APJAI
Asian Pacific Journal of
Allergy and Immunology

ORIGINAL ARTICLE

Effects of a new amino acid, rice glucose polymer-based, and commercial amino acid-based formulas on growth and protein status of infants with cow's milk protein allergy

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Abstract

Background: Infants with cow's milk protein allergy (CMPA) are at risk for nutrient inadequacy and impaired growth.

Objective: To evaluate the effect of a new amino acid-based formula (nAAF) compared with commercial amino acid-based formula (cAAF) on growth and protein status of cow's milk protein (CMP)-allergic infants and to compare their growth with those of healthy infants.

Methods: Infants less than 6 months of age with CMPA were enrolled in the nAAF or cAAF groups. Healthy infants fed breast milk (BM) or infant formula (IF) were controls. They remained on their formula/milk until day 28 of the study. Anthropometric evaluation was performed at birth, day 0 and day 28 of the study and calculated to z-scores of weight-for-age (WAZ), length-for-age (LAZ) and head circumference-for-age (HAZ). Plasma amino acids, albumin, urea nitrogen, and creatinine were assessed for infants with CMPA on day 0 and day 28.

Results: The nAAF and cAAF groups did not differ in increases in WAZ [regression coefficient (95%CI): 0.088 (-0.619, 0.796), p = 0.791], LAZ [0.045 (-0.789, 0.880, p = 0.909], and HAZ [-0.645 (-2.082, 0.793), p = 0.337] between day 0 and day 28. The increases in WAZ and LAZ during 28 days in the nAAF group did not differ from the controls. The changes in the blood chemistry values, except albumin, were not different between CMPA groups.

Conclusion: The nAAF, similar to the cAAF, supports growth and protein status for infants with CMPA, and it might be used as a substitute for the cAAF.

Key words: Amino acid-based formula, cow's milk protein allergy, growth, infants, rice glucose polymer

Citation:
Densupsoontorn, N., Jirapinyo, P., Rukprayoon, H., Kunnangja, S., Chotipanang, K., Pacharn, P., Thaweekul, P., Nanthapisal, S. (0000). Effects of a new amino acid, rice glucose polymer-based, and commercial amino acid-based formulas on growth and protein status of infants with cow's milk protein allergy. *Asian Pac J Allergy Immunol*, 00(0), 000-000. https://doi.org/10.12932/ap-270123-1536

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Introduction

The prevalence of cow's-milk protein allergy (CMPA) in infants and children has been studied worldwide and its determination depends on several factors such as study participants (term vs. preterm infants, general vs. high risk participants, or recruitment rate), age, geographic area, feeding pattern, underlying immune mechanism, and diagnostic criteria.[1] The adjusted incidence of CMPA was 0.74% among European children aged up to 2 years.[2] The prevalence of CMPA was 2.69% in Chinese infants,[3]

1.4% among Irish preterm infants,[4] and 2.2% in Canadian children.[5] The EuroPrevall birth cohort study found an adjusted incidence of immunoglobulin E (IgE)-associated CMPA of 0.59% and that of non-IgE-associated CMPA ranging from 0.13% to 0.72%.[2] The purposes of CMPA management for infants include a decrease in allergic symptoms, the acquisition of tolerance, and their adequate intake of energy and nutrients to promote normal growth and development. Breast milk with maternal cow's milk protein (CMP) avoidance is indispensable for breast-fed infants, whereas a hypoallergenic formula is an essential alternative therapy for formula-fed infants. Hypoallergenic formula refers to an amino acid-based formula (AAF) and an extensively hydrolyzed protein-based formula (EHF). The use of AAF is indicated in the treatment of CMPA with red flag signs (anaphylaxis, severe symptoms of eczema and gastrointestinal tract, faltering growth, and multiple food allergies), or EHF failure.[6-10]

The growth of CMP-allergic infants treated with AAF has been studied. At enrollment, the weight-for-age z-score (WAZ) value of AAF-fed CMP-allergic infants was significantly lower compared to healthy controls, and a 12-month treatment with AAF plus personalized nutritional counseling supported an adequate increase in WAZ and normal ranges for serum urea, total proteins, albumin, retinol-binding protein and insulin-like growth factor 1 among those with CMPA.[11] The use of AAF containing 24% medium-chain triglycerides (MCTs) in combination with complementary foods free from CMP supported the growth of WAZ catch-up in Chinese infants allergic to CMP from 16 weeks to 9 months of age.[12]

We have developed a new amino acid-based formula (nAAF) in which its macronutrients consist of amino acids, glucose polymer hydrolyzed from rice starch as a carbohydrate source for which its protein fraction is eliminated, and vegetable oils. The efficacy of nAAF compared with two commercial amino acid-based formulas (cAAF) in the treatment of CMPA in infants had been confirmed in previous randomized, double-blind controlled studies.[13,14] However, growth data and protein status of CMP-allergic infants who consume nAAF have not been published.

The primary objective of this study was to compare the effect of nAAF *versus* cAAF on growth and protein status of CMP-allergic infants in the absence of the confounding impact of complementary foods. The secondary objective was to compare the growth parameters of CMP-allergic infants receiving nAAF or cAAF with those of healthy infants consuming breast milk or infant formula.

Methods

This nonrandomized control study was conducted in two tertiary centers in Thailand; Siriraj Hospital, Mahidol University, and Thammasat Hospital, Thammasat University. The study protocol was approved by the Institutional Review Board of each institution (COA No. Si 262/2012 and MTU-EC-PE-4-236/63) and was registered on ClinicalTrials.gov (NCT01637688). Each parent of a participant gave written informed consent to participate in this study.

Study participants

All participants were infants under six months of age and did not consume complementary foods. All 19 participants in CMPA groups had severe symptoms compatible with CMPA. Thirteen (68%) CMP-allergic participants had persistent symptoms in spite of EHF consumption, with CMP elimination. All 19 CMP-allergic participants had resolution of symptoms after a provision of cAAF. Then, parents of 14 participants refused to perform an oral food challenge after resolution of symptoms with cAAF, whereas five participants underwent the double-blind, placebo-controlled food challenge (DBPCFC) test to confirm diagnosis. Among five participants with positive DBPCFC test, three performed the test when participating in our previous study[14] that found one with tolerance to a cAAF and two with tolerance to a nAAF, and the other two participants tolerated to a cAAF and a nAAF each. The remaining 14 participants received either a nAAF or cAAF (either Neocate® or Puramino®) on the basis of their financial status and tolerance. If they tolerated either AAF for at least 2 weeks, we enrolled them in this study. Participants allergic to CMP were assigned to the nAAF group or cAAF group according to their previous tolerated formulas. Two control groups, the breast milk and infant formula groups, were composed of healthy babies of the same age who were fed with breast milk and standard infant formula, respectively. Participants who were not a singleton; born prematurely; had chronic illness, metabolic diseases, malignancies, or genetic disorders that affected normal growth or feeding; changed their formula/milk during the study; or consumed complementary foods were excluded.

Anthropometric measurement

Body weight, length, and head circumference were evaluated at birth, enrollment, and a 28-day visit for all participants in four groups. The seca 727 electronic baby scale with an accuracy of 1 gram (Seca GmbH & Co. KG, Hamburg, Germany) was used to measure the body weight of an unclothed infant. A measuring board with an accuracy of 1 mm was used to measure the length of an infant without footwear and headwear. A non-elastic tape with an accuracy of 1 mm was used to measure an infant's head circumference. Growth data were converted to z-scores based on the World Health Organization (WHO) Child Growth Standards using the WHO Anthro Survey Analyser.[15] A weight-for-age z-score (WAZ), length-for-age z-score (LAZ), or head circumference-for-age z-score (HAZ) value between -2 and 2 meant normal growth status.[16,17]

Biochemical measurement

Blood sampling was carried out in only the nAAF and cAAF groups in the morning after withholding one feeding to assess protein status (amino acids, albumin, urea nitrogen, and creatinine) at baseline and during a 28-day visit. Plasma amino acid concentrations were determined using the Biochrom 30 high performance liquid chromatography cation exchange system with ninhydrin detection (Biochrom, Cambridge, UK). Albumin, urea nitrogen, and creatinine

in blood samples were measured using the Cobas 8000® analyzer (Roche Diagnostics, Tokyo, Japan). Plasma albumin was assessed with the colorimetric method (Roche Diagnostics, Mannheim, Germany). Blood urea nitrogen was analyzed with the enzymatic method (Roche Diagnostics, China). Plasma creatinine was analyzed with the enzymatic method (Roche Diagnostics, Mannheim, Germany).

Dietary intake

During the 28-day study, participants in each group were informed to continuously consume their own formula/milk ad libitum. The dietitian informed parents or caregivers of participants in the nAAF, cAAF and infant formula groups how to record the volume of daily formula consumed by their infants.

Statistical analysis

Categorical variables were presented as numbers and percentages and compared between two groups or between four groups using the chi-square test or Fisher's exact test. Normally distributed continuous data, assessed using the Shapiro-Wilk test, were shown as mean ± standard deviation. Differences between groups were assessed using the Student's *t* test without pairs. Four-group differences were calculated using analysis of variance with post hoc multiple comparison (assessed by Scheffe's test).

Non-normally distributed continuous data were presented as median (P25, P75). Two-group differences were assessed using the Mann-Whitney U test, and four-group differences were calculated using the Kruskall-Wallis H test. Multivariate linear regression analysis adjusted for potential confounders was used to assess 28-day changes in WAZ, LAZ, and HAZ between the nAAF group *versus* the cAAF group, the nAAF group *versus* the breast milk group, and the nAAF group *versus* the infant formula group.

Data were analyzed with PASW Statistics for Windows (version 18.0; SPSS Inc., Chicago, IL, USA). All statistical significance tests were two-sided and a *p*-value of less than 0.05 was considered to indicate statistical significance.

Results

Twenty-two infants with CMPA were enrolled. Two infants were excluded because they had coexisting chronic diseases, and another infant was excluded due to later tolerance and the change to EHF (**Figure 1**). Fourteen infants in the nAAF group and five infants in the cAAF completed the study. Ninety-seven healthy infants were enrolled, but fourteen infants were excluded due to their consumption of breast milk and infant formula. Twenty healthy infants were lost to follow-up, and another was excluded due to the change from breast milk to infant formula consumption. Thirty-four infants in the breast milk group and 28 in the infant formula group completed the study.

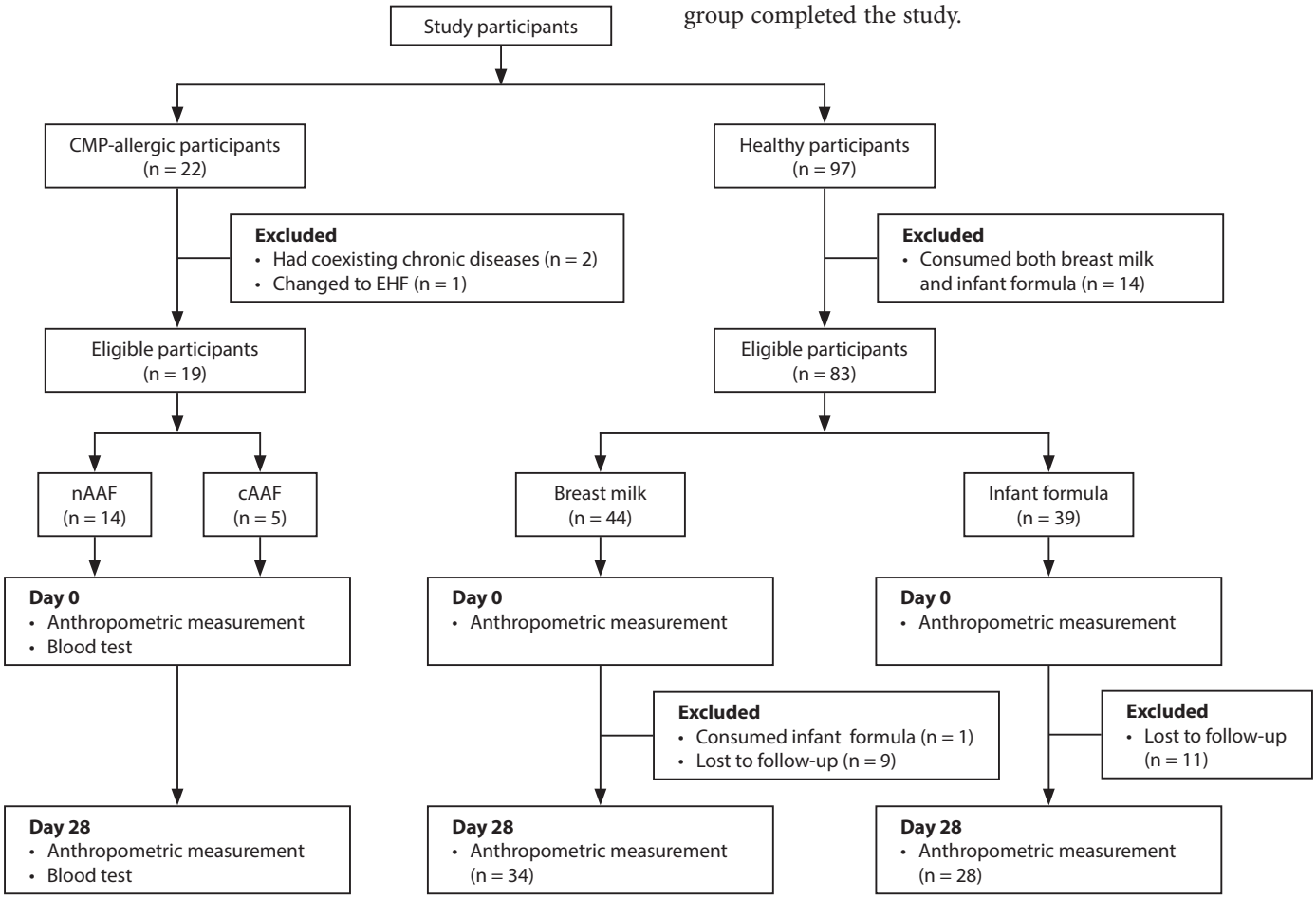

Figure 1. Flow chart of the study.

Abbreviations: CMP: cow's milk protein; EHF: extensively hydrolyzed protein formula; nAAF: new amino acid-based formula; cAAF: commercial amino acid-based formula.

Baseline demographic data

The median age (P25, P75) among the four groups was not significantly different, which was 4.0 (3.0, 4.7) months for nAAF; 2.9 (2.0, 4.1) months for cAAF; 2.6 (2.1, 4.2) months for infant formula; and 3.9 (2.2, 4.2) months for breast milk.

In CMP-allergic infants, sex, growth parameters at birth, age and growth parameters at enrollment, symptoms of CMPA, and percentage of IgE-mediated CMPA of the nAAF group were comparable to those of the cAAF group (**Table 1**). At birth, all growth z-scores were not different among the four groups, except LAZ, which was lower in the infant formula group compared to the nAAF group (**Figure 2**). Prior to enrollment, CMP-allergic infants had been fed more than one food. The nAAF group included infants fed breast milk (n = 12, 86%), cow's milk-based formula (n = 11, 79%), goat milk-based formula (n = 1, 7%), soy protein-based formula (n = 5, 36%), partially hydrolyzed protein formula (n = 3, 21%), extensively hydrolyzed protein formula (n = 10, 71%), or cAAF (Puramino®) (n = 3, 21%) with persistent clinical symptoms. Similarly, the cAAF group consisted of infants fed breast milk (n = 4, 80%), cow's milk-based formula (n = 4, 80%), partially hydrolyzed protein formula (n = 1, 20%), extensively hydrolyzed protein formula (n = 3, 60%), nAAF (n = 2, 40%), or cAAF (Puramino®) (n = 3, 60%) with persisting clinical symptoms. On day 0, the nAAF group had significantly lower WAZ than the breast milk and infant formula groups, lower HAZ than the breast milk group, and lower LAZ than the infant formula group (**Figure 2**).

Formula intake

Daily intake including volume, energy, protein, carbohydrate and lipid during the 4 week period did not differ significantly between the nAAF group and the cAAF group (**Table 1**). Furthermore, when considering the formula groups, no significant differences in daily intake were observed among the nAAF, cAAF, and infant formula groups (data not shown).

Table 1. Characteristics of CMP-allergic infants and enteral nutrition intake during a 28-day period.

	nAAF (n = 14)	cAAF (n = 5)	*p*-value
Male, n (%)	9 (64.3)	5 (100)	0.257
Growth parameters at birth			
Weight-for-age z-score	-0.74 ± 0.99	-0.03 ± 0.50	0.115
Length-for-age z-score	0.63 ± 1.10	0.38 ± 0.60	0.642
Head circumference-for-age z-score[a]	-0.90 ± 1.15	0.03 ± 0.79	0.120
Age at enrollment (months)[a]	4.0 (3.0, 4.7)	2.9 (2.0, 4.1)	0.156
Growth parameters at enrollment			
Weight-for-age z-score[a]	-1.60 (-2.42, -0.84)	-1.29 (-4.63, 0.42)	0.823
Length-for-age z-score[a]	-1.04 (-2.73, -0.26)	-1.75 (-3.61, 0.19)	0.754
Head circumference-for-age z-score[a]	-0.97 (-1.74, -0.36)	-0.70 (-2.87, 0.36)	0.956
Symptoms of cow's milk protein allergy[b]			
Dermatological, n (%)	9 (64.3)	3 (60.0)	1.000
Respiratory, n (%)	4 (28.6)	3 (60.0)	0.305
Gastrointestinal, n (%)	11 (78.6)	2 (40.0)	0.262
Anaphylaxis, n (%)	0 (0)	0 (0)	-
IgE-mediated CMPA, n (%)	5 (41.7)[c]	2 (40.0)	1.000
Volume of formula intake (mL/kg/day)[a]	197 (179, 221)	160 (124, 233)	0.257
Energy intake (kcal/kg/day)	138.52 ± 32.54	117.90 ± 46.63	0.28
Protein intake (g/kg/day)	3.06 ± 0.72	2.94 ± 0.74	0.772
Carbohydrate intake (g/kg/day)	14.26 ± 3.35	12.22 ± 4.43	0.297
Lipid intake (g/kg/day)	8.15 ± 1.91	6.66 ± 2.77	0.200

Abbreviations: CMP: cow's milk protein; nAAF: new amino acid-based formula; cAAF: commercial amino acid-based formula
Data are expressed as mean ± standard deviation, otherwise is indicated.
[a]Data are expressed as median (P25, P75).
[b]Each participant had more than one symptom.
[c]Specific IgE was not evaluated in two subjects.

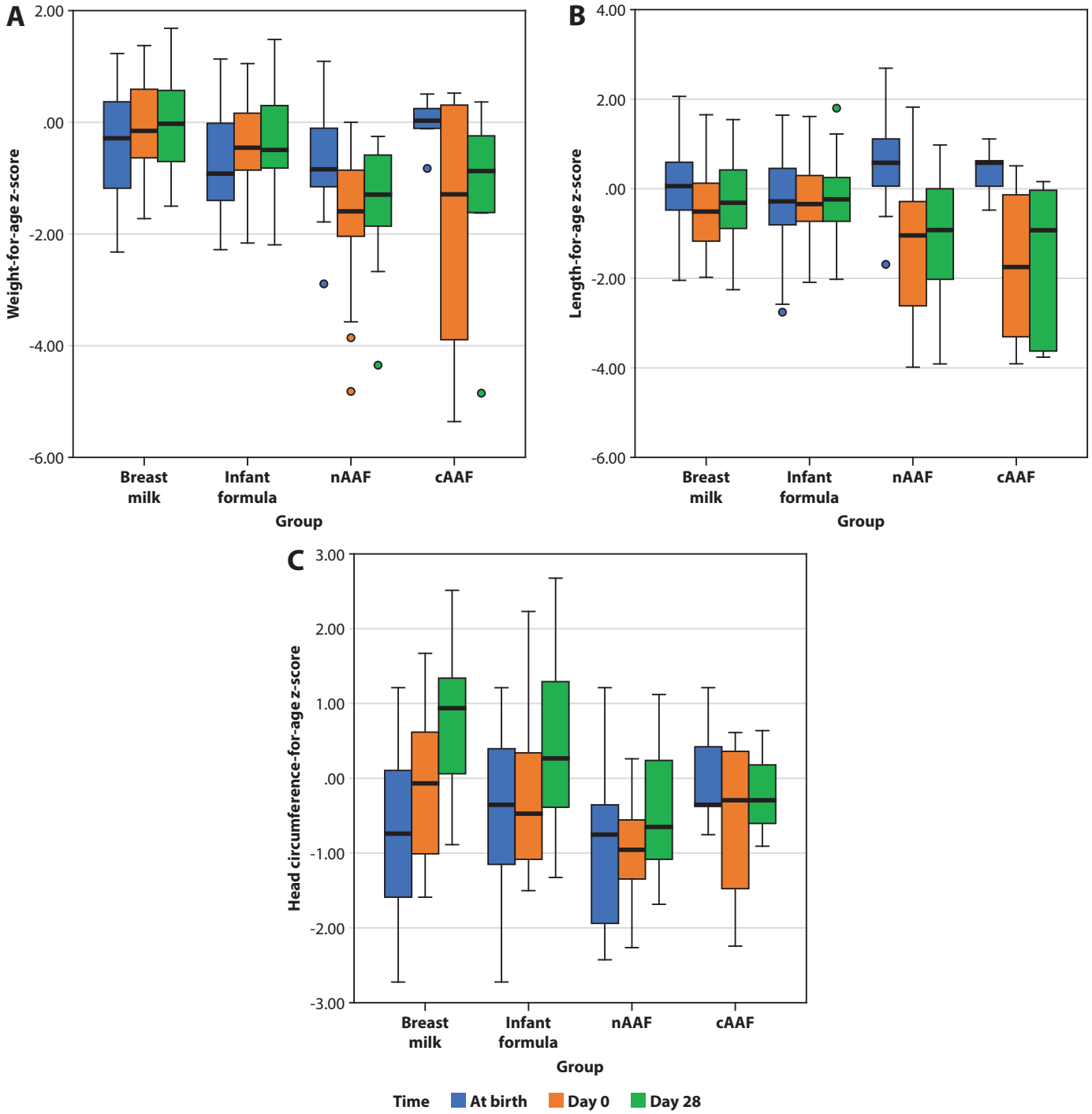

Figure 2. Z-scores of growth parameters at birth and during the study period. Weight-for-age z-score (A), Length-for-age z-score (B), Head circumference-for-age z-score (C). Data are presented as the box-and-whisker plot and median [P25, P75].

Abbreviations: nAAF: new amino acid-based formula; cAAF: commercial amino acid-based formula; BM: breast milk; IF: infant formula

Z-score	Time	Breast milk (median, [P25, P75])	Infant formula (median, [P25, P75])	nAAF (median, [P25, P75])	cAAF (median, [P25, P75])
Weight-for-age	At birth	-0.28 [-1.22, 0.38]	-0.91 [-1.40, 0.01]	-0.85 [-1.23, -0.06]	0.03 [-0.46, 0.38]
	Day 0	-0.15 [-0.63, 0.65]	-0.45 [-0.89, 0.17]	-1.60 [-2.42, -0.84]	-1.29 [-4.63, 0.42]
	Day 28	-0.03 [-0.72, 0.62]	-0.49 [-0.82, 0.32]	-1.30 [-1.90, -0.58]	-0.87 [-2.98, 0.07]
Length-for-age	At birth	0.06 [-0.47, 0.59]	-0.28 [-0.91, 0.46]	0.59 [0.06, 1.39]	0.59 [-0.21, 0.86]
	Day 0	-0.51 [-1.17, 0.16]	-0.33 [-0.74, 0.35]	-1.04 [-2.73, -0.26]	-1.75 [-3.61, 0.19]
	Day 28	-0.31 [-0.91, 0.42]	-0.23 [-0.73, 0.32]	-0.93 [-2.09, 0.01]	-0.93 [-3.78, 0.07]
Head circumference-for-age	At birth	-0.74 [-1.59, 0.10]	-0.36 [-1.15, 0.41]	-0.76 [-1.94, 0.03]	-0.36 [-0.56, 0.82]
	Day 0	-0.07 [-1.01, 0.63]	-0.48 [-1.10, 0.38]	-0.96 [-1.52, -0.17]	-0.30 [-1.86, 0.48]
	Day 28	0.94 [0.30, 1.35]	0.27 [-0.42, 1.32]	-0.66 [-1.17, 0.24]	-0.30 [-0.91, 0.64]

Z-score	Time	BM vs nAAF *p*-value	BM vs cAAF *p*-value	IF vs nAAF *p*-value	IF vs cAAF *p*-value	nAAF vs cAAF *p*-value
Weight-for-age	At birth	0.208	0.334	0.906	0.104	0.087
	Day 0	< 0.001	0.152	< 0.001	0.419	0.754
	Day 28	< 0.001	0.089	0.001	0.207	0.500
Length-for-age	At birth	0.053	0.294	0.011	0.116	0.703
	Day 0	0.054	0.152	0.017	0.142	0.622
	Day 28	0.041	0.178	0.052	0.226	0.687
Head circumference-for-age	At birth	0.667	0.139	0.237	0.338	0.143
	Day 0	0.022	0.535	0.143	0.934	0.624
	Day 28	< 0.001	0.041	0.015	0.429	0.536

Z-score	Day	nAAF *p*-value	cAAF *p*-value	Infant formula *p*-value	Breast milk *p*-value
Weight-for-age	0 vs 28	0.041	0.500	0.025	0.722
Length-for-age	0 vs 28	0.706	0.345	0.891	0.139
Head circumference-for-age	0 vs 28	0.239	0.593	< 0.001	< 0.001

Figure 2. (Continued)

Changes in growth parameters

On day 28, the WAZ, LAZ and HAZ of the nAAF group were lower than those of the breast milk group; and the WAZ and HAZ were lower than the infant formula group (**Figure 2**).

On day 28 compared to day 0, a significant increase in the median (P25, P75) of WAZ was observed only in the nAAF group (-1.30 (-1.90, -0.58) *versus* -1.60 (-2.42, -0.84), $p = 0.041$), whereas no significant change was found in all growth parameter z-scores in the cAAF group. Significant increases in HAZ were observed on day 28 compared to day 0 only in the breast milk and infant formula groups. Regarding CMPA associated with IgE, no differences in WAZ, LAZ, and HAZ were observed at birth, day 0, and day 28 between the IgE-mediated and non-IgE-mediated groups (data not shown).

Both the nAAF and cAAF groups did not differ in terms of increases in WAZ, LAZ, and HAZ between day 0 and day 28, even after adjusting for sex, age at the beginning of AAF, z-scores of growth parameters at day 0, and duration between the beginning of AAF and day 0 (**Table 2A**). The increases in WAZ and LAZ, except for HAZ, between day 0 and day 28 in the nAAF group did not differ from the breast milk or infant formula group, after adjusted for sex, age on day 0, and z-scores of growth parameters on day 0 (**Table 2B**).

Table 2A. Changes in growth parameter z-scores between day 0 and day 28 in the nAAF group compared with cAAF group.

	Change in WAZ		Change in LAZ		Change in HAZ	
	Regression coefficient (95% CI)	*p*-value	Regression coefficient (95% CI)	*p*-value	Regression coefficient (95% CI)	*p*-value
nAAF compared with cAAF	0.088 (-0.619, 0.796)	0.791	0.045 (-0.789, 0.880)	0.909	-0.645 (-2.082, 0.793)	0.337

Abbreviations: nAAF: new amino acid-based formula; cAAF: commercial amino acid-based formula; WAZ: weight-for-age z-score; LAZ: length-for-age z-score; HAZ: head circumference-for-age z-score.
Statistical analysis was performed using multivariable linear regression analysis adjusted for gender, age (in month) at the beginning of AAF, z-scores of growth parameters at Day 0, and duration (in day) between the beginning of AAF and Day 0.

Table 2B. Changes in growth parameter z-scores between day 0 and day 28 of the nAAF group compared with the breast milk or infant formula group.

	Change in WAZ		Change in LAZ		Change in HAZ	
	Regression coefficient (95% CI)	*p*-value	Regression coefficient (95% CI)	*p*-value	Regression coefficient (95% CI)	*p*-value
BM compared with nAAF	-0.172 (-0.455, 0.110)	0.228	0.209 (-0.193, 0.611)	0.303	1.184 (0.535, 1.834)	0.001
IF compared with nAAF	-0.159 (-0.441, 0.122)	0.264	0.072 (-0.363, 0.507)	0.743	0.817 (0.147, 1.487)	0.018

Abbreviations: nAAF: new amino acid-based formula; BM: breast milk; IF: infant formula; WAZ: weight-for-age z-score; LAZ: length-for-age z-score; HAZ: head circumference-for-age z-score.
Statistical analysis was performed using multivariable linear regression analysis adjusted for sex, age (in month) at Day 0, and z-scores of growth parameters at Day 0.

Table 3. Changes in plasma amino acids between nAAF and cAAF groups during a 28-day period.

Plasma amino acids	Reference ranges	nAAF		cAAF		*p*-value		
		Day 0	Day 28	Day 0	Day 28	Day 0[a]	Day 28[a]	Day 28 - Day 0[b]
Essential amino acids (μmol/L)								
Threonine	40-248	150.2 ± 59.9	164.0 ± 53.1	370.2 ± 212.1	154.0 ± 26.6	0.081	0.724	0.057
Tryptophan	16-92	31.1 (27.8, 38.7)	30.6 ± 10.0	29.4 (23.4, 88.1)	31.5 ± 15.6	0.645	0.882	0.407
Lysine	70-258	101.5 (82.2, 111.4)	106.7 ± 34.2	119.3 (100.4, 237.7)	121.8 ± 38.6	0.087	0.422	0.202
Leucine	43-181	78.1 (68.5, 104.6)	84.0 ± 19.6	82.4 (79.3, 141.0)	83.4 ± 36.8	0.343	0.975	0.257
Valine	84-354	163.7 (122.0, 190.8)	156.6 ± 29.4	151.1 (145.2, 244.9)	158.0 ± 39.2	0.823	0.890	0.605
Isoleucine	10-109	42.2 (34.1, 52.6)	37.3 ± 10.2	44.9 (37.5, 81.3)	45.2 ± 12.0	0.444	0.183	0.675
Methionine	12-50	29.1 ± 12.6	22.0 ± 7.1	24.7 ± 5.5	25.3 ± 7.9	0.465	0.539	0.257
Phenylalanine	31-92	56.1 (46.5, 60.0)	49.3 ± 10.6	49.0 (35.0, 56.9)	44.0 ± 16.7	0.343	0.420	0.584
Histidine	42-125	108.0 ± 31.7	68.3 (54.4, 81.4)	83.5 ± 35.9	58.5 (44.0, 74.6)	0.153	0.500	0.679

Table 3. (Continued)

Plasma amino acids	Reference ranges	nAAF		cAAF		*p*-value		
		Day 0	Day 28	Day 0	Day 28	Day 0[a]	Day 28[a]	Day 28 - Day 0[b]
Non-essential amino acids (μmol/L)								
Alanine	119-523	270.8 (231.7, 365.3)	295.2 ± 88.3	215.5 (153.0, 423.7)	243.8 ± 76.9	0.444	0.266	0.770
Aspartic acid	2-14	9.4 (6.5, 12.7)	7.8 ± 2.7	10.40 (9.1, 11.3)	8.2 ± 3.0	0.442	0.830	0.871
Asparagine	20-77	56.0 (52.9, 75.3)	67.4 ± 25.6	83.3 (59.5, 117.1)	103.4 ± 26.8	0.219	0.016	0.116
Arginine	30-147	75.8 ± 24.8	68.1 ± 24.9	91.4 ± 47.1	57.9 ± 22.2	0.355	0.431	0.369
Glutamic acid	32-185	179.3 ± 58.3	103.2 (78.9, 161.4)	157.4 ± 79.0	122.0 (75.2, 155.8)	0.519	0.964	0.864
Glutamine	303-1459	476.9 ± 106.8	495.5 ± 104.0	476.6 ± 226.3	437.7 ± 141.9	0.996	0.347	0.455
Glycine	103-386	225.4 ± 66.3	211.6 ± 56.0	317.5 ± 174.7	217.8 ± 56.6	0.308	0.836	0.302
Proline	104-348	170.1 (131.0, 236.7)	158.5 (120.9, 236.2)	164.6 (133.5, 289.5)	146.5 (111.4, 174.8)	0.964	0.391	0.201
Serine	83-212	128.6 (120.4, 152.2)	133.4 ± 21.7	137.5 (127.4, 227.2)	125.1 ± 26.0	0.298	0.493	0.383
Taurine	26-130	7.7 (6.1, 10.2)	6.3 (4.8, 9.6)	9.1 (4.0, 85.0)	7.9 (4.8, 119.0)	0.754	0.444	0.130
Tyrosine	24-125	67.1 ± 24.3	63.2 (45.4, 70.2)	83.8 ± 55.7	54.1 (42.6, 95.6)	0.365	0.687	0.434

Abbreviations: nAAF: new amino acid-based formula; cAAF: commercial amino acid-based formula.
[a]Independent sample *t*-test or Mann-Whitney U test for the difference between 2 groups at day 0 or day 28.
[b]Independent sample *t*-test or Mann-Whitney U test for the difference between 2 groups on the difference of day 28 to day 0.

Table 4. Changes in biochemical indices of protein status between nAAF and cAAF groups during a 28-day period.

	nAAF		cAAF		*p*-value		
	Day 0	Day 28	Day 0	Day 28	Day 0[a]	Day 28[a]	Day 28 - Day 0[b]
Blood urea nitrogen (mg/dL)	7.4 (6.3, 8.2)	7.9 ± 1.5	11.0 (8.4, 12.1)	11.6 ± 3.8	0.034	0.093	0.754
Plasma creatinine (mg/dL)	0.2 (0.2, 0.2)	0.2 ± 0.0	0.2 (0.2, 0.3)	0.2 ± 0.1	0.391	0.525	0.350
Plasma albumin (g/dL)	4.1 ± 0.3	4.3 ± 0.3	3.7 ± 0.2	4.2 ± 0.1	0.012	0.223	0.026

Abbreviations: nAAF: new amino acid-based formula; cAAF: commercial amino acid-based formula
[a]Independent sample *t*-test or Mann-Whitney U test for the difference between two groups at day 0 or day 28.
[b]Independent sample *t*-test or Mann-Whitney U test for the difference between two groups on the difference between day 0 and day 28.

Changes in protein status

There were no differences between the nAAF and cAAF groups in plasma amino acid concentrations, except for asparagine, which was higher in the cAAF group on day 28 (**Table 3**). The changes in all plasma amino acid concentrations were not different between the two CMPA groups. All plasma amino acids, except taurine, in both groups were within the reference ranges on day 0 and day 28.

On day 0, the nAAF group had fewer BUN but greater albumin than the cAAF group (**Table 4**). After receiving AAF for 28 days, the changes in BUN and creatinine values were not different between the two groups of CMPA. The incremental change in plasma albumin in the cAAF group was significantly greater than in the nAAF group; however, plasma albumins from both groups on day 28 were still within the normal range.

Discussion

We evaluated the effects of nAAF on growth and protein status in the intended participants who were CMP-allergic infants, not healthy infants. Furthermore, participants did not start receiving complementary foods that might have a confounding effect on the results. This ensured the clinical relevance of the study results.

The WAZ of the nAAF group on day 0 had significantly lower values compared to healthy participants, which were in agreement with the results published by Canani et al[11] showing lower WAZ in CMP-allergic participants compared to healthy controls.[11] This impairment of the growth of young infants suffering from CMPA indicated that CMPA may affect infant intake, digestion, and absorption of nutrients, or loss of nutrients, resulting in impaired growth of young infants.[18]

Taking into account WAZ, LAZ, and HAZ at birth and at enrollment of the participants, no differences were observed between the nAAF and cAAF groups indicating similar background characteristics. All anthropometric z-scores in both the nAAF and cAAF groups trended closer to 0 after treatment with their amino acid-based formulas for 28 days, with only a significant increase in WAZ in the nAAF group. However, there were no significant differences in the increase in WAZ, LAZ and HAZ from day 0 to day 28 comparing between the nAAF and cAAF groups after adjusting for potential confounders, suggesting that participants managed with nAAF maintained a similar growth velocity compared to those with cAAF.

The detailed consumption of the formula was not different between the nAAF and cAAF groups, suggesting a similar acceptance of the study formulas.

In this study, no dropout was observed in the nAAF and cAAF groups. A possible explanation might include that participants in both AAF groups, being symptomatic CMPA, had a favorable clinical response after treatment with amino acid-based formulas, resulting in good tolerance and acceptance. Therefore, this argues for taste-influenced acceptance. Compared to previous studies of AAF, the dropout rate was 44%[19] and 37%[20] in healthy full-term infants that was explained by caregivers' dislike of AAF due to the unusual taste or smell. Furthermore, the study of AAF in infants with CMPA had a dropout rate of 18% that was mainly attributed to adverse events and partly due to loss of follow-up and withdrawal of consent.[21] The dropout rate of healthy participants in this study was 23% for the breast milk group and 28% for the infant formula group, which could be due to urban-rural migration of participants and their families, and the inability to continue breastfeeding.

The nAAF used in this study is a nutritionally complete hypoallergenic infant formula containing amino acids, rice starch glucose polymer that is eliminated in its protein fraction, long-chain polyunsaturated fatty acids, minerals, and trace elements. In terms of fat content, the cAAF in this study did not contain MCTs. In comparison, a hypoallergenic formula containing a high content of MCTs (> 50% of total fat) may be associated with impaired growth;[18] but growth impairment was not found with the use of AAF containing 24% MCTs in combination with complementary foods without CMP in infants allergic to CMP from 16 weeks to 9 months of age.[12] Therefore, in this study there is no concern about MCTs content in both AAFs for suboptimal growth.

All plasma amino acid concentrations in both the nAAF and cAAF groups were within the age reference ranges during the study period; except taurine, which is a non-essential amino acid that may not have clinical significance in this age group. After 28 days of treatment, the serum albumin values, but not BUN and creatinine, increased, indicating that both nAAF and cAAF are suitable to improve protein status among infants allergic to CMP.

The strength of this study is that the growth parameters and protein data of infants suffering from isolated severe CMPA of less than 6 months of age were affected by only amino acid-based formulas without the confounding effect of complementary foods and other possible allergenic foods. This study has some limitations. First, the diagnosis of CMPA in most participants was not confirmed by a DBPCFC test. Second, CMP-allergic infants were not randomized into either group of AAF, but they had similar demographic data. Third, there was a small number of participants in the cAAF group, but the amount of participants in the nAAF group (n = 14) was 82% of estimated amounts required to draw conclusions about its findings. However, the results in this study were objective data (anthropometric values) that could not be biased by the preference of parents for formula type. Moreover, we tried to avoid bias by maximizing adherence and blinding data collectors. Fourth, this study did not assess and show the information of daily intake in the breast milk group that was exclusively breastfed; this point may have an impact on the study's findings. However, their z-scores for growth parameters were within normal limits during the study period.

Conclusions

This study demonstrates that the 28-day treatment with nAAF for young infants with CMPA supports growth and protein status, which is comparable to cAAF. Additionally, nAAF has similar weight and length gain to breast milk and infant formula. Our new amino acid-based formula is an alternative formula to treat CMPA that does not respond to EHF in young infants. As growth parameters and protein status were assessed after only 28 days of treatment, a long-term follow-up period may be required to confirm the effect of nAAF in terms of growth parameters and protein status.

Acknowledgments

The authors declare that there is no conflict of interest with respect to the publication of this article. The authors thank all participants and caregivers for their contributions to this study, and Asst. Prof. Dr. Chulaluk Komoltri and Miss Kanokwan Sommai for assistance with statistical analysis. This research project was supported by a grant from the Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand [Grant number (IO) R015633002]. N.D. and P.J. were responsible for study conception and design. P.J., P.P., and S.N. were responsible for data collection. N.D., H.T., S.K., K.C., and P.T. were responsible for data collection, data analysis, and data interpretation. All authors were responsible for preparation of the draft manuscript. N.D. was responsible for critical revision of the manuscript.

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