



Contents lists available at [ScienceDirect](https://www.sciencedirect.com)  
**Clinical Nutrition Open Science**

journal homepage:  
[www.clinicalnutritionopenscience.com](http://www.clinicalnutritionopenscience.com)



## Original Article

# Effects of a high-protein diet and calcium caseinate supplementation on satiety perception and weight in Children with overweight and obesity: a randomized clinical trial

Fernanda Sarahí Fajardo-Espinoza <sup>a</sup>, Karolina Alvarez-Altamirano <sup>b</sup>,  
Alma Nubia Mendoza-Hernandez <sup>c</sup>, Nayeli Garibay Nieto <sup>b</sup>,  
Thamara Falcón-Álvarez <sup>d</sup>, Mónica Bejarano Rosales <sup>b</sup>,  
Anahí Raya-Hernández <sup>d</sup>, Rubens Feferbaum <sup>e</sup>, Vanessa Fuchs-Tarlovsky <sup>b,\*</sup>

<sup>a</sup> Faculty of Health Sciences, Universidad Anáhuac México, Mexico

<sup>b</sup> Clinical Nutrition Ward, Hospital General de México, Dr. Eduardo Liceaga, Mexico

<sup>c</sup> Centro de Investigación en Ciencias de Información Geoespacial, Leon Guanajuato, Mexico

<sup>d</sup> Universidad Iberoamericana, Mexico

<sup>e</sup> Instituto da Criança. Faculdade de Medicina da Universidade de São Paulo, Mexico

## ARTICLE INFO

### Article history:

Received 17 April 2023

Accepted 22 October 2023

Available online 2 November 2023

### Keywords:

High-protein diet

Calcium caseinate

Children

Obesity

Satiety

Body composition

## SUMMARY

**Background & Aims:** Overweight and obesity in children are a public health problem worldwide. There are few studies about nutritional interventions with different diet macronutrient distribution and their potential application in regulating food consumption in this age group. This study aimed to assess the impact of a high protein diet over satiety, energy intake, and body composition in children diagnosed with overweight (Ov) or obesity (Ob).

**Methods:** School-age children (6–10 years) diagnosed Ov or Ob were randomized to receive three different dietary interventions for two months: standard diet (SD), high protein (HP) diet, and HP diet with calcium caseinate (HPC); BMI (Z-score and percentiles), body composition, indirect calorimetry (REE<sup>IC</sup>), energy intake (24-h recall questionnaire) and satiety (visual analog scale) were evaluated before and two-months after intervention.

\* Corresponding author. Clinical Nutrition Ward, Hospital General de México "Dr. Eduardo Liceaga" Dr. Balmis No. 148, Col. Doctores, Alcaldía Cuauhtémoc, CP: 06720, Ciudad de México, Mexico. Tel.: +55 5578 0505.

E-mail address: [vanessafuchstarlovsky@gmail.com](mailto:vanessafuchstarlovsky@gmail.com) (V. Fuchs-Tarlovsky).

**Results:** Sixty-four children diagnosed with overweight (12.5%) and obesity (87.5%) were evaluated. There were no changes in the perception of satiety between groups ( $p>0.05$ ). There was a tendency for a reduction in waist circumference in the HPC group after treatment ( $p=0.05$ ). There were no significant differences in body composition, and children's growth was homogeneous between groups. However, intragroup Z-score values for BMI were significantly reduced in all groups ( $p<0.05$ ).

**Conclusions:** Calcium caseinate as a part of a high protein diet is secure in a short time, but children's perception of satiety did not change between groups. All interventions showed to have a reduction in BMI z-core. More clinical studies evaluating protein consumption and molecular indicators of satiety are needed.

© 2023 The Authors. Published by Elsevier Ltd on behalf of European Society for Clinical Nutrition and Metabolism. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## Introduction

Overweight and obesity continue to be health problems worldwide in the pediatric population. In Mexico, the national combined prevalence of overweight and obesity in children between 5 and 11 was reported as 34.4% in 2012. However, there has been an increase of up to 37.4% for 2021 [1], and it is estimated that around 80% of children with obesity will have this condition during adulthood. [2] Due to this, there is a growing concern in the development of effective and safe nutritional treatments in this age group. [3].

Obesity refers to an abnormal or excessive fat accumulation that has been linked to metabolic complications such as diabetes, hypertension, dyslipidemia, metabolic syndrome, and cardiovascular disease from childhood to adulthood. [4–6]. There is a positive relation between these metabolic conditions and anthropometric parameters such as high body mass index (BMI), waist circumference (WC), and waist-to-height ratio (WtHR) in childhood. [7,8].

Dietary interventions focused on macronutrient diet distribution represent a key area to treat obesity, modifying metabolic response and satiety perception combined with exercise. Studies on children and adolescents have proposed that increasing protein intake percentage in daily diet is a safe and effective treatment for children diagnosed with obesity and overweight to improve fat-free mass and metabolic expenditure [9–11]. Additionally, the evidence in this population suggests an improvement in insulin resistance, C-reactive protein, lipid profile, and waist circumference related to higher protein diets [12,13]. Despite this, there is a lack of studies developed in children evaluating this topic.

According to the Food and Nutrition Board of the US Institute of Medicine, protein intake represents 10–15% of the total energy intake recommendations or 19–34 g/day. Considering 10–30 % is an acceptable range for 4- to 18-year-old patients [14]. Diets rich in protein provide between 20 and 30% of the total energy requirement, in contrast to international recommendations that consider a protein intake of 0.7–1 g/kg of body weight in this population [14].

Dairy protein-rich foods or supplements have also been studied for their biological importance in the growth and development of children [15,16]. Casein or Calcium caseinate is the main protein in milk. It has shown improved satiety perception due to prolonged gastric emptying compared with whey protein in children [17]. Milk consumption and its components are related to linear growth stimulation in children's height, which can be mediated by insulin-like growth factor-I synthesis and insulin secretion [18]. Also, dairy products are considered a good protein source for the adult

population, with potential application in obesity nutritional treatment and satiety, and it has been associated that whey protein intake promotes thermogenesis [19,20].

According to the strategies for treating obesity in children, the aim of this study was to assess the effect of three dietary interventions (high-protein diet (HP), calcium caseinate supplementation as a part of a high-protein diet (HPC), and standard macronutrient distribution diet (SD)) on satiety, caloric intake, and anthropometric variables and body composition (BC).

## Material and methods

### *Sample characteristics*

A randomized clinical trial was conducted in children from the pediatric obesity clinic in a tertiary referral hospital. Patients included in the study were boys and girls between 6 and 10 years with the diagnosis of overweight (Ov) or obesity (Ob) according to CDC (Centers for Disease Control and Prevention) criteria (BMI-for-age  $\geq$ 85th percentile for Ov and BMI-for-age  $\geq$ 95th percentile for Ob) in the absence of pharmacological treatment, and the agreement to participate in the study with the approval of their parents using an informed consent form according to the guidelines of international good clinical practice standards.

All children and their families received physical activity, diet, and other behavioral workshops during the study as a part of standard care. This study was approved by the local ethics and clinical research committees of the “Hospital General de México Dr. Eduardo Liceaga,” with the number ID/13/111/4/18.

### *Diet intervention*

The patients were randomized into three groups: High protein diet (HP), High protein diet with Calcium caseinate supplementation (HPC), and Standard diet distribution (SD), and were followed every two weeks for two months. A stratified randomized recruitment in blocks for sex was used to have the same proportion of boys and girls due to the age-related changes in growth. The macronutrient energy distribution of protein (Pr), carbohydrates (CRB), and lipids (Lp) percentages of each diet was Pr-25%, CRB-25%, Lp-50% for HP group and HPC groups and Pr-15%, CRB-30%, Lp-55% for SD group. The HPC group replaced part of the food with a daily sachet of a supplement containing calcium caseinate as a part of the macronutrient distribution diet for this group. Each sachet of calcium caseinate dried powder with vanilla flavor contained 60 kcal, 14 g of protein, 0.22 g of fat, and 1.5 g of carbohydrates (The supplement used was provided by Evolution Nutraceutical Company®). For compliance with supplementation, each responsible parent received 14 sachets each two weeks for two months, and all of them returned each empty envelope of calcium caseinate supplement.

Energy prescription for all populations was according to the WHO formula that considered age, sex, and weight. [21] The full-day meal plan was distributed in five meals (breakfast, morning snack, lunch, afternoon snack, and dinner). To reduce methodological bias, the same dietitian evaluated each patient and gave nutritional counseling about healthy food.

### *Measurements*

#### *Indirect calorimetry*

Resting energy expenditure was assessed before and after diet interventions with a KORR® REEVUE Model 94690 calorimeter, considering an 8-hour period of fasting previously. For the test, all subjects used a facemask for ten minutes in a sitting position in the same room with the calorimeter calibrated to the temperature and humidity conditions for 15 minutes before the test. This data was used to analyze metabolic changes ( $REE^{IC}$ ) according to intervention groups.

#### *Anthropometry*

Anthropometric data included weight (SECA 750 scale), height (SECA 203 stadiometer), BMI, waist, and hip and abdominal circumferences. Measurements were taken standing with feet together; for the

waist circumference, the mid-point between the lower edge of the last rib and the upper edge of the iliac crest was considered; for abdominal circumference, the metric tape was placed at the level of the navel after the child is completing an unforced expiration; hip circumference was measured at the level of the gluteal prominence. All measurements were registered before interventions and after two months.

BMI z-scores and BMI percentiles were estimated according to the CDC Growth Charts and Ped-Tools calculator with age entered in months, respectively [21]. Cardiometabolic risk was determined by the waist-to-height ratio, defined as the waist circumference divided by the height in cm, with a cutoff point  $\geq 0.5$  as an indicator of central adiposity [5,22].

Body composition was assessed by Bioimpedance Analysis using the Quantum IV RJL systems®, after IC, with the participant laid down over a non-conductive examination table in a supine position. All data was analyzed with BC® software as a part of the equipment using the pediatric equation as the manual equipment indicated. [22].

#### *Satiety perception and adherence to the diet interventions*

Visual analog scales (VAS) are frequently used to assess satiety [10,23]. This study used a VAS, following the methodology of Krebs et al. [10]. This scale has ten levels, ranging from the number zero or “1” as “hungry, feeling weak, dizzy, or presenting other extremely uncomfortable hunger symptoms” to the number ten or “10” as “uncomfortably full or sick, full as in Thanksgiving or Christmas celebrations, completely satiated” (See complementary material). Satiety was evaluated before intervention and at an 8-week follow-up visit.

Adherence to the dietary plan was assessed through the 24-hour recall (R24H) method in order to register the food and beverages that were consumed in the 24 hours before every follow-up visit. Energy and macronutrient intake were estimated, and the diet was considered “adequate” if the calories and macronutrient intake were between 90–110% of the intake recommended according to the study groups; an intake below 90% or superior to 110% was considered “deficient” or “excessive”, respectively.

#### *Statistical analysis*

Statistical analyses were performed using SPSS Version 25 (IBM SPSS Statistics for Windows, 2017, Armonk, NY: IBM Corporation). The Kolmogorov-Smirnov test was used to determine the normality of variables ( $P > 0.05$ ). Variables without normality were expressed in median and interquartile ranges. The participants' baseline characteristics were tested using a one-way ANOVA and Pos Hoc using the Bonferroni test to compare the mean among groups. However, those variables without normality and homoscedasticity (Levene-Test) were analyzed using the Kruskal-Wallis test. For intragroup differences, the ANOVA test was calculated, and Wilcoxon signed the rank-test for nonparametric analysis. Furthermore, for all tests, p-values  $< 0.05$  were considered statistically significant.

## **Results**

### *Study group characteristics*

A total of Sixty-four school-age children were included and grouped according to the intervention treatment in this proportion: HP 34.4% (n=22), HPC 34.4% (n=22), SD 31.3% (n=20). Sixty-six patients were selected, but two patients in the SD group abandoned the follow-up because of the time to stay at the clinic. The proportion of patients with overweight and obesity was 12.5% (n=8) and 87.5% (n=56), respectively. Baseline characteristics of the total population and study group are shown in Table 1.

Energy expenditure in the total population at first nutritional assessment patients showed a  $REE^{IC}$  mean of  $1722.3 \pm 256$  kcal and  $42 \pm 6.6$  kcal adjusted per kilogram of weight (kcal/kg). After treatment,  $REE^{IC}$  was statistically reduced to 1560 kcal ( $p < 0.0001$ ), but this was not observed when adjusted by

**Table 1**  
Descriptive data of all participants before dietetic interventions

Characteristics	All groups	HP	HPC	SD	p-Values
Age in months	102±15	102 ±18	99±13	102±15	0.418
Age in years	8.5±1.2	8.5±1.5	8.2±1.1	8.8±1.1	0.418
Weight (kg)	41.7±7.4	42.8±8.3	38.8±6.1	43.6±7	0.077
Height (m)	1.3±0.1	1.33±0.1	1.31±0.1	1.36±0.1	0.074
BMI (kg/m <sup>2</sup> )	23.2±2.3	23.8±2.5	22.6±2.2	23.3±2.2	0.257
BMI Percentile	98 (88–100)	98.5 (89–99)	97 (88–100)	9700 (91–99)	0.182
BMI Z-score	1.98 ±0.37	2.06 ±0.4	1.95±0.35	1.94 ± 0.35	0.525
Waist Circumference (cm)	76.1±6.8	75.8±5.4	74.8±6.4	77.4±8.4	0.359
Abdominal circumference (cm)	80.2±7.1	81.4±7.8	77.6±6.4	81.7±6.7	0.116
Hip Circumference (cm)	79.1±6.6	80.1±7.1	77.2±6.7	80±5.8	0.283
Waist/Height	0.57 (0.43–0.75)	0.57 (0.5–0.68)	0.56 (0.45–0.71)	0.57 (0.43–0.75)	0.996 <sup>a</sup>

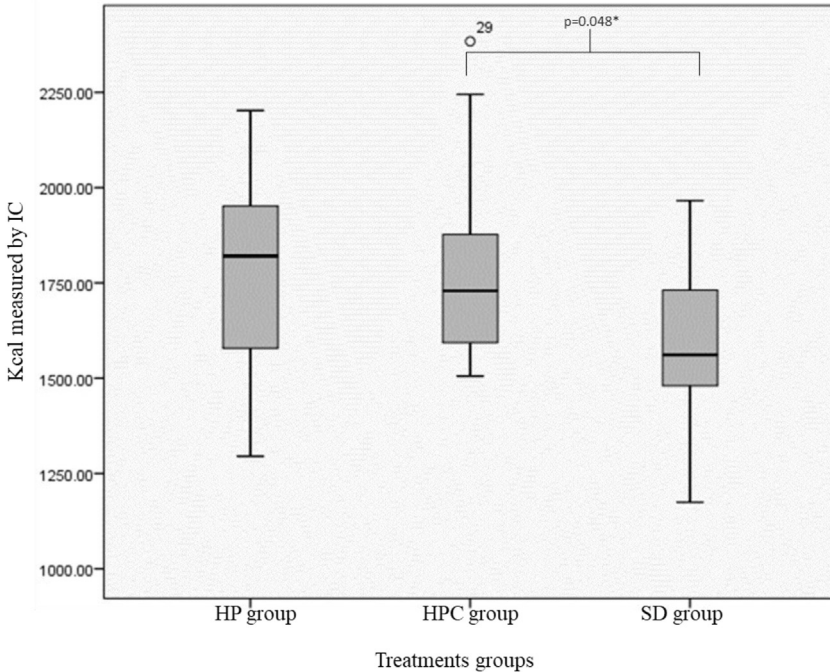
HP: High protein group, HPC: High protein plus caseinate group, SD: Standard diet group.  
p-values using One-way ANOVA test.

<sup>a</sup> p-values using Kruskal-Wallis for non-normal variables expressed in medians.

kg: 39 kcal (p=0.68). **Figure 1** showed that REE<sup>IC</sup> at the first visit, SD group had a lower REE<sup>IC</sup> compared to HP and HPC (p=0.003). After the intervention, the means of REE<sup>IC</sup> were homogenous (p=0.249): HP 1620±406 kcal, HPC 1613.1±364 kcal, SD 1436±417 kcal.

**Satiety**

VAS of satiety expressed in the median of the total population at the baseline assessment was 7 points (min 4; max 10) with no statistical change at the final measurement and all groups (**Figure 2**).



**Figure 1.** Distribution of REE means (kcal) by treatment group at baseline. HP: High protein group, HPC: High protein plus caseinate group, SD: Standard diet group. \*p-values for ANOVA and *Post Hoc* analysis using the Bonferroni test among groups. The means description for REE<sup>IC</sup> were HP:1770±278 kcal, HPC 1787±243 kcal, SD 1599±209 kcal.

Satiety was not different among groups before and after interventions ( $p=0.177$ ). SD group showed to have a hungry-satiety scale stable from the beginning, and calcium caseinate did not influence the HPC group to modify the VAS of satiety ( $p=0.614$ ).

According to the total sample, just one patient with an Ob diagnosis changed to an Ov diagnosis during the intervention (HPC group); however, there were no statistical differences after treatment. Cardiometabolic risk were presented in 89.1% after the study.

### *Anthropometry and body composition*

Changes in anthropometry measurements among groups are shown in [Table 2](#). All patients grew up approximately 1 cm during the intervention. Results showed that the waist circumference was statistically different between groups at the end of the intervention ( $p=0.05$ ), finding that the HPC group had a lower waist circumference. However, *Post Hoc* analysis showed no differences between groups but a tendency for a lower circumference in the HPC group ( $p=0.075$ ). Abdominal circumference had a critical reduction in the HPC and SD groups with no statistical difference between groups ( $p<0.05$ ). Even though groups of interventions had no statistical differences between groups, intergroup results showed a significative reduction of BMI z-score units ( $P<0.05$ ).

Considering body composition data, significant differences were observed between groups in Body Fat Percentage (BFP) and Lean Body Mass (LBM) after the intervention. However, in the pre-post analysis, only the SD group showed a significant BFP reduction ( $p<0.05$ ); HPC and HP groups did not show improvement in these parameters ([Table 2](#)).

### *Dietetic adherence*

The analysis of diet showed that energy intake adequacy (%) compared with recommendations for each group were HP: 87.2% (deficient), HPC: 82.66% (low), and SD: 109.12% (adequate) before intervention. All groups showed similar energy intake adequacy (%) reported by 24-hour recall after intervention but still below the defined requirements for each group ([Table 3](#)).

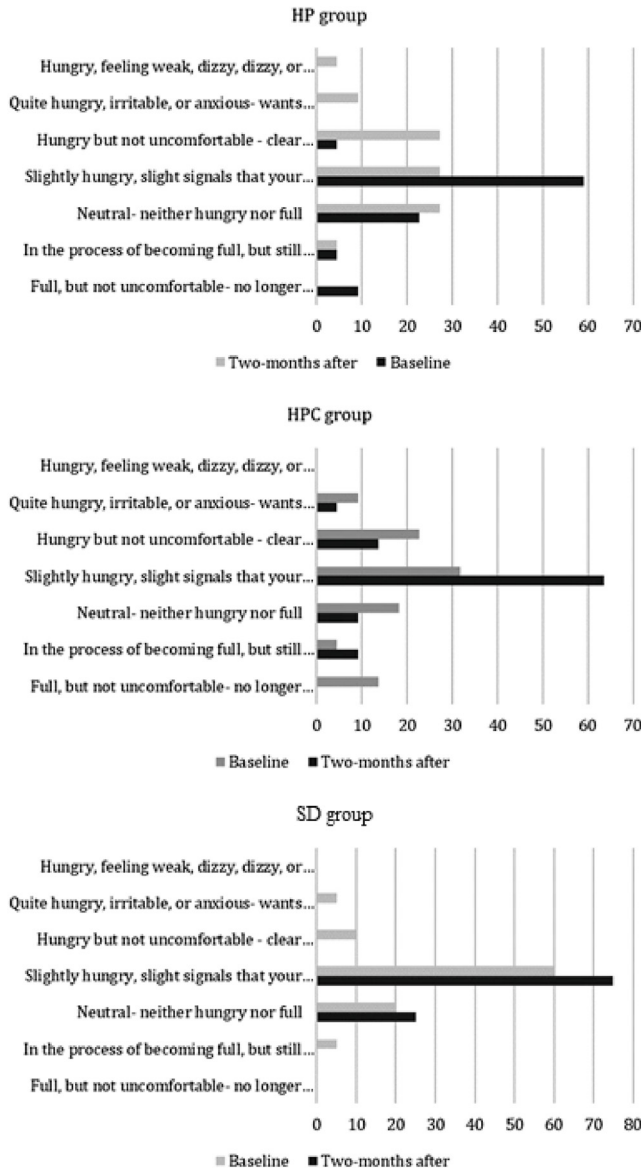
Protein intake was high compared to the standard requirement (~1g/kg weight per day) at baseline and final measures. Poor adherence to the dietetic prescription was observed in the SD and HP groups regarding protein intake. The SD group consumed excessive protein amounts, the HP group did not reach the prescribed quantity, and the HPC group showed a higher protein consumption than the baseline. Nevertheless, protein ingestion did not improve weight loss or reported satiety in any groups ( $p<0.05$ ).

## **Discussion**

### *Satiety*

It is known that the best practices for weight maintenance and loss in the pediatric population are based on a multicomponent intervention [[24,25](#)]. Despite this fact, lower satiety is a remarkable limitation to achieving a negative energy balance. One of our aims was to determine changes in satiety perception after three dietetic treatments in a pediatric population suffering from Ov or Ob. Our results showed that satiety perception had no change after diet intervention in our population. Furthermore, our results showed that VAS reported stable levels (4–10) of satiety throughout the treatment, including in the group of patients with calcium caseinate. We expected this supplement would improve satiety perception in the HPC group due to its digestibility, even though the SD group showed fewer variations in satiety perception from the beginning.

Considering milk or milk derivatives as a part of dairy products for a healthy diet in children, Mehrabani et al. demonstrated the effect of reduced-fat milk consumption as a part of breakfast on short-term satiety, limiting the total intake of an ad libitum buffet in children with obesity [[26](#)]. However, differences with our study, such as implementing a high-protein diet with calcium caseinate as part of breakfast or the need for measurement of food ingestion in this meal, lead to inconclusive results. Another study evaluated the effect of a high-protein–low carbohydrate diet versus a low-fat



**Figure 2.** Frequency (%) of the visual analog scales of Hunger/Satiety for intervention groups. HP: High protein group, HPC: High protein plus caseinate group, SD: Standard diet group.

diet for weight loss in adolescents. The authors found that satiety showed no changes in the pre-and postprandial analysis [10].

Some behavioral studies explain that satiety is more than a decision; it is an adaptation of central satiety and peripheral control hormone response. Children with excess body weight feel more rewarded for eating than lean children [27]. It has been reported that child appetite self-regulation is often linked to cognitive, emotional, biological, and behavioral factors. [28] A study developed on 45 boys and girls between 9–11 years old showed that after family-based behavioral treatment, children

**Table 2**  
Anthropometric changes in participants after diet interventions

Characteristics	HP group			HPC group			SD group			P -values
	Post-diet	Δ	<sup>b</sup> p-values Δ	Post-diet	Δ	<sup>b</sup> p-values Δ	Post-diet	Δ	<sup>b</sup> p-values Δ	
Weight (kg)	42.13± 8.01	-0.69	0.028	38.44± 6.04	-0.41	0.191	42.96± 7.11	-0.68	0.112	0.094
Height (m)	1.34± 0.09	0.01	0.000	1.31± 0.06	0.01	0.000	1.37± 0.07	0.01	0.000	0.057
BMI (kg/m <sup>2</sup> )	23.10± 2.13	-0.76	0.002	22.10± 2.25	-0.57	0.001	22.53± 2.25	-0.77	0.002	0.333
BMI Percentile	97.5 (87–99)		0.207	97 (79–99)		0.694	97 (85–99)		0.263	0.358 <sup>a</sup>
BMI z-score	1.96±0.39	-0.10	<0.0001	1.84 ±0.4	-0.12	0.001	1.80±0.4	-0.14	<0.0001	0.415
Waist Circumference (cm)	74.62± 6.4	-1.13	0.506	70.97± 5.13	-3.85	0.072	75.82± 6.49	-2	0.332	0.050
Abdominal circumference (cm)	79.63± 6.96	-1.73	0.089	75.26± 6.19	-2.35	0.000	79.60± 6.76	-2.26	0.001	0.052
Hip Circumference (cm)	79.19± 6.52	-0.97	0.272	75.55± 6.26	-1.72	0.007	79.47± 7.35	-0.44	0.643	0.111
Waist/Heigh ratio	0.55± 0.03	-0.02	0.281	0.539± 0.03	-0.036	0.038	0.547± 0.03	-0.023	0.208	0.316
Lean Body Mass (LBM, kg)	27.18±4.23	0.15	0.479	25.65±2.96	0.75	0.151	29.3±4.19	0.9	0.295	.012
Body Fat Percentage (BFP, %)	34.99±3.34	0.22	0.767	32.75±4.82	-0.8	0.094	30.1±4.74	-2.57	0.011	.003

HP: High protein group, HPC: High protein plus caseinate group, SD: Standard diet group.  
p-values, One-way ANOVA test.

<sup>a</sup> p-values using Kruskal-Wallis for non-normal variables expressed in medians.

<sup>b</sup> Intragroup p-values, student-t test for paired samples/Wilcoxon rank signed test for variables with non-normal distribution and expressed in medians.



**Table 3**  
Energy and protein intake adequacy analysis after treatment

Adequacy	HP group			HPC group			SD group			P- values
	Final	P- value	Effect size	Final	P- value	Effect size	Final	P- value	Effect size	
Energy intake (% adequacy)	94.15 ±7.69	0.130	0.354	86.98 ±6.27	0.976	0.006	82.18 ±5.4	0.948	0.14	0.258
Protein intake (g/kg weight)	1.67 ±0.12	0.260	-0.26	1.69 ±0.1	0.786	-0.59	1.96 ±1.3	0.042	-0.46	0.087

HP: High protein group, HPC: High protein plus caseinate group, SD: Standard diet group.

p-values, One-way ANOVA test.

Intragroup p-values, Wilcoxon rank, signed test.

with obesity who experienced weight loss had a poor reduction in ghrelin and increased peptide YY and glucagon-like peptide-1 levels associated with meal-induced stimulation [29]. In our study, one of the main limitations resides in the lack of measurement of these parameters, as well as other intestinal hormones related to satiety-hunger signals.

### Anthropometry and body composition

In terms of anthropometric parameters, all interventions showed a general trend toward reduction of measurements. Even though no statistical differences among groups were found for weight or BMI change. However, waist circumference showed a significantly higher reduction in the HPC group (-3.85 cm). This might be explained by the addition of calcium through calcium caseinate supplementation as part of a similar amount of protein intake compared to other groups. The anti-obesity effects of dietary calcium have been previously reported and seem to be related to the modification of fat metabolism and adipogenesis modulation [30]. It has been demonstrated in a human mesenchymal stem cell model that an increment in intracellular calcium levels [Ca<sup>2+</sup>] inhibits adaptogenic differentiation of preadipocytes associated with Wnt5a/β – catenin signaling. Also, it has been reported that with a 300 mg increment of calcium intake in the diet, children might lose up to 1 kg of body fat. [31] Within the anthropometric findings, all three groups exhibited a trend in abdominal circumference reduction; however, only the HPC group showed a significant decrease, which could be associated with a higher calcium intake (164 mg per day) from the supplement. However, we have no records of the total calcium intake of participants to test this hypothesis [32]. Additionally, there were no significant changes in BFP and LBM in the HPC group to support this. Similar results were observed in the study of Veldhorst et al. regarding BFP, where a high-protein diet with a protein milkshake supplementation during four weeks as part of regular diets showed no significant benefit in the short term for fat loss. Although there was no BFP improvement, an increase in LBM was observed [9]. This loss of fat mass does not become significant even when high-protein, low-carbohydrate diets are prescribed to this age group [10]. Considering the positive changes in BFP in the SD group, nutritional guidance on a healthy diet and adequate physical activity promotion might be sufficient to improve body composition in these patients.

Despite the waist circumference reduction and the weight loss trend, children's growth was not different among groups (+1 cm), showing that the proposed intervention is secure to maintain the growth rate in this population.

Regarding the cardiometabolic risk measured through WtHR, we did not observe a significant reduction after two months of intervention, even considering the homogenous height increase in all participants and the homogenous prescription of physical activity as a part of standard care in all groups. In this case, our study found a difference in REE<sup>IC</sup> among groups at the first visit but not after intervention, which could be explained because physical activity was implemented in all groups. However, there was no record of the physical activity to correlate with the REE of the participants.

### Dietetic intake

In our study, the highest adequacy percentage of energy intake was observed in the HP group, followed by the HPC and SD groups; however, energy intake was not statistically different between

groups ( $p < 0.05$ ). The mechanisms involved in promoting a negative energy balance induced by protein consumption have been previously discussed. Thermogenic effect and satiety-inducing properties are commonly attributed to this nutrient, which might be explained through a synergistic effect on the secretion of Glucagon-Like Peptide 1 and Peptide YY hormones in the small intestine. This may also be related to the production of peptides derived from dairy protein during gastric hydrolysis, inducing satiety through the 5-H2C receptor. This receptor is involved in the central regulation of food intake; however, these effects were observed in animal models [20,33,34]. The induction of the thermogenic effect by high protein diets is not clear since it has been observed that even protein restriction might induce this effect. [35] Horner et al. showed that even though the hydrolysis of casein protein did not affect gastric emptying, it did affect the glycemic response, improving metabolic response when this supplement was consumed as a part of a dietary intervention [36]. Moreover, high-protein casein-based diets may contribute to better adiposity and glucose homeostasis compared to other protein sources, as observed in pork-based diets [37].

Additionally, there is a concern about increased protein consumption in early life and certain foods, such as dairy products, with a potential obesity-promoting effect. However, studies are not conclusive about this possible association [38,39]. Adequate protein intake early in life is of major importance to guarantee insufficient growth and development. Nevertheless, excessive protein intake may have adverse effects when protein intake exceeds metabolic requirements through stimulation of insulin secretion and insulin-like growth factor 1, thereby enhancing weight gain. This phenomenon is known as the early protein hypothesis: a high protein intake during childhood is related to modified endocrine responses and an increased risk of obesity [40].

Previous studies have demonstrated that standard-protein diets comprising approximately 15% of total energy expenditure (TEE) may not achieve the dietary intake recommendation of 1 g/kg/day for children and adolescents from 2- to 16-year-old. There is evidence that protein intake is quickly replaced by carbohydrates or fats in the diet of subjects with obesity at this age [12,40]. The results observed in the present study showed that the amount of protein (25% of TEE) prescribed in the HP and HPC groups did not induce a significant difference in the BMI; however, the increase in protein consumption also showed by the SD group in the present study should be considered. However, protein is a crucial nutrient during this stage of development, and its adequate consumption should be a key consideration when devising dietary strategies for children. The evidence regarding the role of macronutrient distribution is convincing in the treatment of obesity in adulthood [41]; however, it is still unclear in pediatric populations, where there is no optimal macronutrient distribution for obesity treatment and long-term outcomes have been observed to be better with interventions focused on behavioral modification, physical activity or addressed to environmental factors [24,42,43].

Although we did not observe significant differences between the three dietary groups, we consider that a healthy diet-focused intervention could benefit overeating management in this population. There is not enough evidence considering a high protein diet as beneficial for appetite control, energy intake, or energy expenditure, as reported in other studies. In conclusion, the use of calcium caseinate as a part of a high protein diet is secure in a short time, although it has no apparent impact on satiety or anthropometric parameters in children with obesity. Improvements in metabolic risk parameters, such as abdominal fat or insulin sensitivity, could potentially occur due to calcium consumption, though. Further clinical studies to assess protein effects on satiety and metabolic status for the treatment of obesity are needed, considering gut hormone levels, physical activity, and other behavioral strategies in the long term.

## Ethical statement

The authors declare that this study was conducted on children by the Declaration of Helsinki, and all procedures were carried out with the adequate understanding and written consent of the parents of each child and the child himself.

## Author contributions

FSFE: Patient follow-up, writing, draft preparation, data curation, and statistical analysis; ANM: Statistical analysis; ARH and TFA: Study design and patient follow-up; MPBR: Reviewing language and writing; KAA: Writing, reviewing, and editing; NGN and VFT: Conceptualization, validation, supervision, reviewing, and editing.

## Funding

None. This research did not receive any specific grant from the public, commercial, or not-for-profit funding agencies or laboratories. The calcium Caseinate used was donated by Evolution Nutraceutical Company.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

The authors thank Evolution Nutraceutical Company for accepting our request to sponsor the calcium caseinate supplement used in this study.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.nutos.2023.10.008>.

## References

- [1] Reveles F, Reyes R, Tlapale Vázquez Diseño formación A, Pablo Luna J, Montiel A. Encuesta Nacional de Salud y Nutrición 2021 sobre Covid-19. Resultados nacionales. Morelos: Cuernavaca; 2022.
- [2] Simmonds M, Llewellyn A, Owen CG, Woolcott N. Predicting adult obesity from childhood obesity: a systematic review and meta-analysis. *Obesity Reviews* 2016;17:95–107. <https://doi.org/10.1111/obr.12334>.
- [3] Cuda SE, Censani M. Pediatric Obesity Algorithm : A Practical Approach to Obesity Diagnosis and Management. *Front Pediatr* 2019;6. <https://doi.org/10.3389/fped.2018.00431>.
- [4] Arnaiz P, Marín A, Pino F, Barja S, Aglony M. Índice cintura estatura y agregación de componentes cardiometabólicos en niños y adolescentes de Santiago. *Rev Med Chil* 2010;138:1378–85. <https://doi.org/10.4067/S0034-98872010001200006>.
- [5] Ashwell M, Gunn P, Gibson S. Waist-to-height ratio is a better screening tool than waist circumference and BMI for adult cardiometabolic risk factors: Systematic review and meta-analysis. *Obesity Reviews* 2012;13:275–86. <https://doi.org/10.1111/J.1467-789X.2011.00952.X>.
- [6] Marrodan M, Álvarez J, Espinoza M. Predicting percentage body fat through waist-to-height ratio (WtHR) in Spanish schoolchildren. *Public Health Nutr* 2013;17:870–6. <https://doi.org/10.1017/S1368980013000888>.
- [7] Simmonds M, Burch J, Llewellyn A, Griffiths C, Yang H, Owen C, et al. The use of measures of obesity in childhood for predicting obesity and the development of obesity-related diseases in adulthood: a systematic review and meta-analysis. *Health Technol Assess (Rockv)* 2015;19:1–336. <https://doi.org/10.3310/hta19430>.
- [8] Yoo E-G. Waist-to-height ratio as a screening tool for obesity and cardiometabolic risk. *Korean J Pediatr* 2016;59:425. <https://doi.org/10.3345/kjp.2016.59.11.425>.
- [9] Veldhorst MAB, Verbruggen SCAT, Van Harskamp D, Vermes A, Schierbeek H, Van Goudoever JB, et al. Effects of a high-protein intake on metabolic targets for weight loss in children with obesity: a randomized trial. *Obes Sci Pract* 2018;4: 347–56. <https://doi.org/10.1002/osp4.277>.
- [10] Krebs N, Gao D, Gralla J, Collins J. Efficacy and safety of a high protein, low carbohydrate diet for weight loss in severely obese adolescents. *J Pediatrics* 2010;157:252–8. <https://doi.org/10.1016/j.jpeds.2010.02.010>.
- [11] Gately PJ, King NA, Greatwood HC, Humphrey LC, Radley D, Cooke CB, et al. Does a high-protein diet improve weight loss in overweight and obese children? *Obesity* 2007;15:1527–34. <https://doi.org/10.1038/oby.2007.181>.
- [12] Markides BR, Crixell SH, Page J de G. High Protein Intake Among Preschoolers in Childcare in a Region at Elevated Risk for Obesity. *Matern Child Health J* 2020;24:1008–18. <https://doi.org/10.1007/s10995-020-02935-1>.
- [13] Diogenes. Higher protein diets consumed ad libitum improve cardiovascular risk markers in children of overweight parents from eight European countries. *J Nutr* 2013;143:810–7. <https://doi.org/10.3945/jn.112.173427>.
- [14] Joint WHO/FAO/UNU Expert Consultation. Protein and amino acid requirements in human nutrition. *World Health Organ Tech Rep Ser* 2007;935:1–265.

- [15] Onvani S, Haghghatdoost F, Surkan P, Azadbakht L. Dairy products, satiety and food intake: A meta-analysis of clinical trials. *Clinical Nutrition* 2017;36:389–98. <https://doi.org/10.1016/j.clnu.2016.01.017>.
- [16] Batista MA, Cruz N, Campos A, Pinto M, Andrade Batista M, Silvestre C. Whey and protein derivatives: Applications in food products development, technological properties and functional effects on child health. *Cogent Food & Agriculture* 2017;4. <https://doi.org/10.1080/23311932.2018.1509687>.
- [17] Meyer R, Foong RXM, Thapar N, Kritas S, Shah N. Systematic review of the impact of feed protein type and degree of hydrolysis on gastric emptying in children. *BMC Gastroenterol* 2015;15. <https://doi.org/10.1186/S12876-015-0369-0>.
- [18] Mølgaard C, Larnkjær A, Arnberg K. Milk and growth in children: effects of whey and casein. *Milk and Milk Products in Human Nutrition* 2011;67:67–78. <https://doi.org/10.1159/000325576>.
- [19] Kassia A, Godin J, Moille S, Nielsen-Moennoz C. Effects of protein quantity and type on diet induced thermogenesis in overweight adults: A randomized controlled trial. *Clinical Nutrition* 2019;28:1570–80. <https://doi.org/10.1016/j.clnu.2018.08.004>.
- [20] O'Halloran F, Bruen C, McGrath B, Schellekens H. A casein hydrolysate increases GLP-1 secretion and reduces food intake. *Food Chem* 2018;252:303–10. <https://doi.org/10.1016/j.foodchem.2018.01.107>.
- [21] Estephanje Becerril-Sánchez M, Flores-Reyes M, Ramos-Ibáñez N, Ortiz-Hernández L. Ecuaciones de predicción del gasto de energía en reposo en escolares de la Ciudad de México. *Acta Pediatr Mex* 2015;36:147–57.
- [22] Rjl Systems. User's guide body composition. 2.1. Michigan: Clinton Township; 2011.
- [23] Lee JJ, Brett NR, Wong VCH, de Zepetnek Jot, Fiocco AJ, Bellissimo N. Effect of potatoes and other carbohydrate-containing foods on cognitive performance, glycemic response, and satiety in children. *Applied Physiology, Nutrition and Metabolism* 2019;44:1012–9. <https://doi.org/10.1139/APNM-2018-0792>.
- [24] Hernández-Cordero S, Cuevas-Nasu L. Overweight and obesity in Mexican children and adolescents during the last 25 years. *Nutr Diabetes* 2017;7:e247. <https://doi.org/10.1038/nutd.2016.52>.
- [25] Jelalian E, Evans E. Behavioral intervention in the treatment of obesity in children and adolescents: implications for Mexico. *Nutr Rev* 2017;75:79–84.
- [26] Mehrabani S, Safavi SM, Mehrabani S, Asemi M, Feizi A, Bellissimo N, et al. Effects of low-fat milk consumption at breakfast on satiety and short-term energy intake in 10- to 12-year-old obese boys. *Eur J Nutr* 2016;55:1389–96. <https://doi.org/10.1007/S00394-015-0956-4>.
- [27] Carnell S, Wardle J. Appetite and adiposity in children: evidence for a behavioral susceptibility theory of obesity. *Am J Clin Nutr* 2008;88:22–9.
- [28] Papaioannou MA, Micheli N, Power TG, Fisher JO, Hughes SO. Associations Between Independent Assessments of Child Appetite Self-Regulation: A Narrative Review. *Front Nutr* 2022;8. <https://doi.org/10.3389/fnut.2021.810912>.
- [29] Roth C, Melhorn S, De Leon M. Impaired Brain Satiety Responses After Weight Loss in Children with Obesity. *J Clin Endocrinol Metab* 2022;107:2254–66.
- [30] Zhang F, Ye J, Zhu X, Wang L, Gao P, Shu G, et al. Anti-obesity effects of dietary calcium: The evidence and possible mechanisms. *Int J Mol Sci* 2019;20:3072. <https://doi.org/10.3390/ijms20123072>.
- [31] Heaney RP, Davies KM, Barger-Lux MJ. Calcium and weight: clinical studies. *J Am Coll Nutr* 2002;21:152S–5S. <https://doi.org/10.1080/07315724.2002.10719213>.
- [32] Ross AC, Taylor CL, Yaktine AL, Del Valle HB. Dietary reference intakes for calcium and vitamin D. 2011.
- [33] Schellekens H, Nongonierma A, Clarke G. Milk protein-derived peptides induce 5-HT<sub>2C</sub>-mediated satiety in vivo. *Int Dairy J* 2014;28:55–64. <https://doi.org/10.1016/j.idairyj.2014.04.004>.
- [34] Astrup A, Raben A, Geiker N. The role of higher protein diets in weight control and obesity-related comorbidities. *Int J Obes* 2015;39:721–6.
- [35] Pezeshki A, Chelikani PK. Low Protein Diets and Energy Balance: Mechanisms of Action on Energy Intake and Expenditure. *Front Nutr* 2021;8:655833. <https://doi.org/10.3389/fnut.2021.655833>.
- [36] Horner K, Drummond E, O'Sullivan V, Sri Harsha P SC, Brennan L. Effects of a casein hydrolysate versus intact casein on gastric emptying and amino acid responses. *Eur J Nutr* 2019;58:955–64. <https://doi.org/10.1007/S00394-018-1610-8>.
- [37] Myrrel LS, Fauske KR, Fjaere E, Bernhard A, Lissberg U, Hasselberg AE, et al. The Impact of Different Animal-Derived Protein Sources on Adiposity and Glucose Homeostasis during Ad Libitum Feeding and Energy Restriction in Already Obese. *Nutrients* 2019;11:1153. <https://doi.org/10.3390/nu11051153>.
- [38] Dougkas A, Barr Suzanne, Reddy Sheela, Carolyn D, Summerbell. A critical review of the role of milk and other dairy products in the development of obesity in children and adolescents. *Nutr Res Rev* 2018;32:106–27. <https://doi.org/10.1017/S0954422418000227>.
- [39] Lind MV, Larnkjær A, Mølgaard C, Michaelsen KF. Dietary protein intake and quality in early life: impact on growth and obesity. *Curr Opin Clin Nutr Metab Care* 2017;20:71–6.
- [40] Patro-Gołąb B, Patro-Gołąb Bernadeta, Bartłomiej M Zalewski, Kouwenhoven Stefanie MP, Karaś Jacek, Koletzko Berthold, et al. Protein concentration in milk formula, growth, and later risk of obesity: a systematic review. *J Nutr* 2016;146:551–64. <https://doi.org/10.3945/jn.115.223651>.
- [41] Freire R. Scientific evidence of diets for weight loss: Different macronutrient composition, intermittent fasting, and popular diets. *Nutrition* 2020;69:110549. <https://doi.org/10.1016/j.nut.2019.07.001>.
- [42] Rajjo T, Mohammed K, Alsawas M, Ahmed T, Farah W, Asi N, et al. Treatment of pediatric obesity: an umbrella systematic review. *J Clin Endocrinol Metab* 2017;102:763–75.
- [43] Styne DM, Arslanian SA, Connor EL, Farooqi IS, Murad MH, Silverstein JH, et al. Pediatric Obesity—Assessment, Treatment, and Prevention: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* 2017;102:709–57. <https://doi.org/10.1210/clinem.2016-2573>.