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Amino acid-based formula in cow's milk allergy: long-term effects on body growth and protein metabolism. A randomized trial.

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Abstract

Objectives. The long-term effects of amino acid–based formula (AAF) in the treatment of cow's milk allergy (CMA) is largely unexplored. This study comparatively evaluates body growth and

protein metabolism in CMA children treated with AAF or with extensively hydrolysed whey formula (eHWF), and healthy controls (HCs).

Methods. A 12-month multicentre randomised control trial (RCT) was conducted in outpatients with CMA (aged 5–12 m) randomised in two groups, treated with AAF (Group 1) and eHWF (Group 2), and compared with HCs (Group 3) fed with follow-on (if age <12 m) or growing-up formula (if age >12 m). At enrolment (T0), after 3 (T3), 6 (T6) and 12 months (T12) a clinical evaluation was performed. At T0 and T3, were dosed in CMA subjects serum levels of albumin, urea, total protein, retinol binding protein, and insulin-like growth factor 1 (IGF-1).

Results. 21 subjects in Group 1 (61.9% male, aged 6.5 ±1.5 m), 19 in Group 2 (57.9% male, aged 7 ±1.7 m) and 25 subjects in Group 3 (48% male, aged 5.5±0.5 m) completed the study. At T0, the weight z-score was similar in Group 1 (−0.74) and 2 (−0.76), with differences compared to Group 3 (−0.17, $p < 0.05$). At T12, the weight z-score value was similar between the three groups without significant differences. There were no significant changes in protein metabolism in children in Groups 1 and 2.

Conclusions. Long-term treatment with AAF is safe and allows adequate body growth in CMA children without alterations in protein metabolism.

Keywords: Body length, body weight, extensively hydrolysed whey formula, retinol binding protein, serum albumin

What Is Known:

- The epidemiological pattern of cow's milk allergy has changed during the last decades with increased risk of persistence and severity of clinical manifestations.
- Cow's milk exclusion diet is the only available treatment, and may lead to nutritional deficiencies and poor growth.
- Amino acid–based formula is currently used in patients that are non-responsive to or intolerant of other treatments, but data on long-term use are limited by short-term observation periods.

What Is New:

- The long-term use of amino acid–based formula is able to stimulate a growth pattern similar to extensively hydrolysed whey formula in children with cow's milk allergy.

Introduction

Cow's milk allergy (CMA) is one of the most common food allergies in childhood. The epidemiological pattern of the disease has changed during the last decades with an increased severity of clinical manifestations and risk of persistence (1,2). The actual prevalence in children under 3 years of age is up to 3% in industrialised countries (3,4). CMA is the leading cause of food-induced anaphylaxis requiring hospitalisation in the Italian paediatric population (5,6). Recent data shows that only about a half of these patients acquire oral tolerance 2 years after CMA diagnosis (2,7).

A diet that excludes cow's milk is the only available treatment (8,9). There are two main goals of nutritional intervention for patients with CMA: preventing allergic reactions through allergen avoidance and ensuring optimal nutrition and body growth on the restricted diet. Cow's milk exclusion diets without appropriate substitution may lead to nutritional deficiencies and poor growth (10). A summary report from an international consensus panel of allergy specialists recommends substituting a formula "of adequate nutritional value" or breast milk until 2 years of age. Suitable formulas include extensively hydrolysed whey formula (eHWF) and amino acid–based formula (AAF), which are considered to be hypoallergenic. The total protein content and composition of these two formulas are similar, but the form in which the amino acids are delivered to the infant is different, with the latter containing only free amino acids. These differences may have implications in protein metabolism (11).

Amino acid–based formula is currently used in all cases that are non-responsive to or intolerant of treatment with eHWF and in all cases characterised by severe allergic reactions and multiple food allergies because this formula is by definition nonallergenic (12). AAF is able to ensure a rapid recovery from clinical symptoms (12–14), but data on long-term use are limited by short-term observation periods (up to 6–9 months) and by a lack of healthy controls evaluation (8,14–16).

Considering the changing scenario of CMA, it is important to better define the long-term effect of AAF strategy on body growth and protein metabolism.

In this study, we aimed to comparatively evaluate the effects on body growth and protein metabolism of AAF and eHWF in CMA children with a parallel age-matched healthy group on an unrestricted diet. The primary study outcome was the evaluation of possible differences in body weight z-score after 12 months of treatment among the groups. Secondary study outcomes include possible differences in length and head circumference z-scores after 12 months of treatment, and possible differences in protein metabolism biomarkers.

Methods

Study design

This multicentre randomised control trial was conducted in consecutive outpatient infants (aged 5–12 months) referred to three Italian tertiary centres for Pediatric Allergy and Nutrition (located in Naples, Rome, and Milan) for strongly suspected CMA, but still receiving cow's milk proteins. The study design was discussed. According to a centralised randomisation list, subjects were allocated to one of the two groups of dietary interventions: Group 1, patients receiving AAF (Neocate®, Nutricia, Milan, Italy for children up to the age of 12 months, switched to Neocate® Advance, Nutricia, Milan Italy after the completion of the 12th month of age); and Group 2, patients receiving an eHWF (Hypolac DMF srl, Limbiate, Italy). When full and stable remission of CMA symptoms was achieved, a double-blind placebo-controlled food challenge (DBPCFC) was performed as previously described (4,17,18). Briefly, every 20 minutes, successive doses (0.1, 0.3, 1, 3, 10, 30, and 100 mL) of fresh pasteurised cow's milk (CM) containing 3.5% fat or an amino acid–based formula were administered. Full emergency equipment and medications (epinephrine,

antihistamines, and steroids) were available. The results were assessed simultaneously by three experienced paediatric allergists. Study subjects were scored for 9 items divided into 4 main categories: i. General (lowered blood pressure plus tachycardia); ii. Skin (rash, urticaria/angioedema); iii. Gastrointestinal (nausea/repeated vomiting, crampy-like abdominal pain, diarrhoea); and iv. Respiratory (sneezing/itching, nasal congestion/rhinorrhea, stridor deriving from upper airway obstruction or wheezing) on a 0- to 3-point scale (0, none; 1, light; 2, moderate; and 3, severe). If at least 2 of the 3 physicians independently scored any item at level 3, or 2 (or more) items at level 2, the test result was considered positive. Clinical symptoms occurring within 2 h of administering the highest dose were defined as "immediate reactions." The infants were observed for 2 h after the final dose and then discharged. In the case of a positive DBPCFC at any testing dose, the patient remained under observation until symptom resolution. If the patient did not show any symptoms within the first 24 hours, parents were advised to give one single feed of 100 ml of the tested formula (verum or placebo) every day at home for 7 days. If any symptoms occurred during this period, the patients returned to the outpatient clinic on the same day. After 7 days of verum or placebo administration, the patients were examined and the parents interviewed at the centre. To rule out false-negative challenge results, parents were asked to contact the centre if any symptoms occurred in the following 7 days after the DBPCFC procedures. The challenge was considered negative if the patient tolerated the entire challenge, including the observation period. Only infants with DBPCFC-based CMA diagnoses continued the trial. During the same period, a third parallel group of age-matched healthy children on an unrestricted diet was consecutively evaluated at the centres while undergoing minor surgical procedures and enrolled as controls (Group 3). These children assumed a follow-on formula up to the age of 12 months and were switched to growing-up formula after the completion of the 12th month of age.

The nutrient composition of the formulas consumed by study subjects is reported in Table 1.

We excluded patients with a history of prematurity; CMA-induced anaphylaxis; other concomitant food allergies; eosinophilic disorders of the gastrointestinal tract; chronic systemic diseases; chronic infections; immunodeficiencies; inflammatory bowel diseases; primary gastroesophageal reflux disease; celiac disease; cystic fibrosis; metabolic diseases; malignancies; chronic pulmonary, respiratory, cardiac, and renal diseases; malformations; chronic neuropsychiatric diseases; renal failure; or moderate to severe malnutrition at enrolment (body weight for age z-score: <−2 SD).

Written informed consent was obtained from the parents/tutors of each study subject. The study was approved by the ethics committee of each institution and was registered in the Clinical Trials Protocol Registration System (ID number NCT02379598).

All subjects were evaluated not by the investigators, but by a multidisciplinary team (unaware of the study aims and in charge at the three centres), comprised of a paediatrician, a nurse, and a dietitian at enrolment (T0) and at 3- (T1), 6- (T2), and 12-month (T3) control visits. The medical record of each child was recorded on a clinical chart.

At each visit, the following variables were determined:

I. Weight, length or height, and head circumference were measured using standard procedures (11).

II. Anthropometric indices (*z*-score for weight, *z*-score for length/height, *z*-score for head circumference) were determined using the Euro-Growth References (19,20).

III. Seven-day food record was analysed using ad hoc software based on the Italian food composition tables (Winfood Pro 3.7, released 2012, Medimatica Srl, Teramo, Italy) (21).

IV. Food allergy–related signs and symptoms were recorded.

At each visit, the dietitian explained to parents how to record the amount and type of foods and drinks consumed by the child over a period of 7 consecutive days, including 5 weekdays and 2 weekend days. The chart also contained instructions about how to record the food consumed and

how to measure food using graduated bowls, cups, dishes, and spoons. Seven days after enrolment and at each subsequent visit, the dietitian examined the dietary history and reviewed the food records. Starting from the enrolment visit, study subjects underwent a personalised dietary counselling session with the dietitian, as previously described (22). Briefly, dietary counselling was based on the evaluation of (i) body weight, length/height, weight-to-height ratio, and head circumference; (ii) protein and energy requirements. At each visit, the diet of study subjects was carefully assessed with the aim to give 400–600 ml/day of formula together with correct total energy and nutrient intake. The key words and phrases used during dietary counselling were emphasised to encourage discussion about food-related topics at home.

At T0 and T3, venous blood sampling (2 ml) was performed after an overnight fast in subjects with CMA to determine serum biomarkers of protein metabolism (urea, total proteins, albumin, retinol binding protein, insulin-like growth factor 1 (IGF-1)). Samples were labelled with an anonymous identification code and stored at −20°C until analysis.

All samples were analysed during the same session using the same lot of reagents by personnel unaware of the study aims and group assignment. In particular, retinol binding protein was measured using Dimension Vista, an automated immunoturbidimetric platform (Siemens Healthcare Diagnostics Products, Milan, Italy).

Urea, total proteins, and albumin were measured using Cobas 8000 c702, a fully automated UV kinetic and colourimetric platform (Roche/ Hitachi Manufacture, Holliston, Massachusetts).

IGF-1 was measured using Liason XL (DiaSorin Manufacture, Saluggia, Italy), a fully automated chemiluminescence analyser.

Sample Size

To detect a possible difference between Groups 1 and 2, up to 0.2 in the z-score for body weight at 12 months, 19 subjects per group were requested (type I error of 5%, power 81%). Considering that

patients were enrolled before diagnostic challenge for CMA, and possible dropouts, this number was increased to 25 per group.

Statistics

Intention-to-treat (ITT) and per-protocol (PP) analyses were conducted. For continuous variables, groups were compared using equality of means testing. The $\chi 2$ test and Fisher's exact test were used for categorical variables. When necessary, comparisons were performed with nonparametric tests (Wilcoxon–Mann–Whitney U test). Results were reported as means and 95% confidence interval (CI) and as median and interquartile range (IQR) due to nonnormal distribution (established by the Kolmogorov test). The level of significance for all statistical tests was 2-sided, $p < 0.05$. All data were collected in a dedicated database and analysed by a statistician who was unaware of patients' group assignment, with IBM SPSS Statistics version 19.0 for Windows (SPSS Inc, Chicago, IL).

Results

The flow of the study population is reported in Figure 1 (see also Supplemental Digital Content, *http://links.lww.com/MPG/A745*). 56 consecutive subjects were evaluated for the study; 6 children were excluded because the presence of exclusion criteria. Fifty were randomized into 2 study groups and 10 were excluded after negative DBPCFC for cow's milk.

Main baseline features of the study groups are described in Table 2. Table 3 reports main baseline features of subjects with positive diagnostic DBPCFC who continued the treatment with the dietary products: 21 subjects in Group 1 and 19 in Group 2. CMA signs and symptoms recovered within the first 2 weeks of treatment in all patients without differences between subjects enrolled in Groups 1 and 2. No patient had other food allergies during the study period.

Per-protocol analysis revealed that at T0, body weight z-score values were not significantly different between the two intervention groups (Groups 1 and 2), but were significantly lower if compared to Group 3 (Figure 2, Panel a). Use of both hypoallergenic formulas resulted in similar weight gain for CMA subjects during the 12-month study period. At T3, a difference in body weight z-score was observed in Groups 1 and 2 compared to healthy children (Group 3). But at T6, no significant differences in body weight z-score were observed comparing CMA subjects and healthy controls. The effect was sustained until T12, when body weight z-score values remained similar among the 3 groups (Figure 2, Panel a). Slight but not significant differences were found at T0 for length z-score when CMA subjects (Groups 1 and 2) and healthy controls (Group 3) were compared. In both CMA groups, comparable increase in length was also observed. At T3, a difference in length z-score was observed between CMA patients and healthy children (Group 3). At T6, differences in length z-score were observed only in children treated with AAF, but not in children treated with eHWF. At T12, length z-score values were not significantly different among the 3 groups (Figure 2, Panel b). No differences at any time point were found for the three groups regarding the head circumference z-score (Figure 2, Panel c).

The intake of energy and proteins at each study point for the three study groups are reported in Table 4. Study formulas were well accepted and tolerated by all CMA patients, and energy and protein intake levels were comparable in Groups 1 and 2. A higher protein intake was found for CMA subjects at 12 months compared to Group 3.

The median values of protein metabolism biomarkers are reported in Table 5. At T0 and at T3, all CMA subjects showed protein metabolism biomarkers within normal range. No significant differences between Groups 1 and 2 were observed, with the exception of IGF-1 at T0 and urea at T3 median values, which were significantly higher in subjects receiving eHWF, but still within the normal range.

There was no centre effect.

Discussion

Although studies have demonstrated that AAF is effective for treating symptoms in virtually all infants with CMA, few studies have focused on the long-term efficacy of this type of formula in supporting adequate growth during infancy. Most studies on AAF were conducted over a relatively short period of observation in infants with CMA or in healthy subjects, and most studies were not designed to measure effects on growth (14–16, 23–25). The safety of long-term AAF use to support growth in CMA infants is an important issue, especially during the last decade when increased disease clinical severity and risk of persistence have been observed (13,23). As the persistence of CMA continues to increase, AAF use will likely increase, especially for infants with severe CMA and for specific subsets of infants (12). The present study was designed to comparatively assess the long-term effect on body growth of CMA infants fed an AAF versus an eHWF in a randomised controlled trial that met the robust proposed criteria (24). Our results show that long-term use of AAF is safe and able to stimulate a growth pattern similar to eHWF in CMA children. Both formulas, despite substantial differences in the form of protein components, were able to stimulate a progressive normalisation of anthropometric parameters in these patients without relevant alteration in protein metabolism as demonstrated by the serum biomarkers analysed in the study. These data are well in line with previous observations that AAF supports normal growth of healthy infants comparable to that of subjects fed eHWF (24). Small different body growth effects of AAF and eHWF have been described in two previous studies evaluating CMA infants during a 6- or 9-month intervention (26,27). The concomitant enrolment of patients affected by multiple food allergies, the

lack of detailed information on dietary intake, and the lack of concomitant evaluation of healthy controls make it difficult to interpret these data.

According to our findings, we can confirm that CMA is an at-risk condition for body growth. All CMA paediatric patients in our study presented with a negative body weight z-score at recruitment. Nutritional intervention with AAF or eHWF was equally effective in normalising these parameters. Beginning 3 months after randomisation, the two formulas led the body weight z-score toward the scores of healthy controls and 0 value of normality. The trajectories of these two interventions were very similar for all the examined parameters. Weight-for-age and length-for-age tracking were similar, which indicates that equal weight-gain velocity is achievable with either of these two different dietary approaches in CMA paediatric patients. A strength of our study is that diet and formula intake were assessed systematically. Parents completed 7-day intake records at enrolment and at 3, 6, and 12 months. The volume of formula consumed and the energy intake were similar between groups throughout the study. The similarity of these effects could derive from similar effects on satiation signals and metabolism. Thus, as previously reported by others (28), the detection of small peptides or free amino acids in the infant's gut after feeding may induce similar satiation signals and stimulate earlier meal termination and increased energy expenditure for infants who consumed these formulas.

Conclusion

We observed that after the 6th month of life, and within the support of an individualised dietary approach, AAF and eHWF supplied for a 12-month period are associated with a progressive recovery of body growth and normal protein metabolism. The choice between the two dietary strategies in CMA children should rely mainly on clinical considerations.

Supplemental Digital Content

Supplemental Digital Content 1, Figure: CONSORT 2010 Flow Diagram.

Supplemental Digital Content 2, Table: CONSORT 2010 Checklist.

References

1. McBride D, Keil T, Grabenhenrich L, et al. The EuroPrevall birth cohort study on food allergy: baseline characteristics of 12,000 newborns and their families from nine European countries. Pediatr Allergy Immunol 2012;23:230-9.
2. Schoemaker AA, Sprikkelman AB, Grimshaw KE, et al. Incidence and natural history of challenge-proven cow's milk allergy in European children--EuroPrevall birth cohort. Allergy 2015;70:963-72.
3. Sicherer, S.H. Epidemiology of food allergy. J Allergy Clin Immunol 2011;127:594–602
4. Boyce, J.A., Assa'ad, A., Burks, A.W., et al. Guidelines for the diagnosis and management of food allergy in the United States: summary of the NIAID-Sponsored expert panel report. J Allergy Clin Immunol 2010;126:1105–18.
5. Berni Canani R, Nocerino R, Terrin G, Leone L, Troncone R. Hospital admissions for food-induced anaphylaxis in Italian children. Clin Exp Allergy 2012;42:1813-4.
6. Nocerino R, Leone L, Cosenza L, Berni Canani R. Increasing rate of hospitalizations for food-induced anaphylaxis in Italian children: An analysis of the Italian Ministry of Health database.J Allergy Clin Immunol 2015;135:833-5.e3.
7. Burks AW, Tang M, Sicherer S, et al. ICON: food allergy. J Allergy Clin Immunol 2012;129:906-20.
8. De Greef E, Hauser B, Devreker T, Veereman-Wauters G, Vandenplas Y. Diagnosis and management of cow's milk protein allergy in infants. World J Pediatr 2012;8:19-24.
9. Frank Kneepkens C. M., Yolanda Meijer. Clinical practice. Diagnosis and treatment of cow’s milk allergy. Eur J Pediatr 2009;168:891–6.
10. Isolauri E1, Sütas Y, Salo MK, Isosomppi R, Kaila M. Elimination diet in cow's milk allergy: risk for impaired growth in young children. J Pediatr 1998;132:1004-19.

11. Academy of Pediatrics. Assessment of nutritional status. In: Pediatric Nutrition Handbook, 6th Academy of Pediatrics. United States of America. AAP Committee on Nutrition and Ronald E. Kleinman MD FAAP; 2009, p. 559.
12. Sicherer SH1, Noone SA, Koerner CB, Christie L, Burks AW, Sampson HA. Hypoallergenicity and efficacy of an amino acid-based formula in children with cow's milk and multiple food hypersensitivities. J Pediatr 2001;138:688-93.
13. de Boissieu D, Dupont C. Allergy to extensively hydrolyzed cow's milk proteins in infants: safety and duration of amino acid-based formula. J Pediatr 2002;141:271-3.
14. Niggemann B, Binder C, Dupont C, Hadji S, Arvola T, Isolauri E. Prospective, controlled, multi-center study on the effect of an amino-acid-based formula in infants with cow's milk allergy/intolerance and atopic dermatitis. Pediatr Allergy Immunol 2001;12:78-82.
15. Flammarion S, Santos C, Guimber D, et al. Diet and nutritional status of children with food allergies. Pediatr Allergy Immunol 2011;22:161–5.
16. Koletzko S, Niggemann B, Arato A, et al. Diagnostic approach and management of cow's-milk protein allergy in infants and children: ESPGHAN GI Committee practical guidelines. J Pediatr Gastroenterol Nutr 2012: 55: 221–9.
17. Bock SA, Sampson HA, Atkins FM, et al. Double-blind, placebo-controlled food challenge (DBPCFC) as an office procedure: a manual. J Allergy Clin Immunol 1988;82:986-97.
18. Bindslev-Jensen C, Ballmer-Weber BK, Bengtsson U, et al. Standardization of food challenges in patients with immediate reactions to foods—position paper from the European Academy of Allergology and Clinical Immunology. Allergy 2004;59:690-7.
19. Van't Hof MA, Haschke F. Euro-Growth references for body mass index and weight for length. Euro-Growth Study Group. J Pediatr Gastroenterol Nutr 2000;31(Suppl 1):S48-59.

20. Haschke F, Van't Hof MA. Euro-Growth references for length, weight, and body circumferences. Euro-Growth Study Group. J Pediatr Gastroenterol Nutr 2000;31(Suppl 1):S14-38.
21. Italian Society of Human Nutrition (SINU). Recommended levels of intake of energy and nutrients for the Italian population (LARN). Third revision. Medical Publishing and New Media (EDRA). Italy. SICS Editor 1996, p. 32.
22. Berni Canani R, Leone L, D'Auria E, et al. The effects of dietary counseling on children with food allergy: a prospective, multicenter intervention study. J Acad Nutr Diet 2014;114:1432-9.
23. Dupont C, Kalach N, Soulaines P, et al. Safety of a New Amino Acid Formula in Infants Allergic to Cow's Milk and Intolerant to Hydrolysates. J Pediatr Gastroenterol Nutr 2015;6:456-63.
24. Borschel MW, Ziegler EE, Wedig RT, Oliver JS. Growth of healthy term infants fed an extensively hydrolyzed casein-based or free amino acid-based infant formula: a randomized, double-blind, controlled trial. Clin Pediatr (Phila) 2013;52:910-7.
25. Taylor RR, Sladkevicius E, Panca M, Lack G, Guest JF. Cost-effectiveness of using an extensively hydrolysed formula compared to an amino acid formula as first-line treatment for cow milk allergy in the UK. Pediatr Allergy Immunol 2012;23:240-9.
26. Isolauri E, Sütas Y, Mäkinen-Kiljunen S, Oja SS, Isosomppi R, Turjanmaa K. Efficacy and safety of hydrolyzed cow milk and amino acid-derived formulas in infants with cow milk allergy. J Pediatr 1995;127:550-7.
27. Niggemann B, Binder C, Dupont C, Hadji S, Arvola T, Isolauri R. Prospective, controlled, multi-center study on the effect of an amino acid-based formula in infants with cow's milk allergy/intolerance and atopic dermatitis. Pediatr Allergy Immunol 2001;12:78-82.

28. Mennella JA, Ventura AK, Beauchamp GK. Differential growth patterns among healthy infants fed protein hydrolysate or cow-milk formulas. Pediatrics 2011;127:110-8.

Figure legends

Figure 1. The flow of children through the study.

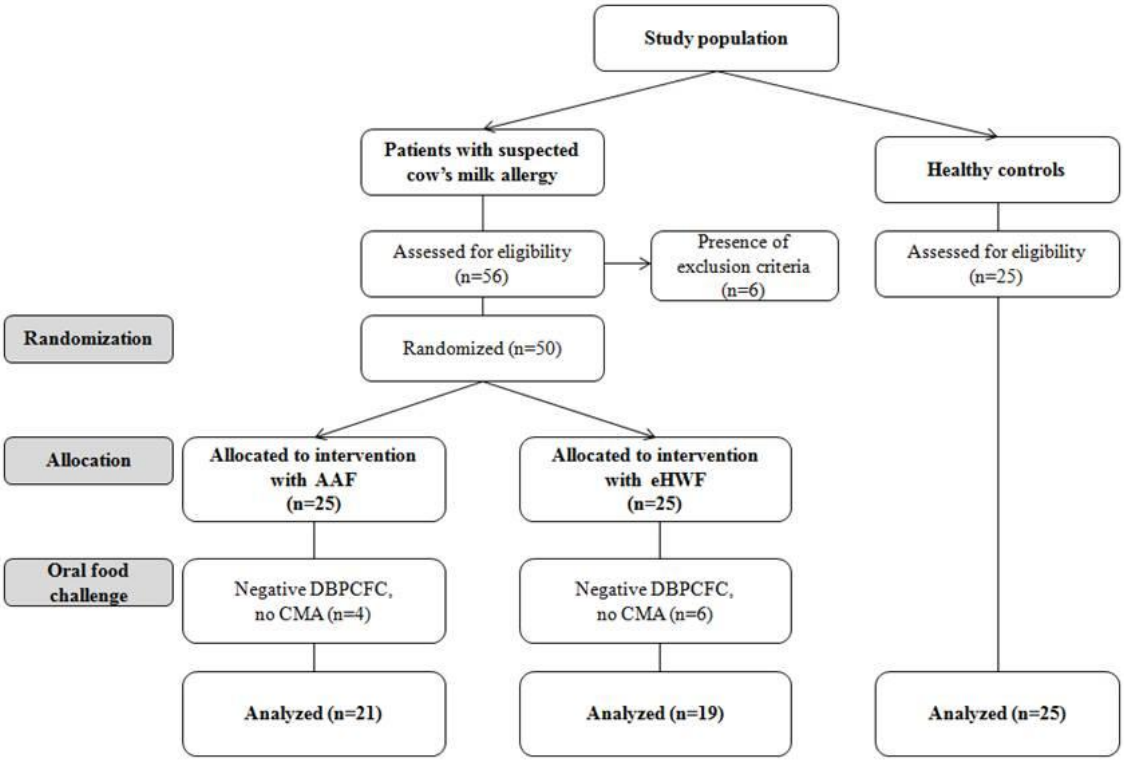
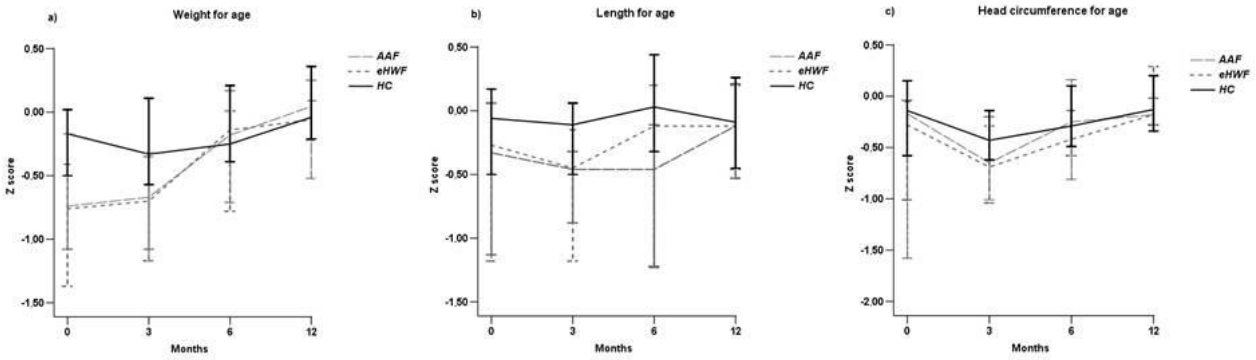


Figure 2. Study subject z-scores during the study period. Weight for age (Panel a), length for age (Panel b), head circumference for age (Panel c).

HC = healthy controls; eHWF = extensively hydrolysed whey formula; AAF = amino acid–based formula. Data are expressed as median. Error bars are 95% CI.



Z score	Months	HC *p*	eHWF *p*	AAF *p*
Weight for age	0 *vs* 3	0.216	0.573	0.575
	0 *vs* 6	0.786	<0.0001	0.03
	0 *vs* 12	0.313	0.001	0.005
	3 *vs* 6	0.042	0.001	0.004
	3 *vs* 12	0.065	0.003	0.002
	6 *vs* 12	0.677	0.177	0.179
Length for age	0 *vs* 3	0.201	0.178	0.099
	0 *vs* 6	0.851	0.227	0.931
	0 *vs* 12	0.700	0.159	0.370
	3 *vs* 6	0.019	0.028	0.144
	3 *vs* 12	0.404	0.009	0.04
	6 *vs* 12	0.510	0.405	0.478
Head cirumference for age	0 *vs* 3	0.002	0.085	0.664
	0 *vs* 6	0.493	0.793	0.614
	0 *vs* 12	0.119	0.036	0.370
	3 *vs* 6	0.122	0.099	0.058
	3 *vs* 12	0.001	0.001	0.001
	6 *vs* 12	0.071	0.004	0.332

Z score	Months	AAF vs HC *p*	eHWF vs HC *p*	AAF vs eHWF *p*
Weight for age	0	0.046	0.003	0.456
	3	0.03	0.007	0.745
	6	0.494	0.448	0.560
	12	0.698	0.265	0.509
Length for age	0	0.142	0.105	0.745
	3	0.05	0.045	0.850
	6	0.027	0.158	0.350
	12	0.430	0.561	0.866
Head cirumference for age	0	0.238	0.092	0.559
	3	0.251	0.104	0.616
	6	0.494	0.135	0.498
	12	0.451	0.522	0.944

Table 1. Nutrients composition of the study formulas

		Neocate LCP 100 ml (13.8% p/v)	Neocate Advance 100 ml (15%p/v)	Hypolac 100 ml (13.5%p/v)	Follow-on formula 100 ml (14%p/v)	Growing-up milk 100 ml (13.4%p/v)
Energy	kcal	67	60	62.5	68	65
Proteins	g	1.8	1.5	1.6	1.4	1.6
Carbohydrate	g	7.2	8.8	6.5	8.2	8
Sugar	g	0.65	0.8	1.3	6.1	6.6
Total fat	g	3.4	2.1	3.4	3.2	3
SFA	g	1.2	0.8	-	1	1.5
MUFA	g	1.3	0.92	-	1.6	0.9
PUFA	g	0.66	0.29	-	0.6	0.6
Linoleic acid (LA)	mg	579	225	440	532	500
Linolenic acid (ALA)	mg	57.8	60	50	49	60
Arachidonic acid	mg	11.3	-	-	-	-
DHA	mg	11.3	-	-	-	-
MCT	(% total fat)	4	35	-	-	-
LCT	(% total fat)	96	65	-	-	-
LA/ALA ratio		10:1	4:1	-	-	-
Nucleotides	g	3.22	-	-	-	-
Fiber	g	0	0	-	0.4	0
Na	mg	26.1	36	25	32	34
K	mg	72.5	70.2	75	90	100
Cl	mg	53.3	55.2	50	60	71
Ca	mg	65.6	30	50	65	90
P	mg	47.1	23.3	30	43	59
Mg	mg	7	7.5	5	8.1	9.5
Fe	mg	1	0.372	0.7	1.2	1.2
Cu	µg	56.6	0.04	0.04	44	45
Zn	mg	0.73	0.3	0.6	0.6	0.9
Mn	mg	0.03	0.03	0.02	0.03	9
I	µg	13.8	4.2	7.5	20	20
Mo	µg	1.6	2.1	<5	-	-
Se	µg	2	1.5	1	1.6	1.6
Cr	µg	1.5	0.75	<5	-	-
Vit. A	µg-RE	56	22.2	63	72	75
Vit. D_3	µg	1.2	0.49	0.94	1.2	1.6
Vit. E	µg-α-TE	0.67	0.35	1.3	1.6	2
Vit. C	mg	7.1	1.98	9.4	10	15
Vit. K_1	µg	5.9	2.1	3.8	7.4	5
Thiamin	mg	0.07	0.036	0.05	0.125	0.16
Riboflavin	mg	0.07	0.048	0.08	0.17	0.18
Niacin	mg-NE	1.4	0.57	0.63	0.602	0.495
Pantothenic acid	mg	0.4	0.15	0.31	0.7	0.6
Vit. B_6	mg	0.07	0.05	0.04	0.06	0.1
Folate	µg	8.8	6	7.5	18	15
Vit. B_{12}	µg	0.18	0.042	0.19	0.2	0.25
Biotin	µg	2.6	1.2	1.5	2.5	3
Choline	mg	13.2	11.52	6.3	-	-
Inositol	mg	14. 9	1.13	4	13	-
Osmolarity	mOsm/l	310	520	175	-	-

Table 2. Main features of the study population at enrolment

	Subjects with CMA treated with AAF ***n=25***	**Subjects with CMA treated with eHWF** ***n=25***	**Healthy controls** ***n=25***
Male, n (%)	15 (60)	14 (56)	12 (48)
Age, months (±SD)	6.5 (1.4)	6.8 (1.6)	5.5 (0.5)
Duration of breastfeeding, months (±SD)	4.4 (1.4)	4.9 (1.7)	4.8 (1.1)
Age of weaning, months (±SD)	5 (0.9)	5.3 (0.6)	5.1 (0.7)
Energy intake, Kcal/kg/day*	98.2 (5.9)	96.8 (6.4)	101.3 (15.1)
Protein intake, gr/kg/day*	2.2 (0.4)	2.2 (0.3)	2.3 (0.3)
Suspected IgE mediated mechanism, n (%)	13 (52)	15 (60)	---
Gastrointestinal symptoms, n (%)	12 (48)	16 (64)	---
Cutaneous symptoms, n (%)	13 (52)	16 (64)	---
Respiratory symptoms, n (%)	4 (16)	4 (16)	---
Urea, mmol/L*	3.5 (1.9)	3.8 (1.8)	---
Total protein, g/L*	65.6 (7.3)	65 (10.6)	---
Albumin, g/dl*	4 (0.6)	4.1 (0.4)	---
Retinol binding protein, mg/L*	22.3 (28.7)	30.5 (30)	---
Insulin-like growth factor 1, µg/L*	52 (30)	64 (65)	---

AAF = amino acid–based formula; eHWF = extensively hydrolysed whey formula.

**Data are reported as median and (IQR)*

Table 3. Main features of subjects with positive diagnostic double-blind placebo-controlled food challenge

	Subjects with CMA treated with AAF ***n=21***	**Subjects with CMA treated with eHWF** ***n=19***	**Healthy controls** ***n=25***
Male, n (%)	13 (61.9)	11 (57.9)	12 (48)
Age, months (±SD)	6.5 (1.5)	7 (1.7)	5.5 (0.5)
Duration of breastfeeding, months (±SD)	4.3 (1.6)	5 (2)	4.8 (1.1)
Age of weaning, months (±SD)	4.9 (0.9)	5.3 (0.6)	5.1 (0.7)
IgE mediated mechanism, n (%)	10 (47.6)	14 (73.7)	---
Gastrointestinal symptoms, n (%)	11 (52.4)	11 (57.9)	---
Cutaneous symptoms, n (%)	10 (47.6)	12 (63.2)	---
Respiratory symptoms, n (%)	4 (19)	4 (21.1)	---

AAF = amino acid–based formula; eHWF = extensively hydrolysed whey formula.

Table 4. Energy and protein intake in study subjects during the study period

	Months	Subjects with CMA treated with AAF *n=21*	Subjects with CMA treated with eHWF *n=19*	Healthy controls *n=25*
Energy intake, *Kcal/kg/day*				
	0	98.4 (8.8)	96.0 (7.8)	101.3 (15.1)
	3	102.9 (7.8)	96.9 (12.5)	98.2 (9)
	6	94.8 (6.9)	95.2 (12.5)	99.5 (9.2)
	12	89.7 (5.8)	90.6 (8.8)	96.4 (8.7)
Protein intake, *gr/kg/day*				
	0	2.1 (0.3)	2.3 (0.3)	2.3 (0.3)
	3	2.2 (0.4)	2.3 (0.4)	2.1 (0.4)
	6	2.2 (0.2)	2.2 (0.1)	2.1 (0.3)
	12	2.3 (0.1)[a]	2.3 (0.1)[a]	2.1 (0.3)[b]

Data are reported as median and (IQR)
Different superscript indicate a significant difference (p=0.04 and p=0.015, respectively)
AAF = amino acid–based formula; eHWF = extensively hydrolysed whey formula.

Table 5. Biomarkers of protein metabolism

	Months	Subjects with CMA treated with AAF *n=21*	Subjects with CMA treated with eHWF *n=19*
Urea, mmol/L	**0**	3.1 (1.68)	3.8 (2.1)
	3	3.7 (1.9)[a]	4.3 (2)[b]
Total protein, g/L	**0**	64.1 (6.6)	63 (10)
	3	64.9 (10.7)	65.6 (7.5)
Albumin, g/dl	**0**	3.9 (0.6)	4.1 (0.4)
	3	4.3 (0.7)	4 (0.6)
Retinol binding protein, mg/L	**0**	16.6 (23.5)	26.6 (9.7)
	3	27.9 (14.3)	27.2 (11.4)
Insulin-like growth factor 1, µg/L	**0**	56 (30.5)[a]	77 (58)[b]
	3	65.5 (52.2)	71 (66)

Data are reported as median and (IQR)
Different superscript indicate a significant difference (p=0.033 and p=0.016, respectively)
AAF = amino acid–based formula; eHWF = extensively hydrolysed whey formula.