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Original Article

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Growth and Nutritional Biomarkers in Brazilian Infants with Cow's Milk Allergy at Diagnosis and 18-Month Follow-Up: A Prospective Cohort Study

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ABSTRACT

Purpose: This study aimed to describe the growth, body protein status, and micronutrient biomarkers of Brazilian infants with cow's milk allergy (CMPA) at baseline and at 18 months of follow-up in comparison with their healthy peers.
Methods: Thirty infants with CMPA younger than six months of age were included in this longitudinal study, and their nutritional status was compared with that of 24 non-allergic age-matched children. Anthropometric measurements were used to assess growth, and blood and urine samples were analyzed for protein and micronutrient status. Mixed linear models adjusted for birth weight, socioeconomic status, infant feeding at baseline, weight-for-age, C-reactive protein, serum albumin, micronutrient dietary supplementation, and salt consumption were employed to evaluate the evolution of nutritional parameters throughout the follow-up period.
Results: Overall, the mean age of the children at enrolment was 2.9 (standard deviation 1.7) months, and 29 children (53.7%) were male. Infants with CMPA showed a higher prevalence of functional iron depletion (transferrin saturation <20) (p=0.027), lower serum ferritin (p=0.009), and lower urinary iodine (p=0.034) levels than non-allergic children at baseline. Patients with CMPA showed a higher increment in weight-for-age and length-for-age over time than those in the control group (p<0.01). Mixed linear analyses showed a significantly lower increase in serum vitamin B12 (s-B12) (p=0.001) and urinary iodine (p<0.001) concentrations over time compared to the control group.
Conclusion: Infants with CMPA on a cow's milk elimination diet had a higher weight and length at 18 months of follow-up but showed signs of inadequate iron, iodine, and B-12 vitamin status.

Keywords: Food hypersensitivity; Anthropometry; Micronutrients; Nutritional status; Vitamin B12 deficiency; Iron deficiencies; Growth; Infant

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Conflict of Interest

The authors have no financial conflicts of interest.

INTRODUCTION

Cow milk protein allergy (CMPA) is one of the most common food allergies in childhood affecting 0.5–3% of infants in developed countries [1]. Although there is a paucity of epidemiological food allergy (FA) data in Brazil, in reference centers 1.9% of children aged 4–23 months have oral food challenge (OFC)-confirmed FA, of whom 1.0% present with CMPA [2].

Some studies on CMPA in developed countries have found that children with food allergies have lower growth parameters, in particular, height-for-age, than healthy infants and a lower intake of micronutrients, which may not necessarily translate into deficiencies [3]. However, studies on height growth in children with FA have produced conflicting results [4-7]. This discrepancy may be attributed to differences in studied populations, including those with IgE- and non-IgE-mediated CMPA, presence of comorbidities, type of elimination diet, duration of follow-up, and age at which CMPA was diagnosed [8-12].

To date, only one study has published a broad evaluation of nutritional biochemical parameters in children aged 0–2 years- on a cow's milk elimination diet. An increased risk of B12 deficiency in breastfeeding infants on a cow's milk elimination diet and iron, zinc, and vitamin D deficiencies was found in all feeding groups [13]. Additionally, Thomasson et al. [14] found that one-third of Norwegian children aged <2 years with suspected CMPA had urine iodine concentrations indicating iodine deficiency. However, as these studies evaluated children from developed countries, extrapolation of these data for children from developing countries may not be reflective, because nutritional deficiencies may also be a result of food insecurity. Therefore, we performed a longitudinal observational study to compare the growth, protein, and micronutrient biomarkers of Brazilian infants with CMPA at diagnosis and 18 months of follow-up with those of a non-allergic control group.

MATERIALS AND METHODS

Study design

A prospective cohort study comparing the growth, protein, and micronutrient biomarkers of Brazilian infants with CMPA with those of a non-allergic control group was conducted (**Supplementary Fig. 1**). Infants up to six months of age with symptoms of IgE and/or non-IgE-mediated CMPA were prospectively recruited at the Reference Center for Food Allergy of Sergipe (RCFAS), Federal University of Sergipe, Northeastern Brazil, over a 12-month period (February 2019-February 2020). A total of 150 symptomatic children met the inclusion criteria, of whom 30 had OFC-confirmed CMPA. Healthy infants were recruited from a maternity clinic where the mother did not experience any prenatal complications between September 2019 and March 2020, and were placed in the non-allergic control group. A total of 42 healthy children met the inclusion criteria. Of these, 24 agreed to participate.

The exclusion criteria were prematurity, inflammatory bowel disease, growth hormone deficiencies, celiac disease, cystic fibrosis, autoimmune disease, chromosomal abnormalities, developmental delay, and any other presumed or diagnosed chronic diseases.

The RCFAS is a public service of the Unified Health System (Sistema Único de Saúde- SUS) and has a multidisciplinary team and is responsible for the diagnosis of CMPA in all children

from the Sergipe state, with a suspected clinical history based on the recognition of symptom patterns and OFC.

Infants with symptoms suggestive of CMPA underwent open OFC after resolution of the symptoms with an elimination diet performed after 4–8 weeks on a cow's milk exclusion diet, which consisted of extensively hydrolyzed formula (eHF) or amino acid formula (AAF), and/or advising a breastfeeding mother to exclude all cow's milk proteins (CMP) from her diet and to take daily calcium and vitamin D supplementation [15,16]. The OFC was performed under medical supervision according to the PRACTALL guidelines [17]. OFC were not performed in patients with a clinical history of IgE-mediated CMPA with specific serum IgE ≥5,17 kUA/L. This cutoff has been found to have an 88% predictive value for positive OFC [18]. Children who did not present with immediate reactions after the OFC were asked to regularly consume cow milk protein for 30 days after the OFC, followed by a physician evaluation. Patients were also advised to return to our allergy center at any time if they presented with delayed CMPA symptoms for clinical evaluation. Children with clinician-confirmed delayed symptoms were also classified as positive for OFC based on clinical criteria.

In the RCFAS, all formula-fed children with OFC-proven CMPA have eHF or AAF through medical prescriptions reimbursed by the local health authority.

The scheduled time for the follow-up study visit at 18 months coincided with that during the COVID-19 pandemic. Although the study participants were still followed-up, this resulted in a larger follow-up loss than expected.

Data collection

Demographic and socioeconomic information, gestational age, mode of delivery, family history of allergies, breastfeeding, use of infant formula, and complementary food and salt consumption were collected using structured questionnaires. These data were included in the multivariate model to adjust for the analyses as appropriate.

Information on ownership of household assets, parental income, water and sanitation were used to construct a wealth index, by using the method of ABEP (Brazilian Association of Research Companies), where 'A' is the highest socioeconomic level and 'E' is the lowest [19].

Weight and length measurements were collected by a trained pediatric dietitian using regularly calibrated equipment according to the anthropometric standardized method defined by the World Health Organization (WHO) [20]. The child's length was measured using a supine length measurement board (Alturexata) with 0.1-cm precision. Weight was measured in children without clothing using a pediatric scale (Welmy) with 5-g precision.

Z-scores were calculated using WHO Anthro software to obtain the child length (LAZ), weight-for-age (WAZ), and weight-for-length (WLZ). Children were classified as stunted if their LAZ was <−2 and were graded as underweight and wasted if their WAZ and WLZ had z-scores <−2, respectively. In addition, children were classified as having a high weight-for-age if WAZ >2 and overweight if WLZ >2 [21].

Blood samples were collected in the morning, following a 4 hours fasting period, upon enrolment and at 18 months of follow-up, in accordance with routine procedures. Nutritional biomarkers were collected as shown in **Supplementary Table 1**, and the reference values

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used for this study are listed in the same table. Vitamin B12 deficiency was assessed by the algorithm s-B12 <250 pmol/L and Homocysteine (tHcy) >6.5 μmol/L OR s-B12 <250 pmol/L, and Methylmalonic acid (MMA) >0.28 μmol/L [13,22].

Children with iron and/or vitamin D deficiencies were supplemented according to the Brazilian Pediatric Association guidelines [23-25]. Vitamin B12 deficiency was treated with a high oral dose of cyanocobalamin (1 mg every day for 1 week, then 1 mg every week for 4 weeks) [26-28]. Other nutritional deficiencies were treated individually.

Data analysis

Statistical analyses were performed using the R Core Team 2021 (Version 4.1.0) software, and a 5% significance level was used. The difference-in-differences (DID) estimator in mixed linear models was employed to evaluate the evolution of nutritional parameters from baseline to 18 months of follow-up. The models were adjusted for covariates according to each nutritional biomarker to provide robust results, indicating the true effect of CMPA on nutritional parameters over the 18 months of follow-up. Mixed linear models were adjusted for birth weight; socioeconomic status; infant feeding at baseline; weight-for-age; C-reactive protein, iron, vitamin D, and vitamin B12 dietary supplementation (for children with vitamin B12 deficiency); and salt consumption in complementary foods (for iodine analysis). Serum albumin levels were also included in the adjusted longitudinal analysis of serum calcium levels.

The sample size was established based on repeated measures ANOVA [29] with G*Power 3.1.9.7 software [30]. Considering two groups, two measurements, a 5% significance level, 80% power, and a medium effect size (f=0.236) [31], the calculation yielded a requirement for 108 observations, resulting in 27 participants per group.

Ethical approval

This study was approved by the Research Ethics Committee of the Federal University of Sergipe (CAAE 97662918.0.0000.5546), and followed the ethical principles of the Declaration of Helsinki. Consent and participation were voluntary, and caregivers were also reassured of the confidentiality of the research.

RESULTS

Description of participants

One hundred and fifty children attended the RCFAS were eligible for inclusion in the study. Of these, 30 children had OFC-confirmed CMPA. We also recruited 24 healthy age-matched infants in the control group, following a frequency-matching protocol according to age. At the end of the study, 30 (55.6%) children were lost to follow-up and 24 children completed the study: 13 (54.2%) from the CMPA group and 11 (45.8%) from the control group (p=0.536).

No statistically significant differences were detected at baseline for age, sex, liquid feed including breastmilk and formula, socioeconomic status, and nutritional parameters between children who completed the 18 months of follow-up and those who were lost to follow-up.

More children had non-IgE-mediated gastrointestinal (GI) manifestations of CMPA, with food Protein-Induced Allergic Proctocolitis and food protein-induced Gastroesophageal

Reflux Disease diagnosed in 19 (63.3%) and 10 (33.3%) children, respectively. Acute urticaria/angioedema and anaphylaxis were observed in 2 (6.7%) children and 1 (3.3%) child, respectively.

Overall, the mean age of the children at enrolment was 2.9 (standard deviation [SD] 1.7) months, and 29 children (53.7%) were male (**Supplementary Table 2**). The cow's milk elimination diet was commenced in symptomatic infants at a mean age of 3.2 (1.5) months, and the formal diagnosis occurred at a mean age of 5.4 (SD 2.5) months. In children with a positive OFC for delayed CMPA, the onset of symptoms occurred at a median of 13 days (interquartile range [IQR]: 4–31 days) after the OFC. By the end of the 18-months follow-up, the mean age of the children was 21±3.6 months, and no significant differences in age were detected among the studied groups (p=0.267).

The children in both groups predominantly belonged to middle- to low-income families. Twenty-five (46.3%) families earned less than $200 per month and 42 (77.8%) earned less than $600 per month. Socioeconomic status and family income of the recruited children did not differ significantly among the study groups (p=0.086 and p=0.076, respectively). Three (5.6%) recruited children were small for gestational age (1,000–2,499 g); however, it was decided to keep those children in the study, as they had already reached a normal weight and length-for-age when they first attended the RCFAS at baseline.

Infant feeding and anthropometry

One-third of the children with CMPA were not breastfeeding at the beginning of the study, whereas 96% of the non-allergic children were breastfeeding (p=0.008) (**Table 1**). At the end of the study, five (29.4%) and 10 (76.9%) children were still breastfeeding in the CMPA and control groups, respectively (p=0.013).

At baseline, the daily median intake of infant formula by non-breastfed children was 850 mL (IQR: 760–1,110) in the CMPA group and 758 mL (IQR: 675–840) in the control group (p=0.533). By the second year of life, there was still no statistically significant difference (p=0.703) in the daily median intake of infant formula in the non-breastfed children in the CMPA group (600 mL [IQR: 420–660]) vs. the control group (430 mL [IQR: 190–960]).

Of the total study population, three children (5.7%) were stunted (LAZ <−2) and three (5.6%) were underweight (WAZ <−2) at baseline. Five (9.3%) children were overweight with a weight-for-length >2 z-score. No significant differences in baseline anthropometric status were detected between CMPA and non-allergic children (**Table 1**).

By the end of the follow-up, one child (1.9%) was stunted, none were underweight, and three (5.6%) were overweight. Comparing the z-scores from baseline to 18 months at follow-up, a significant increase was observed in weight-for-age and length-for-age in children with CMPA compared with non-allergic children (p<0.01) (**Fig. 1**, **Table 2**).

Nutritional biomarkers

1. Protein and iron status

The baseline total protein, albumin, prealbumin concentrations, and iron biomarkers are shown in **Table 3**. Comparing the protein and iron biomarkers from the baseline to the 18-months follow-up, there was no significant difference between the groups in the DID-adjusted analyses (**Fig. 1**, **Table 2**).

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Table 1. Infant feeding description and anthropometry of studied children at baseline

Infant feeding and anthropometry	All (n=54)	CMPA (n=30)	Control group (n=24)	*p*-value
Formula feeding at maternity				0.147
Yes	16 (30.2)	11 (37.9)	5 (20.8)	
No	37 (69.8)	18 (62.1)	19 (79.2)	
Breastmilk cessation				0.008
Yes	11 (20.4)	10 (33.3)	1 (4.2)	
No	43 (79.6)	20 (66.7)	23 (95.8)	
Infant nutrition in the beginning of study				0.120
Exclusive breastmilk	31 (57.4)	15 (50.0)	16 (66.7)	
Infant formula*	4 (7.4)	2 (6.7)	2 (8.3)	
Soy formula	1 (1.9)	1 (3.3)	0 (0.0)	
Substitute formula†	5 (9.3)	5 (16.7)	0 (0.0)	
Whole cow's milk	3 (5.6)	2 (6.7)	1 (4.2)	
Breastmilk and infant formula*	7 (13.0)	2 (6.7)	5 (20.8)	
Breastmilk and substitute formula†	3 (5.6)	3 (10.0)	0 (0.0)	
Number of complementary foods				0.935
None	41 (75.9)	22 (73.3)	19 (79.2)	
One food item	5 (9.3)	3 (10.0)	2 (8.3)	
2 to 5 food items	6 (11.1)	4 (13.3)	2 (8.3)	
≥6 food items	2 (3.7)	1 (3.3)	1 (4.2)	
Age of introduction of complementary foods	80 (59.0)	73 (63.0)	112 (11.0)	0.007
None	42 (77.8)	20 (66.7)	22 (91.7)	0.151
<1 mo	3 (5.6)	3 (10.0)	0 (0.0)	
1 to 3 mo	4 (7.4)	3 (10.0)	1 (4.2)	
4 to 5 mo	5 (9.3)	4 (13.3)	1 (4.2)	
Weight-for-age	−0.22 (−0.9/0.5)	−0.5 (−1.0/0.4)	−0.03 (−0.5/0.9)	0.074
Underweight	3 (5.6)	2 (6.7)	1 (4.2)	0.259
Eutrophy	49 (90.7)	28 (93.3)	21 (87.5)	
High weight-for-age	2 (3.7)	0 (0.0)	2 (8.3)	
Length-for-age	−0.64 (1.1)	−0.75 (0.84)	−0.50 (1.4)	0.113
Stunting	3 (5.7)	2 (6.7)	1 (4.3)	0.600
Eutrophy	50 (94.3)	28 (93.3)	22 (95.7)	
Weight-for-length	0.54 (−0.4/1.1)	0.46 (−0.43/0.86)	0.57 (−0.18/1.3)	0.273
Eutrophy	49 (90.7)	28 (93.3)	21 (87.5)	0.393
Overweight	5 (9.3)	2 (6.7)	3 (12.5)	

Values are presented as number (%) or median (interquartile range).
CMPA: cow's milk allergy group.
*Standard formula and partially hydrolyzed cow's milk formula, †Extensively hydrolyzed cow's milk formula, amino acid formula, and hydrolyzed rice formula.

Nineteen (35.2%) children with CMPA received iron supplements at the baseline. No significant differences in iron supplementation were found between the study groups (p=0.486) at enrolment or at the end of follow-up (p=0.556).

2. B12 and folate status

The baseline serum B12 vitamin, folate, and their biochemical markers are described in **Table 3**. We found B12 deficiency (assessed by the algorithm s-B12 <250 and tHcy >6.5 OR s-B12 <250 and MMA >0.28) in three (12.5%) children with CMPA and none in the control group at baseline. None of the children diagnosed with B12-vitamin deficiency presented with clinically significant neurological signs and symptoms. Only one (1.9%) child was receiving a vitamin B12 supplement at baseline and at the end of follow-up (4%), at a dose of 0.24 and 2 mcg/day, respectively. None of the children received folic acid supplements throughout the study period.

Children in the CMPA group showed a significantly lower increase in serum vitamin B12 concentration over time than those in the control group in the adjusted and non-adjusted DID analyses (p=0.001 and 0.008, respectively) (**Fig. 1**, **Table 2**).

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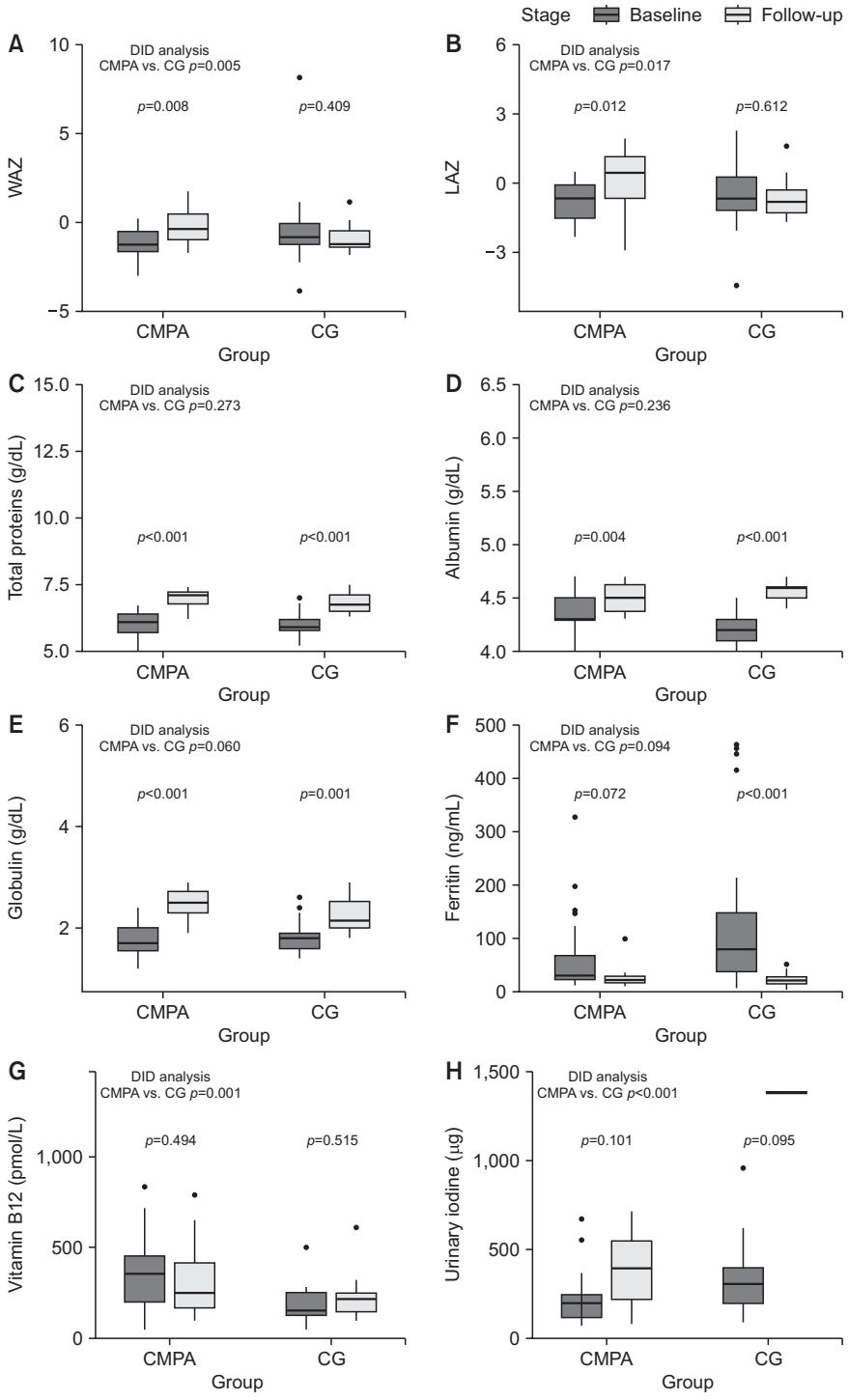

Fig. 1. Longitudinal analyses of anthropometric and nutritional biomarkers. (A) Weight-for-age distribution at baseline and follow-up. (B) Length-for-age distribution at baseline and follow-up. (C) Total protein distribution at baseline and follow-up. (D) Albumin distribution at baseline and follow-up. (E) Globulin distribution at baseline and follow-up. (F) Ferritin distribution at baseline and follow-up. (G) Vitamin B12 distribution at baseline and follow-up. (H) Urinary iodine distribution at baseline and follow-up.
DID: difference-in-difference, CMPA: cow's milk protein allergy, CG: control group, WAZ: Weight-for-age z-score, LAZ: length-for-age z-score.

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Table 2. Adjusted and unadjusted DID analyses to describe differences in nutritional biomarkers and anthropometry from baseline to 18 months follow-up in cow's milk allergic children compared to control children

Nutritional parameter	Unadjusted (95% CI)	*p*-value	Adjusted (95% CI)	*p*-value
Total proteins (g/dL)	0.498 (−0.008–1.005)	0.054	0.374 (−0.295–1.044)	0.273
Albumin (g/dL)	−0.098 (−0.265–0.069)	0.25	−0.128 (−0.341–0.084)	0.236
Globulin (g/dL)	0.609 (0.193–1.025)	0.004	0.531 (−0.022–1.085)	0.06
Prealbumin (mg/dL)	−1.191 (−5.596–3.213)	0.596	−3.265 (−8.268–1.739)	0.201
Hemoglobin (g/dL)	0.460 (−0.586–1.507)	0.389	0.521 (−0.730–1.772)	0.414
Ferritin (ng/mL)	58.727 (−23.455–140.909)	0.161	92.614 (−15.894–201.123)	0.094
Transferrin saturation (%)	0.082 (−14.148–14.313)	0.991	1.377 (−16.958–19.712)	0.883
b-MCV (fL)	2.601 (−3.808–9.011)	0.426	1.436 (−5.707–8.580)	0.694
b-MCH (pg)	1.250 (−0.890–−3.390)	0.252	0.654 (−1.799–3.108)	0.601
Vitamin B12 (pmol/L)	−233.306 (−405.508–−61.103)	0.008	−325.739 (−523.784–−127.694)	0.001
Homocysteine (μmol/L)	0.732 (−2.403–3.867)	0.647	−2.843 (−7.056–1.370)	0.186
Methylmalonic acid (μmol/L)	0.502 (−0.279–1.284)	0.208	0.795 (−0.577–2.166)	0.256
Folate (nmol/L)	3.141 (−1.194–7.475)	0.156	2.886 (−2.442–8.215)	0.288
25-hidroxi-vitamin D (nmol/L)	1.979 (−12.320–16.278)	0.786	4.848 (−13.972–23.667)	0.614
Alkaline phosphatase (U/L)	−136.252 (−565.572–293.068)	0.534	−248.716 (−740.323–242.891)	0.321
Serum calcium (mg/dL)	−0.336 (−1.013–0.341)	0.331	−0.464 (−1.210–−0.282)	0.210
Serum phosphorus (mg/dL)	0.618 (−0.243–1.478)	0.159	0.225 (−0.797–1.248)	0.666
Serum magnesium (mg/dL)	−0.082 (−0.237–0.074)	0.305	−0.107 (−0.312–0.097)	0.304
Urinary iodine (μg)	−848.603 (−1,102.455–−594.751)	<0.001	−981.948 (−1,371.464–−592.432)	<0.001
Weight-for-age	1.168 (0.354–1.983)	0.005	1.511 (0.388–2.634)	0.008
Length-for-age	1.143 (0.208–2.077)	0.017	2.120 (0.850–3.391)	0.001
Weight-for-length	0.796 (−0.022–1.614)	0.056	0.429 (−0.281–1.139)	0.236

DID: difference-in-difference, CI: confidence interval, b-MCV: mean corpuscular volume, b-MCH: mean corpuscular hemoglobin.

3. Bone metabolism biomarkers

The mean baseline 25(OH)D, alkaline phosphatase, calcium, and magnesium levels were normal and no significant differences were observed between the CMPA and control groups (**Table 3**). Forty-two (77.8%) and 11 (45.8%) children received vitamin D supplements at baseline and follow-up assessment, respectively, and no significant differences in this supplementation were found between the studied groups (p>0.05).

There were no significant differences in the evolution of bone metabolism biomarkers between the groups from the baseline to the end of the study in the DID analyses (**Table 2**).

4. Iodine status

The median baseline urinary iodine level was normal (218 μg) in both groups, and the control cohort showed significantly higher levels than the children with CMPA (296 vs. 198 μg; p=0.034) (**Table 3**). None of the children received iodine supplements either at baseline or at the end of follow-up, except for iodinated salt added to the child's complementary foods from one year of age.

The urinary iodine concentration remained stable throughout the follow-up period in both groups (**Fig. 1**), although children with CMPA presented a significantly lower increase in urinary iodine concentration over time than control children in the DID analyses (p<0.001) (**Table 2**).

DISCUSSION

To the best of our knowledge, this is the first prospective longitudinal study to compare anthropometric measurements and protein/micronutrient biomarkers between infants with

Table 3. Nutritional biomarkers in infants with and without cow's milk allergy at baseline

Nutritional biomarker	All (n=47)		CMPA (n=24)		Control group (n=23)		*p*-value
	N	95% CI	N	95% CI	N	95% CI	
Total proteins (g/dL)	6.0	0.4	6.0	0.5	5.9	0.4	0.251
Low (<6,0)	22	47.8	9	39 (22–59)	13	57 (37–75)	0.188
Albumin (g/dL)	4.2	4.0–4.3	4.3	4.2–4.5	4.2	4.40–4.3	0.044
Globulin (g/dL)	1.8	0.3	1.7	0.3	1.8	0.2	0.213
Low (<2.3)	43	93.5	22	96 (79–99)	21	91 (73–98)	0.500
Prealbumin (mg/dL)	15.2	3.7	14.9	4.1	15.5	3.4	0.264
Low (<20)	42	91.3	21	91 (73–98)	21	91 (73–98)	0.696
C reactive protein (mg/L)	0.5	0.2–0.9	0.5	0.4–1.0	0.4	0.1–0.8	0.159
Elevated (>3.0)	3	6.7	0	0 (0–15)	3	13 (5–32)	0.125
Hemoglobin (g/dL)	11.2	10.7–11.7	11.2	10.9–11.5	11.2	10.5–12.2	0.624
Anemia	10	21.3	4	17 (7–36)	6	26 (13–46)	0.333
Ferritin (ng/mL)	49.2	23.1–117.2	27.6	20.4–74.2	74.1	36.8–142.9	0.009
Iron deficiency	14	29.8	10	42 (24–61)	4	17 (7–37)	0.066
Transferrin saturation	21.0	14.0–26.5	18.5	14.5–26.0	22	14.0–36.5	0.282
Low (<20)	22	46.8	15	63 (43–79)	7	30 (16–51)	0.027
b-MCV (fL)	81.1	8.6	78.4	7.2	83.9	9.1	0.165
Low	15	31.9	9	38 (21–57)	6	26 (13–46)	0.393
Elevated	1	2.1	1	4 (1–20)	0	0 (0–14)	
b-MCH (pg)	27.0	3.2	26.1	2.8	27.9	3.3	0.359
Low	18	38.8	10	42 (24–61)	8	35 (19–55)	0.427
Vitamin B12 (pmol/L)	314	178–487	354	199–453	146	124–250	0.005
Low (<250)	27	58.7	10	43 (26–63)	17	74 (54–87)	0.036
Homocysteine (μmol/L)	7.2	5.2–8.9	6.2	4.9–7.8	7.9	6.2–9.9	0.039
Hyperhomocysteinemia (>6.5)	28	60.9	11	48 (29–67)	17	74 (54–87)	0.065
Methylmalonic acid (μmol/L)	0.38	0.21–0.60	0.22	0.16–0.42	0.48	0.32–0.79	0.001
Elevated (>0.28)	25	62.5	7	41 (22–64)	18	78 (58–90)	0.019
Vitamin B12 deficiency (algorithm)	3	6.5	3	13 (5–32)	0	0 (0–14)	0.117
Folate (nmol/L)	23.5	17.0–24.8	59.7	45–62	57.9	41–62	0.642
25-hidroxi-vitamin D (nmol/L)	54.0	17.4	143.8	44.3	126.0	41.4	0.747
Deficiency (25 to 50)	1	2.1	0	0 (0–14)	1	4 (1–21)	0.489
Alkaline phosphatase (U/L)	831	696–980	832	726–929	752	682–1,009	0.774
Low (<250)	1	2.1	1	4 (1–20)	0	0 (0–14)	0.378
Elevated (>950)	13	27.7	5	21 (9–40)	8	35 (19–55)	
Serum calcium (mg/dL)	10.8	10.5–11.1	10.7	10.4–11.6	10.8	10.5–10.9	0.724
Elevated (>10.5)	32	68.1	16	67 (47–82)	16	70 (49–85)	0.540
Serum phosphorus (mg/dL)	6.3	6.0–7.0	6.2	5.9–6.4	6.5	6.1–7.5	0.031
Elevated (>7.0)	10	22.2	1	5 (1–22)	9	39 (22–59)	0.006
Serum magnesium (mg/dL)	2.4	2.3–2.5	2.4	2.3–2.5	2.4	2.3–2.4	0.204
Elevated (>2.5)	6	13.0	4	17 (7–37)	2	9 (2–27)	0.333
Urinary iodine (μg)	218	161–341	198	117–245	296	193–397	0.034
Low (<100)	5	12.5	4	19 (8–40)	1	5 (1–25)	0.204

Values are presented as mean (standard deviation) or median (interquartile range).
CMPA: cow's milk protein allergy, CI: confidence interval, b-MCV: mean corpuscular volume, b-MCH: mean corpuscular hemoglobin.
N=46 for total proteins, albumin, globulin, prealbumin, vitamin B12, homocysteine, folate, and serum magnesium; n=45 for serum phosphorus; and n=40 for methylmalonic acid and urinary iodine.

CMPA and nonallergic infants in a developing country. We found that children with CMPA who received a CMP elimination diet had higher rates of both weight gain and length gain than non-allergic children. In addition, children with proven CMPA showed a significant increase in total serum protein, albumin, and globulin levels over the measurement period, similar to that in non-allergic children.

Several cross-sectional studies have demonstrated concerns about longitudinal growth in children with CMPA on an elimination diet [4,6,32]. A feasible explanation for this is a decreased intake of nutrients, especially low intake of energy, protein, calcium, phosphorus, magnesium, zinc, riboflavin, and niacin [4,9,10]. However, in this study, children with CMPA

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had a growth status similar to that of the control group at baseline and significantly higher weight-for-age and length-for-age z-scores at the 18-month follow-up.

One possible explanation for this finding is the higher levels of protein in hypoallergenic formulas (1.7–1.9 g/100 mL) when compared to breastmilk and to standard formula (1.2–1.4 g/100 mL). This implies that the protein energy contribution is approximately 10–11%, which is similar to that suggested for catch-up growth [33]. There is a paucity of longitudinal studies describing the anthropometry of children with CMPA compared to that of healthy children from developing countries. A longitudinal study enrolling Thai infants with CMPA, but without a control group, demonstrated a significantly more pronounced improvement in growth in CMP-allergic infants fed with hypoallergenic formula than in those who were breastfed [34]. This finding reinforces our hypothesis that higher protein intake through substitute formulas contributes to higher weight and height increments. In contrast, longitudinal studies of children with CMPA from developed countries that included healthy controls showed that children with CMPA using hypoallergenic formula reached similar (but not higher) weight and height z-scores from baseline to 2 years of age [8,9]. In our study, the higher anthropometric z-scores in CMP-allergic children than in non-allergic children may also be explained by the lower socioeconomic status of both groups of children, as formula-fed children from the CMPA group had free access to substitute formula by a social program for CMPA treatment, which did not occur in healthy children.

Regarding micronutrient biomarkers, children in the control group had significantly lower concentrations of vitamin B12 and higher concentrations of t-Hcy and MMA than those in the CMPA group at baseline. This result may be a reflection of the higher rates of breastfeeding in the control group, as vitamin B12 in breast milk has been found to be positively associated with maternal B12 intake [35], and in lower socioeconomic groups, animal protein consumption is lower because of cost [36,37]. Moreover, infant formulas are enriched with cobalamin and have been found to yield higher serum vitamin B12 and lower t-Hcy levels in formula-fed children [38]. Although not statistically significant, the fact that the formula-fed infants from the control group consumed 430 mL vs. 600 mL in the CMPA group could make this difference.

However, when vitamin B12 status was evaluated using an algorithm that considered vitamin B12 and its biochemical markers t-Hcy and MMA, none of the children in the control group showed B12 deficiency at baseline. In contrast, 13% of the children with CMPA had vitamin B12 deficiency when this algorithm was used. A similar prevalence of vitamin B12 deficiency (12%) was observed by Kvammen et al. [13] in 0–2-year-old Norwegian children undergoing cow milk elimination diet.

This study could not ultimately attribute baseline vitamin B12 deficiency to CMPA, because these levels could also reflect lower newborn endogenous stores associated with mothers. Several studies have shown that the most common etiology of vitamin B12 deficiency in infancy is maternal deficiency in strictly vegetarian mothers or even in non-vegetarian mothers from low socioeconomic populations [39,40]. Maternal food preferences were recorded in this study, with no mothers in either group consuming a vegetarian diet. Although the control group presented with prolonged breastfeeding in the second year of life, children with CMPA showed a significantly lower increase in s-B12 over time, which may be explained by unnoticed and sustained intestinal inflammation, which in turn may affect vitamin B12 absorption [16,41]. This finding underscores the need to consider vitamin B12 deficiencies in young children with CMPA.

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The present study also demonstrated that children with CMPA had a higher rate of functional iron depletion and lower ferritin concentrations at baseline than those in the control group. Functional iron depletion, defined as low transferrin saturation, is characterized by the presence of adequate iron stores as defined by conventional criteria, but with insufficient iron mobilization for adequate support, which is commonly related to chronic inflammation [42,43].

The CMPA group presented with a lower initial ferritin concentration over time despite consuming sufficient hypoallergenic iron-fortified formula in non-breastfed children. These findings suggest that bowel permeability dysfunction due to inflammatory processes in children with predominantly non-IgE-GI manifestations of CMPA may contribute to infant iron store depletion prior to treatment [44,45]; however, the elimination diet per se does not guarantee iron store repletion. The volume of iron-fortified formula intake decreased throughout follow-up as the infants' acceptance of complementary foods increased, which may in low-income populations lead to lower intake of iron-rich protein due to the cost of some iron-rich foods [36]. The present study revealed a lower prevalence (19%) of urinary iodine content <100 μg in the CMPA group than that (31%) in Norwegian children younger than 2 years on a cow's milk protein-free diet [14]. Urinary iodine is subject to daily variations according to individual diet and hydration, which limits the interpretation of the nutritional status [14]. Cow milk is an important source of iodine, particularly in young children, where the use of fortified salt is not promoted due to concerns about excessive sodium intake. Further research is required in children with CMPA to establish the iodine status in larger populations.

A limitation of this study is that nutrient intake was not quantified to establish comparisons with the control group. However, the analysis of the amount of daily infant formula intake did not demonstrate significant differences between formula-fed children with and non-allergic. Another limitation of this study was the loss of a relevant number of participants to follow-up, which was mostly due to the COVID-19 pandemic. However, the proportion of children lost to follow-up was similar between groups.

The practical implications of this study are that children with CMPA from a resource-constrained setting may show adequate anthropometric achievement, although they may be at risk of specific micronutrient deficiencies such as iron, vitamin B12, and iodine, which may compromise growth, immune function, and neuromotor development [41,46,47]. This highlights the need for thorough nutritional evaluation as part of clinical practice, including blood assays and dietetic assessment, to investigate micronutrient deficiencies.

In conclusion, Brazilian infants with CMPA who consumed a cow's milk protein elimination diet showed signs of inadequate iron and vitamin B12 nutrition and presented a higher increment in weight and length scores from 0-6 months to 17-22 months of age than nonallergic children predominantly breastfed at a similar age and socioeconomic status.

SUPPLEMENTARY MATERIAL

Supplementary Fig. 1

Flow diagram of the study design.

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Supplementary Table 2

Cut-offs for the nutritional biomarkers

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Supplementary Table 3

Demographic and birth characteristics of the study population

Click here to view

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