

REGULAR ARTICLE

Treating cow's milk protein allergy: a double-blind randomized trial comparing two extensively hydrolysed formulas with probiotics

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ABSTRACT

Aim: The treatment for cow's milk protein allergy (CMPA) is a diet with an extensive hydrolysate. This study aimed to determine whether a whey (eWH) or casein hydrolysate (eCH) is the best option.

Methods: Infants with suspected CMPA were treated with an eWH or eCH, and efficacy was assessed with a symptom-based score developed by the authors. Diagnosis of CMPA was based on a positive challenge. If positive, the same eHF/eCH was continued. If negative, a standard starter and follow-up formula were given up to the age of 12 months.

Results: An open challenge was performed on 85/116 (73%) infants suspected of CMPA on clinical grounds and was positive in 59/85 (69%). After 1 month, the symptom-based scores in both groups showed significant statistical and clinical reductions, and total and specific IgE and skin prick test results were similar. Both hydrolysates were enriched with probiotics, which were recovered in the gastrointestinal flora. The eWH-Standard Formula sequence led to better growth at the age of 1 year than the other three feeding regimens tested.

Conclusion: The eWH and eCH are equally effective. The symptom-based score is a useful tool to evaluate the efficacy of dietary treatment in infants with CMPA.

INTRODUCTION

Cow's milk protein (CMP) is a major food allergen in infants (1–3). Symptoms of CMP-allergy (CMPA) are not specific. The most frequent symptoms involve the skin (such as atopic dermatitis), the gastrointestinal tract (vomiting, diarrhea, constipation), the airways (wheezing, sneezing) are more general (colic) (1,4). Diagnosing CMPA remains the subject of debate and controversy (5). According to the definition proposed by the World Allergy Organization, CMPA is a hypersensitivity reaction brought on by specific immunologic mechanisms to cow's milk (6). CMPA is defined as an adverse health effect arising from a specific immune response that occurs after exposure to a cow's milk allergen (7). This immune reaction may be IgE or non-IgE-mediated or mixed, and diagnosis requires a food challenge, which is often refused by the parents. Only IgE testing, in the form of total and specific IgE skin prick tests (SPT), is available as a matter of routine. A symptom-based

score (SBS) was developed by the authors covering the most frequent presentations of mild to moderate CMPA.

A correct diagnosis of CPMA ensures that an appropriate diet is given to affected infants and thus supports their normal growth and development. The guidelines define a therapeutic hypoallergenic formula as one that is tolerated by at least 90% of CMPA infants (8). These criteria are met by extensively hydrolysed formulas (eHF) based on whey or casein. The aim of this study was to compare the efficacy of a whey eHF and a casein eHF, both enriched with different probiotic strains.

Abbreviations

BMI, body mass index; CMP, cow's milk protein; CMPA, cow's milk protein allergy; eCH, extensive casein hydrolysate; eHF, extensive hydrolysate formula; eWH, extensive whey hydrolysate; GI, gastrointestinal; SBS, symptom-based score; SPT, skin prick test.

Key notes

- The recommended treatment for cow's milk protein allergy (CMPA) is an elimination diet with an extensive hydrolysate.
- Our study of 85 infants showed that both whey and casein hydrolysates were equally effective and resulted in significant statistical and clinical reductions.
- It also showed that using a specially designed symptom-based score, developed by the authors, helped to select infants who face a high risk of cow's milk protein allergy.

METHODS

Exclusively formula fed infants who presented with symptoms suggesting mild to moderate CMPA were eligible for inclusion in this multicentre, prospective, randomized, double-blind trial. The eligibility criteria were as follows: singleton birth, gestational age 37–42 weeks, birthweight of 2500–4500 g, age range 0.5–6 months, both sexes and any ethnicity.

Suspicion of mild to moderate CMPA was based on the presence of a combination of the following symptoms: general discomfort in the form of persistent distress or colic (wailing or irritable $\geq$ three hours per day, at least 3 days a week over a period of >3 weeks), gastrointestinal (frequent regurgitation, vomiting, diarrhoea, constipation – with/without perianal rash), blood in stools, respiratory (runny nose, otitis media), chronic cough, wheezing (unrelated to infection) and dermatological (atopic dermatitis, angio-oedema, urticaria unrelated to acute infections, drug intake etc.) manifestations (1,4).

Since different guidelines recommend amino acid-based formula as the first option for children presenting with

severe symptoms of CMPA (anaphylactic reaction), the goal of this study was to select patients with mild to moderate CMPA (1,4). A symptom-based score (SBS), quantifying the number and severity of symptoms, was developed by the 18 study participants from 14 different hospital sites, including six paediatric gastroenterologists and three from university hospitals (Table 1). The Bristol stool scale was applied to evaluate the consistency of the stools: types 1 (separate hard lumps, like nuts) and 2 (sausage-shaped but lumpy) indicated constipation; types 3 (like a sausage but with cracks on its surface) and 4 (like a sausage or snake, smooth and soft) were 'ideal consistencies', type 5 (soft blobs with clear-cut edges) and type 6 (fluffy pieces with ragged edges, a mushy stool) tended to indicate diarrhoea and type 7 (watery, no slide pieces, entirely liquid) indicated overt diarrhoea. The paediatricians attributed the SBS to the patients at inclusion. The SBSs ranged from 0 to 33, as five of the symptoms had a maximum score of six, and respiratory symptoms had a maximum score of three. An arbitrary cut-off value of >12 was selected as the criterion to pick-up infants 'at risk of CMPA'. A score of 12 necessitated

Table 1 Symptom-based clinical score

Symptom	Score			
Crying*	0	≤ 1 h/day		
	1	1–1.5 h/day		
	2	1.5–2 h/day		
	3	2–3 h/day		
	4	3–4 h/day		
	5	4–5 h/day		
	6	≥ 5 h/day		
Regurgitation†	0	0–2 episodes/day		
	1	≥ 3 – ≤ 5 of small volume		
	2	>5 episodes of >1 coffee spoon		
	3	>5 episodes of $\pm$ half of the feedings in $<$ half of the feedings		
	4	Continuous regurgitations of small volumes >30 min after each feeding		
	5	Regurgitation of half to complete volume of a feeding in at least half of the feedings		
	6	Regurgitation of the 'complete feeding' after each feeding		
Stools (Bristol scale)‡	4	Type 1 and 2 (hard stools)		
	0	Type 3 and 4 (normal stools)		
	2	Type 5 (soft stool)		
	4	Type 6 (liquid stool, if unrelated to infection)		
	6	Type 7 (watery stools)		
	0–6	Atopic eczema		
Skin symptoms			Head–neck–trunk	Arms–hands–legs–feet
		Absent	0	0
		Mild	1	1
		Moderate	2	2
		Severe	3	3
	0 or 6	Urticaria (no 0/yes 6)		
Respiratory symptoms	0	no respiratory symptoms		
	1	Slight symptoms		
	2	Mild symptoms		
	3	Severe symptoms		

*Crying was only considered if the child was crying for 1 week or more, assessed by the parents, without any other obvious cause.

†Vandenplas Y, Hachimi-Idrissi S, Casteels A, Mahler T, Loeb H. A clinical trial with an 'antiregurgitation' formula. *Eur J Pediatr*. 1994;153:419–423.

‡Lewis SJ, Heaton KW. Stool form scale as a useful guide to intestinal transit time. *Scand. J. Gastroenterol*. 1997; 32: 920–924.

the presence of at least two symptoms, if they were severe. A score >12 necessitated the presence of at least three symptoms and the involvement in two organ systems. IgE (total, cow milk, alpha-lactalbumin, beta-lactoglobulin, casein) and skin prick tests (SPTs) were performed at baseline, before randomization.

The exclusion criteria were as follows: breastfeeding at enrolment, having been treated before enrolment with an extensive hydrolysed formula, disease impairing a normal gut transit (e.g. pyloric stenosis, oesophageal atresia), known intolerance to lactose, antibiotics at enrolment, severe or chronic diarrhoea, failure to thrive, neurological disease, surgical intervention, receiving a medical treatment that could interfere with the protocol (e.g. antihistaminics, corticoids), parents or caregivers who could not be expected to comply with the treatment and having participated, or be currently participating, in another clinical trial.

The objective of the trial was to compare the efficacy of an extensive whey hydrolysate (eWH) to that of an extensive casein hydrolysate (eCH). Both of the hydrolysates were enriched with different probiotic strains. The eWH was marketed as Althera® (Nestlé, Vevey, Switzerland) and enriched with *Bifidobacterium lactis* (10^7 CFU/g powder) for the purpose of this study. The eCH is marketed as Nutramigen lactobacillus GG (10^7 CFU/g powder) + Lipil® (Mead Johnson, Evansville, IN, USA). The energy density of the eWH and eCH was 68 and 67 kcal/100 mL, and the protein content was 2.48 and 2.80 g/100 kcal, respectively.

A computer programme randomized the patients for each physician, allocating them to either the product A (eWH) or the product B (eCH) group. Random allocation was made in blocks of ten to obtain groups of a similar size and concealed until the treatments were assigned. Participating physicians had to open a neutral envelope to find out which group the patient had been allocated to. Both formulas were packed in identical tins and had a neutral label, indicating the 'group' and providing information on how to prepare the feed. Every participating physician received ten blinded treatments.

We did not expect that the eWH would have better efficacy than the eCH, which has been on the market for many years. Therefore, a noninferiority hypothesis was stated. The trial was powered by 80% in order to show noninferiority of the eWH to the eCH on a significance level of 0.025. Discussions with the clinicians established that a difference in SBS of >3 would matter in practice. Therefore, a noninferiority boundary of three points was chosen. Since the SBS was designed specifically for this study, the standard deviation for calculating the sample size was based on an estimate of seven points, and we chose a group-sequential design, with an interim analysis carried out on 50% of the children, in order to reassess the standard deviation. The interim data were made available to an independent data monitoring committee consisting of two clinicians and one statistician. The recommendations available to them were stopping the trial, because it had proved futile or successful, or

continuing the trial in order to demonstrate the objective of the trial. The critical values and the test characteristics of the group sequential test design were calculated for a Wang and Tsatis design with boundary shape parameter $\Delta = 0.41$. This Δ minimizes the average sample size under the alternative hypothesis. The total sample size needed was calculated at 188. These calculations were performed with ADDPLAN 4.0. (AptivSolutions, Reston, VA, USA) In order to compensate for dropouts, we planned to include 250 children. The interim analysis was conducted when data became available on 100 children after 1 month.

Patients were randomly assigned to one of the two treatment groups stratified by centre, age and Kjellman-score. They were fed exclusively with the study products, on demand, for 1 month, and no other food was given to them during that period. After 1 month, an open challenge was performed in the hospital, under medical supervision, with a standard starter formula, NanPro1® (Nestlé, Vevey, Switzerland). The procedure for the open challenge test has been published elsewhere (4). The challenge started with a small quantity of NanPro1® that was increased every 30 min if no reaction occurred. If at the end of the challenge the child was asymptomatic, the parents were told to give the child at least 250 mL of NanPro1® each day for the next week (4). Daily telephone contact took place during the first week following the 'in-hospital challenge'. If the parents mentioned a reaction, the child was seen again by the physician, who made the final decision about a 'positive versus negative' challenge. If the challenge was positive, the infant was fed the same test-formula as before up to the age of 1 year. If the challenge was negative, the infant was fed a standard starter and follow-up formula (NanPro 1® and 2®). The follow-up study ended when the child reached the age of 1 year. The SBS was used to measure efficacy and was determined at inclusion, before the challenge and at each visit up to the age of 10 months. Parents were asked to fill in a paper diary in the week before the next visit.

Secondary outcomes were anthropometric data, crying time, regurgitation, stool composition, eczema, urticaria and respiratory symptoms. Gastrointestinal flora was analysed using the FISH technique for concentrations (CFU/g) of *Bifidobacteria*, *Lactobacilli*, *Bacteroides* and *Clostridia* at baseline and after 1 month in a subgroup. Anthropometry measures were weight, length, head-circumference, body mass index (BMI) and the z-scores. Data were obtained at 0, 1, 2, 4, 6, 8 and 10 months. The measurement at 0 months was considered as baseline (randomization). The covariate analysis was performed by a mixed model. Fixed effects were as follows: baseline, gender, visit and treatment. Random effect was subject. Inference was obtained by a likelihood ratio test between the mixed model, as explained above, and a reduced mixed model without treatment as a fixed effect. The likelihood ratio test was a formal hypothesis test for different curves.

Eighteen investigators from 14 different centres took part in the trial, and the study was approved by the Ethical

Committee of UZ Brussel, the leading study centre, and by each participating centre. Informed consent was obtained from both parents. The trial was registered in the Clinical-Trial.gov database with the number NCT01727115. Nestle Nutrition provided the formula up to the age of 1 year and an unrestricted grant to perform the study.

RESULTS

The 18 paediatricians enrolled 116 infants in the study because their clinical symptoms suggested mild to moderate CMPA. Their characteristics are presented in Table 2. All infants presented with at least three different symptoms involving at least two organ systems. Although 'acceptance to perform a challenge after 1 month of the elimination diet' was one of the inclusion criteria, 26% of the parents refused the challenge. As a result, the open challenge was performed in 85 (74%) of the infants. Patient characteristics were comparable between the children whose parents refused or agreed to the challenge. The challenge was positive in 26/41 (63%) children in the eWH group and in 33/44 (75%) in the eCH group. Just under a third of the children (26/85, 31%) experienced a 'late' reaction when the parents had returned home: 8/41 (20%) children in the eWH group and 18/44 (41%) in the eCH group. The difference between the eWH and eCH group is statistically significant ($p = 0.037$) (Table 3). Because the difference at baseline in total-IgE may influence this finding, we repeated the analysis by logistic regression correcting for total-IgE at baseline. Again we found a statistically significant effect: OR = 0.22, 95% CI 0.06, 0.87 and $p = 0.031$. The symptoms that developed in the infants who had a late positive challenge were as follows: crying (in 50%), regurgitation (65%), liquid stools (58%), atopic eczema (35%) and

Table 3 Challenge test results analysed by exact chi-square test

Challenge	eWH	eCH	OR (95%CI)	p
Negative	37% (15/41)	25% (11/44)	1.73 (0.62, 4.92)	0.35
Positive early	44% (18/41)	34% (15/44)	1.51 (0.58, 3.99)	0.38
Positive late	20% (8/41)	41% (18/44)	0.35 (0.11, 1.02)	0.04

Note the p-value results from the permutation test does not correspond to the 95% confidence interval. In this case, the 95% confidence interval is too conservative. (ITT data set). eWH = extensive whey hydrolysate; eCH = extensive casein hydrolysate; OR = Odds ratio; 95%CI = 95% confidence interval.

respiratory symptoms (42%) (Table 4). The sensitivity and specificity of IgE and SPT, in relation to the challenge, test are listed in Table 5.

All the inclusion and challenge procedures were performed in the hospital, but for practical reasons some follow-up visits were performed by the participating physicians in their private practices. Baseline patient characteristics were comparable. No relevant or significant differences were encountered, except that significantly more infants in the eWH group had a total IgE level >2 kU/L than in the eCH group ($p < 0.05$) (Table 2). The age in days at inclusion was similar in both groups (P25, P50, P75: 57.0, 80.0, 136.0 days, respectively, in the eWH group and 48.0, 64.0, 114.0 days, respectively, in the eCH group). The results of the SPTs were also comparable, but total IgE was positive in 55% of the children in the whey group and only in 36% of the children of the casein group. Total baseline IgE was >20 kU/L (range, 20–1180) in 12 infants and above 100 kU/L in a further four. None of these children were challenged.

Table 2 Patient characteristics

	Extensive whey hydrolysate (eWH)		Extensive casein hydrolysate (eCH)	
	Median or %	Lower/Upper Q	Median or %	Lower/Upper Q
Male/Female	64/36%		51/49%	
Birthweight (kg)	3.35	3.04–3.60	3.29	3.04–3.56
Length birth (cm)	50.0	49.0–52.0	50.0	48.0–51.5
Gestational age (weeks)	39	38–40	39	38–40
Delivery vaginal/C sect	85/15%		87/13%	
Family history atopy				
0–1/2–3/ ≥ 4	45/50/5%		47/43/10%	
Age diagnosis (days)	80	57–136	64	48–114
IgE cow milk >0.35 kU/L	8%		7%	
IgE α -lactalb >0.35 kU/L	6%		3%	
IgE β -lacto >0.35 kU/L	6%		3%	
IgE casein >0.35 kU/L	4%		5%	
IgE tot >2 kU/L*	55%		36%	
IgE tot (kU/L)	2.89	2.00–4.54	2.00	2.00–3.48
SPT positive rash	17%		17%	
SPT positive papulla	12%		9%	

Q = quartile; C sect = caesarean section; SPT = skin prick test (positive if diameter is ≥ 3 mm greater than negative control).

*Significantly more infants in the eWH group had a positive total IgE than in the eCH group (55% vs 36%, $p < 0.05$).

Table 4 Symptoms that developed during (positive) challenge

Symptom	Early pos challenge		p	Late pos challenge		p
	eWH	eCH		eWH	eCH	
N (%)	18 (44)	15 (34)	0.38	8 (20)	18 (41)	0.04
Crying, n (%)	5 (28)	7 (47)	0.26	6 (75)	7 (39)	0.09
Regurgitation, n (%)	7 (39)	13 (87)	0.005	5 (62)	12 (67)	0.84
Liquid stools, n (%)	8 (44)	6 (40)	0.80	4 (50)	11 (61)	0.60
Atopic eczema, n (%)	9 (50)	7 (47)	0.85	3 (38)	6 (33)	0.84
Resp symptoms, n (%)	4 (22)	(33)	0.47	2 (25)	9 (56)	0.23

resp = respiratory; statistics = *t*-test.**Table 5** Sensitivity, specificity, positive predictive and negative predictive value of IgE and skin prick test regarding challenge test

IgE	Sensitivity, %	Specificity, %	Pos pred value, %	Neg pred value, %
Cow milk	5	100	100	31
Alfa-lactalbumin	2	100	100	30
Beta-lactoglobulin	4	100	100	31
Casein	2	100	100	31
Total	54	82	88	42
Skin prick test				
Rash	13	92	78	32
Papula	4	100	100	30

The SBS was determined in all included infants. At inclusion, it was 13.58 (2.20; range, 5–21) (mean (SD; min–max)) in the eWH and 13.79 (1.47; range, 12–17) in the eCH group (NS) as shown in Table 6 (the subject with a value of 5 was a protocol deviation). The percentage of infants with a positive IgE did not increase in relation to a higher score. Blood sampling was performed in 106 of the 116 children. Fifteen dropped out: two because the parents refused their consent (one in each group) and 13 because the infant refused to drink the hydrolysate (four in the eWH group and nine in the eCH group). The blood scores in the remaining 106 children showed that 25 children had a score of 12, and IgE was positive in 12 (48%); 30 children had a score of 13 and IgE was positive in 12 (40%); 23 children

had a score of 14 and IgE was positive in 13 (57%); 28 children had a score of ≥ 15 and IgE was positive in 10 (36%). The SBS decreased significantly after the first month by -8.07 (95% CI $-8.74, -7.40$; $p < 0.001$) if both treatment groups were pooled. The decrease was -8.32 in the eWH group and -7.82 in the eCH group. The treatment difference between both groups (whey minus casein) was -0.879 with a 95% CI ($-2.79, 1.03$). The noninferiority boundary was set at below three, giving a one-sided *p*-value on the noninferiority hypothesis of <0.0001 (Table 6). The difference was calculated in such a way that a negative value pointed towards a beneficial effect. As a result, the trial was stopped prematurely, as it had already successfully shown its objective. This was possible because the standard deviation was overestimated by seven at the planning stage of the trial. The actual standard deviation was approximately three. During the following months, the SBS showed further reductions in both groups: to 1.04 (range, 0–3) in the eWH group and to 1.96 (range, 0–9) in the ECH group (Table 6).

All the secondary outcomes in the SBS (crying time, regurgitation, stool characteristics, eczema, urticaria and respiratory symptoms) decreased significantly and equally in both groups during the 1-month diet, but they were not different between the groups at baseline or after 1 month (Table 7). The decrease in the SBS did not differ according to the results of the challenge test, the total/specific IgE levels and the skin prick tests.

Anthropometry was analysed according to the actual feeding, resulting in four possible feeding sequences,

Table 6 Evolution of the symptom-based scores between inclusion and 10 months of dietary treatment (ITT data set)

	Extensive whey hydrolysate (eWH)			Extensive casein hydrolysate (eCH)		
	N	Mean (SD)	Range	N	Mean (SD)	Range
Baseline	55	13.58 (2.20)	5–21	61	13.79 (1.47)	12–17
1 month	50	5.16 (3.16)	0–13	51	5.98 (3.22)	0–18
2 months	48	3.98 (2.92)	0–12	47	4.64 (2.82)	0–13
4 months	48	2.79 (2.63)	0–10	46	3.13 (2.86)	0–12
6 months	47	2.11 (2.17)	0–9	43	2.91 (2.65)	0–12
8 months	42	1.33 (1.79)	0–9	44	1.91 (1.79)	0–8
10 months	23	1.04 (1.02)	0–3	23	1.96 (2.62)	0–9

Table 7 Secondary outcomes contributing the symptom-based score (ITT data set)

	Extensive whey hydrolysate (eWH)			Extensive casein hydrolysate (eCH)		
	N	Mean (SD)	Range	N	Mean (SD)	Range
Crying time						
Baseline	55	4.2 (2.0)	0–6	61	4.6 (1.8)	0–6
1 month	50	1.1 (1.4)	0–6	51	1.5 (1.7)	0–6
Regurgitation						
Baseline	55	2.6 (1.7)	0–6	61	3.0 (1.6)	0–6
1 month	50	1.1 (1.2)	0–4	51	1.8 (1.2)	0–4
Stool consist						
Baseline	55	3.5 (1.5)	0–6	61	3.5 (1.3)	0–6
1 month	50	1.8 (1.8)	0–6	51	1.4 (1.6)	0–6
Eczema						
Baseline	55	2.1 (2.0)	0–6	61	1.8 (1.9)	0–6
1 month	50	0.8 (1.1)	0–4	51	1.0 (1.5)	0–6
Urticaria						
Baseline	55	0.4 (1.6)	0–6	61	0.1 (0.5)	0–3
1 month	50	0 (0)	0–0	51	0 (0)	0–0
Resp sympt						
Baseline	55	0.8 (1.0)	0–3	61	0.8 (0.9)	0–3
1 month	50	0.4 (0.8)	0–3	51	0.4 (0.6)	0–2

Consist, consistency = resp symp, respiratory symptoms. All parameters decreased significantly during diet with whey and casein eHF; no difference between the groups.

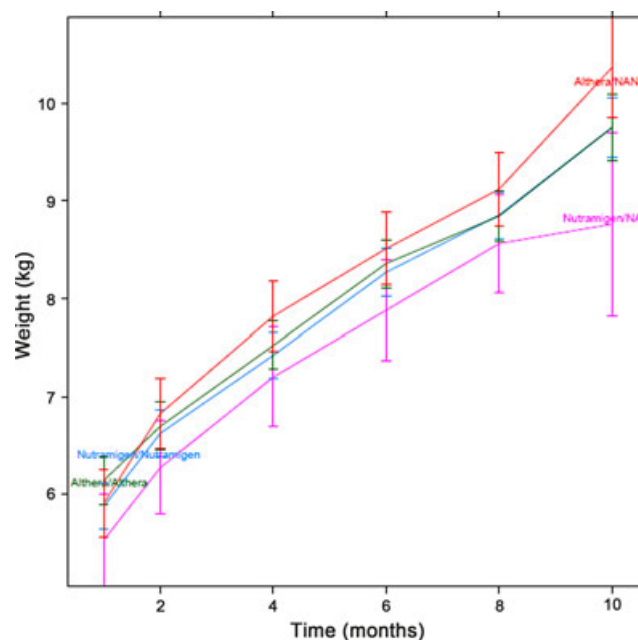
depending on the outcome of the challenge test (eWH-eWH, eWH - NanPro1/2[®], eCH-eCH, eCH - NanPro1/2[®]). We could not detect a statistical difference in weight, length, head circumference, BMI and the z-scores, respectively, between the curves over time during the first month. However, at the age of 1 year, the eWH-NanPro1/2[®] sequence led to better growth (weight and weight for age z-scores) than the three other feeding regimens, and the eCH-eCH sequence led to smaller head circumferences (Figure 1).

Gastrointestinal (GI) flora was comparable at inclusion (Table 8). Since the eWH was enriched with bifidobacteria, it was expected that the number of bifidobacteria would increase after 1 month's feeding, and this was actually the case: the bifidobacteria counts increased by 1.34 (95% CI 0.57, 2.11) on a log10 scale ($p = 0.001$). The eCH was enriched with a lactobacillus strain and, as a result, lactobacilli increased by 2.17 (95% CI 1.62, 2.71) on a log10 scale ($p < 0.001$). Enterobacteriaceae were increased by 0.61 (95% CI 0.14, 0.99), $p = 0.012$. There were no differences between both groups when it came to the other bacteriological parameters.

There were no differences in adverse events between both groups. During the 1-year follow-up period, 62% of the children in the eWH group and 64% of those in the eCH group experienced at least one adverse event.

DISCUSSION

We achieved the primary objective of this trial and demonstrated that the eWH is equally as efficacious as the eCH. We consider the two hydrolysates differently in many

**Figure 1** 1-year weight evolution of the study groups.

respects, and the research we carried out is a comparison of two nutritional strategies. The eWH, without the probiotics, had previously been shown to be safe and well tolerated in infants diagnosed with CMPA (9), and the eCH has been on the market for many years. The primary objective was shown with the help of a noninferiority hypothesis. The trial was planned with a group sequential

Table 8 Summary statistics and inferential statistics on bacteriology.

	Extensive whey hydrolysate(eWH)			Extensive casein hydrolysate(eCH)			p-value
	N	Mean (SD)	Range	N	Mean (SD)	Range	
Bifidobacteria							
Baseline	16	9.36 (1.36)	5.91–10.80	17	9.57 (1.06)	6.79–10.70	0.001
1 month	15	9.85 (1.00)	7.51–10.75	16	8.55 (1.22)	5.91–10.21	
Lactobacilli							
Baseline	16	6.92 (1.24)	6.02–9.36	17	6.90 (1.13)	6.02–8.89	<0.001
1 month	15	6.47 (0.95)	6.02–8.87	16	8.65 (0.44)	7.81–9.32	
Enterobacteriaceae							
Baseline	16	8.60 (0.74)	7.20–9.62	17	8.51 (0.68)	7.20–9.48	0.012
1 month	15	8.90 (0.62)	7.20–9.99	16	8.32 (0.53)	7.20–9.00	
Bacteroides & Prevotella							
Baseline	16	7.00 (1.20)	6.40–9.95	17	7.64 (1.31)	6.40–9.57	0.152
1 month	15	7.75 (1.35)	6.40–9.69	16	7.12 (0.95)	6.40–8.91	
Clostridium & Eubacterium							
Baseline	16	8.39 (1.38)	6.77–10.30	17	8.59 (1.38)	6.77–10.11	0.127
1 month	15	9.09 (1.07)	6.77–10.24	16	9.63 (0.79)	6.87–10.43	
Total bacteria							
Baseline	16	10.27 (0.67)	7.99–10.89	17	10.32 (0.43)	9.05–11.07	0.039
1 month	15	10.46 (0.37)	9.49–11.01	16	10.22 (0.28)	9.78–10.94	

Bacteria counts after log₁₀ transformation were analyzed by ANCOVA correcting for baseline. Values below the detection limit were replaced by the detection limit (ITT data set).

design, and the primary objective had already been convincingly demonstrated by the interim analysis. We used an independent data monitoring committee for the interim review in order to achieve the highest level of evidence for such a setting, in line with the FDA guidance on Adaptive Design Clinical Trials for Drugs and Biologics (<http://www.fda.gov/downloads/Drugs/.../Guidances/ucm201790.pdf>).

The patients were selected based on their presenting clinical symptoms. All patients had more than three symptoms involving at least two organ systems. A specially designed symptom-based score measured the severity of the symptoms, and this SBS was used to quantify the efficacy of the intervention. At baseline, the SBS in each infant was ≥ 12 , and within 4 weeks of treatment, it had improved strongly in both groups. However, we are aware that this strong reduction in SBS can be partially attributed to regression towards the mean since we were selecting a diseased subpopulation. Under certain assumptions (mean and standard deviation of the general population, 2–6 and 3–7 score points, respectively), we estimated that the regression towards the mean could be between one and four SBS points.

The eWH led to fewer late reactions (8/41; 20%) than the eCH (18/44; 41%) ($p = 0.037$). However, the imbalance in total IgE positives at baseline, 55% for whey versus 36% for casein, may influence this outcome. Therefore, we repeated the analysis by logistic regression, correcting for total IgE at baseline. But again we found a statistically significant effect ($p = 0.031$). The patch test was not used in this trial, as it is not used in clinical routine in Belgium, but it could have provided interesting information (10).

We were able to show a beneficial effect in anthropometry for the eWH when applying the per protocol principle, as the eWH-NanPro1/2[®] sequence led to better growth (weight and weight for age z-scores). However, we are aware that this finding is exploratory and requires experimental proof in another trial. Previous analysis in a prospective, population-based cohort (the GINI study) reported reduced weight gain in children fed the same eCH (without probiotics) during the first year of life, but showed no effect on growth between the ages of one and six (11). The findings in our trial are in line with the findings of this study. However, when the GINI study follow-up period was extended from 6 to 10 years, no long-term consequences on BMI could be shown for the four infant formulas (12). In another trial, carried out on a double-blind basis, anthropometric measures and 24-hour dietary recalls obtained at 30, 60, 90, 120 and 150 days showed no difference in a group fed the same eCH, without and with lactobacillus GG, or partially whey/casein (60/40%) hydrolysate, also with lactobacillus GG (13). But, there are some data in the literature that suggest that a whey hydrolysate provides better nutrition than a casein hydrolysate (14).

Since the SBS we used was specially designed for this study by the authors, we also tried to validate the score, according to 'Validating scales and indexes' (15). We showed face, content and construct validity. Test-retest reliability was not part of our investigation.

The challenge test is considered as the gold standard for diagnosing CMPA. So we were interested to see how measuring the specific IgE and total IgE at baseline could help to predict the outcome of the challenge test 1 month

later. Since specific IgE (cow-milk, alpha-lactalbumin, beta-lactalbumin, casein) was only positive in one in three children, it makes no sense to evaluate these outcomes with respect to their sensitivity and specificity. Total IgE was positive in 33 children and allows us make reasonable estimates of sensitivity and specificity, which were 53% and 82, respectively. In many situations, IgE-mediated allergy is relatively easy to recognize because of its rapid onset. The more rapid the reaction, the easier it is to relate ingestion of certain foods to the appearance of symptoms. It is reasonable to question whether a challenge test needs to be performed to confirm the diagnosis if one of these specific IgE-determinations tests is positive. But, on the other hand, the sensitivity is low. The same is true for the SPT (rash diameter after NanPro1® challenge 3 mm greater than rash diameter of the negative control (NaCl)): the sensitivity and specificity were 15% and 92%, respectively. This raises the question of whether it is worthwhile performing these investigations as the results are only positive in a small minority of cases.

Many delayed allergic reactions are non-IgE-mediated (1,4), and it would be worthwhile to have a symptom-based validated scoring system specially for non-IgE-mediated allergy. The SBS that we developed contributed to the diagnosis of CMPA, as 70% of the infants with a score ≥ 12 had a positive challenge.

Analysing the composition of the GI-flora provided the expected results: more bifidobacteria in the eWH group (which was enriched with bifidobacteria) and more lactobacilli in the eCH group (which was enriched with lactobacilli). Data were available that the eCH enriched with the same amount of lactobacillus GG was well tolerated and successfully colonized the intestinal tracts of healthy, term infants (16). Enrichment of hydrolysates with probiotics may induce a more rapid development of tolerance as was shown for the lactobacillus strain that was present in the eCH (17). The addition of lactobacillus GG to eCH was shown to increase the decrease faecal calprotectin more than eCH without lactobacillus GG in infants with allergic colitis (18). A detailed discussion on the advantages of bifidobacteria versus lactobacilli is beyond the scope of this paper. The goal was to demonstrate the viability of the strains in both formulas.

In conclusion, the SBS developed by the authors for this study provided an additional parameter in the diagnosis of CMPA, especially in non-IgE-mediated CMPA. The therapeutic efficacy of the eWH and eCH formulas we tested was comparable. Therefore, the choice of hydrolysate will depend on other variables such as availability, palatability, cost and, last but not least, nutrition. However, the most important factor is that the hydrolysate chosen has been evaluated for its efficacy, in vitro and in vivo, in scientifically sound studies (4).

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CONFLICT OF INTEREST

Y. Vandenplas is consultant for United Pharmaceuticals, Biocodex and Rongis AC.

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