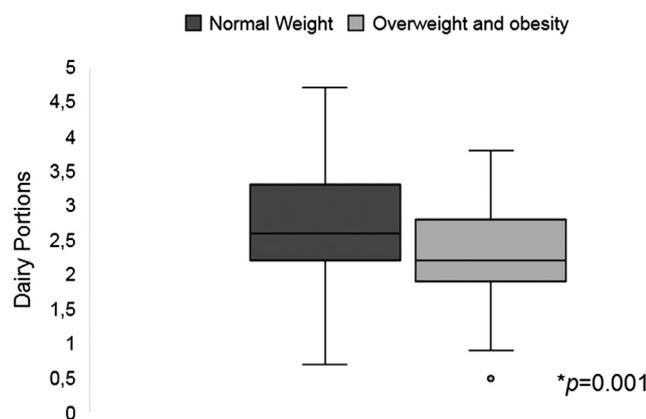
However, this effect was not observed in T allele carriers, where the consumption of 100 mg of calcium was not associated with the BMI (β = -1.3e<sup>-4</sup> (-0.0022, 0.0024), p = 0.93) (Fig. 3).

**4. Discussion**

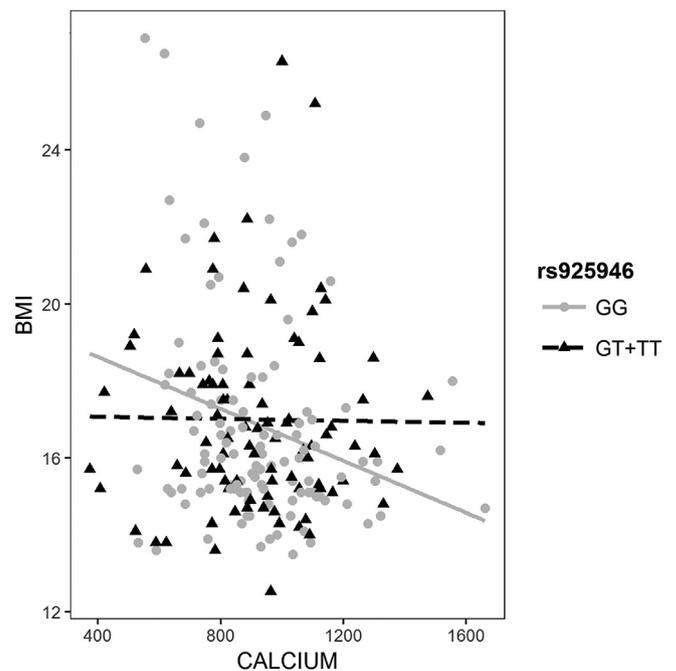
The high prevalence of childhood overweight and obesity, as well as the associated comorbidities, mean that it is necessary to find personalized nutritional recommendations to provide new solutions to this global problem. Considering the importance of calcium during the school age and the implications that this nutrient seems to have on the nutritional status, the present study aimed to determine whether the intake of calcium/dairy products and the presence of the rs925946 genotype of the *BDNF-AS* gene could influence the nutritional status in a group of schoolchildren from the Community of Madrid enrolled in the *GENYAL study to childhood obesity prevention*.



**Fig. 1.** Association between schoolchildren's BMI and calcium consumed per day.



**Fig. 2.** Differences between the schoolchildren's nutritional status and the number of dairy portions per day.



**Fig. 3.** Interaction between rs925946 genotypes and the consumption of calcium (mg) and BMI (kg/m<sup>2</sup>). Carriers of the GG genotype showed an inverse significant association between calcium consumption and BMI (β = -0.003 (-0.006, -0.001), p = 0.004), which was not observed in T allele carriers (β = -1.3e<sup>-4</sup> (-0.0022, 0.0024), p = 0.93).

Regarding the body composition, one out of four of the children evaluated presented overweight or obesity according to the international reference standards [31]. These numbers are similar to those reported in other national studies [4,33].

The average calcium intake was  $922.02 \pm 220.22$  mg/day, although 28.86% of the children did not cover the daily recommendation of 800 mg/day [28]. Similarly, 21.40% consumed less than 2 portions of dairy products per day (despite the broad definition of this group: milk, yogurt, cheese, commercial milk shakes, and other products containing milk, such as flan or ice creams). This intake is far from the childhood recommendation of 2–3 portions/day of milk products [34]. These findings are similar to the results of other cohorts including Spanish children [13,35] or children from other countries (37).

Moreover, the intakes of dairy products are generally higher in boys than in girls, as has also been reflected in this study, although without reaching statistical significance [36]. However, it should be noted that controversies exist regarding the cut-off point used for the calcium intake [13]. Thus, there are some authors that use the Recommended Dietary Allowances defined by the Institute of Medicine [37], which establishes daily 1000 mg for children aged 4–8 years.

In the present study it was observed that overweight children presented a lower consumption of calcium than those presenting a normal weight. Besides, the higher the BMI (normal weight, overweight, obesity), the lower the calcium intake. These results may be related to different functions associated with calcium and body weight control. Firstly, an adequate intake of calcium has been associated with a decrease in parathyroid hormone and 1,25-dihydroxycholecalciferol, thus decreasing the concentration of intracellular calcium that promotes lipolysis [38]. Secondly, it has been observed that the adequate intake of calcium produces an increase in the fecal excretion of fatty acids by the formation of insoluble soaps of long-chain fatty acids plus calcium in the intestine, reducing their solubility and therefore, their absorption [39]. Finally, other studies have focused on the role of calcium in the regulation of appetite [40].

In the present study it has also been observed a highly significant association between the number of dairy portions consumed and the children's BMI, as the analysis admitted multiple-test adjustment for more than 50 variables associated with dietary, anthropometric and socio-sanitary factors included in the study. The consumption of dairy products as a source not only of calcium but of other nutrients and bioactive compounds, has already been studied for its association with the body weight. Previous research has been focused on the effects of the conjugated linoleic acid on body weight [41] and on the effects of branched-chain amino acids (valine, leucine, isoleucine) since they have an anabolic function through the insulin-like growth factor system and act within the signaling pathway of the rapamycin 1 complex [42]. Likewise, peptides present in dairy products have been also studied due to their involvement in the appetite control [43]. Besides, it has also been reported that schoolchildren who consume 3 dairy portions/day have a more adequate overall diet and a better score in the questionnaires of adherence to the Mediterranean diet pattern [14] and, on the contrary, lower dairy intakes are associated with a rise in the caloric consumption [44]. However, these associations were not identified in the present study.

The inverse relationship between calcium/dairy consumption and the body weight status has been reported in numerous investigations carried out both in adults and in the infant population [45–48], but with controversial results. In a study on 548 Brazilian children it was observed that milk and dairy products consumption was strongly correlated with the risk of excess body weight [49]. However, in a meta-analysis of 29 clinical trials that evaluated the

effects of an increased dairy intake on the nutritional status of adult volunteers, a significant reduction in fat mass was shown, but only in short-term studies (with a duration of less than one year) and with a caloric restriction intervention [50], whereas in an intervention study with children and adolescents aged 8 to 16, the increase of dairy products intake was not associated with any adiposity measures [51].

Furthermore, this study showed a statistically significant association between calcium intake and the blood pressure of schoolchildren. This relation has already been reported in other studies in adults [46,52,53] and children [9,54]. Hence, the Nutrition and Dietetics Academy suggests that adults with hypertension consume adequate amounts of dietary calcium to control blood pressure levels, as well as the supplementation if the recommended amounts cannot be achieved through diet [55]. Calcium may influence blood pressure as a hormone regulator and as a controller of the sympathetic nervous system and electrolyte interactions. Besides, it has been shown to suppress the renin activity, which is responsible for the arterioles constriction [51].

Moreover, a different behavior depending on the rs925946 allele was observed in the association between the calcium consumed by the schoolchildren and their BMI. This SNP is located in an intronic region of non-coding ARN, in which there is a substitution of a G allele for a T allele [56]. This genetic variant has already been reported in a recent meta-analysis as a determinant of obesity in adults [56], as well as in studies with children and adolescents [24,25,57]. Its mechanism of action is not well known, however, it has been postulated that by acting as an antisense transcript, it might be implicated in the regulation of the *BDNF* gene [23], a key gene in the energy homeostasis [58]. Regarding its relationship with calcium consumption, the existing data in the literature are very scarce. Dušátková L et al. observed that the rs925946 allele carriers presented a lower calcium intake; however, they did not study if there could be any relationship with the children nutritional status [25]. Given that this gene plays a vital role in the control of the energy balance by promoting satiety and energy expenditure [19,59], the presence of the SNP may cause any of these mechanisms to fail, conditioning the effect of calcium on satiety or on other mechanisms involved in body weight control [40,60]. To our knowledge, this is the first time that the association between this genotype and calcium intake as potential modulators of the nutritional status has been described, which opens the avenue for future investigations in this area. Nevertheless, these results are close to the significance and should be confirmed in future prospective studies with a larger sample size.

A response to calcium influenced by the presence of certain SNPs, such as the presented in this study, or by other genetic factors, may explain the controversial results obtained to date on the nutritional status according to calcium and dairy consumption [45–51].

One of the weakness points of this study is the sample size used, being necessary to include new schools to expand the number of children included in it. However, as it was commented previously, from this first phase of the study we expect to calculate the sample size needed to increase the statistical power to find strong associations between the studied variables.

Another limitation was the use of dietary questionnaires to estimate calcium and food intake. Nevertheless, in the absence of better tools with low cost and high throughput to estimate the intake, consumption record can offer valuable information, although it should be interpreted with caution. Finally, the sunlight exposure was not assessed in this study despite the relevance of this aspect on calcium absorption.

Regarding the gene-diet interaction, it is important to consider that a single SNP is unlikely to be the unique factor in complex

conditions like obesity and gene-diet interactions, and many other genetic and environmental factors are likely to contribute to the phenotype.

## 5. Conclusion

The results presented in this study show the children vulnerability from the nutritional point of view, where the dietary pattern plays a fundamental role in the children's health, being reflected in their body weight and blood pressure. Given the high prevalence of overweight and obesity in children in the studied population and the need to search for tools that help in its prevention, consumption of calcium and the adherence to the daily requirements become of high importance and may be a key prevention tool. The consumption of milk and dairy products during infancy makes the schoolchildren's diet more complete and helps to comply with the recommendations of calcium intake. Thus, a proper promotion of the consumption of this type of products as well as the design of controlled long-term studies to assess their effects on child's health become necessary. Likewise, the results associated with the rs925946 genotype should be further studied to confirm their availability as may contribute to the personalization of future nutritional advice.

## Statement of authorship

The authors' responsibilities were as follows: VLK was the principal investigator and was responsible for the study design. HMP made the interpretation of results and drafting the manuscript; HMP, EAA, RI and IES were responsible for data collection; GC conducted statistical analysis of the data; SM contributed to genetic samples management; GRR and ARM supervised the final compilation of the manuscript and provided scientific advice and consultation.

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## Conflict of interest

None of the authors report a personal conflict of interest. Helena Marcos-Pasero has no conflict of interest. Elena Aguilar-Aguilar has no conflict of interest. Rocío de la Iglesia has no conflict of interest. Isabel Espinosa-Salinas has no conflict of interest. Mónica Gómez-Patiño has no conflict of interest. Gonzalo Colmenarejo has no conflict of interest. Ana Ramírez De Molina has no conflict of interest. Guillermo Reglero has no conflict of interest. Viviana Loria-Kohen has no conflict of interest.

## CRediT authorship contribution statement

**Helena Marcos-Pasero:** Writing - original draft, Investigation. **Elena Aguilar-Aguilar:** Investigation, Writing - review & editing. **Rocío de la Iglesia:** Investigation. **Isabel Espinosa-Salinas:** Investigation. **Mónica Gómez-Patiño:** Investigation. **Gonzalo Colmenarejo:** Formal analysis, Writing - review & editing. **Ana Ramírez de Molina:** Supervision. **Guillermo Reglero:** Supervision. **Viviana Loria-Kohen:** Conceptualization, Methodology, Project administration, Supervision, Writing - review & editing.

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## Appendix A. Supplementary data

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