Received: 6 September 2021 | Revised: 10 May 2022 | Accepted: 17 May 2022

DOI: 10.1111/pai.13814

ORIGINAL ARTICLE

WILEY

Hypoallergenicity assessment of an extensively hydrolyzed whey-protein formula in cow's milk allergic infants

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Funding information
The study was funded by Danone Nutricia Research. Alessandro Fiocchi received funds for research, fees for consulting, speaker fees, and reimbursement for attending a symposium from Danone.

Editor: Rachel Peters

Abstract

Background: Extensively hydrolyzed formulas are recommended for the dietary management of infants with cow's milk allergy (CMA).

Objectives: Hypoallergenicity, growth, and gastrointestinal (GI) tolerability of a new extensively hydrolyzed whey-protein formula (eHWF) in CMA children were assessed.

Methods: In this prospective, randomized, international, multi-center study (Trial NL3889), 34 children with confirmed CMA (74% IgE-mediated) underwent a double-blind, placebo-controlled food challenge (DBPCFC) with an eHWF developed with non-porcine enzymes, supplemented with prebiotic short-chain galacto- and long-chain fructo-oligosaccharides (0.8 g/L, ratio 9:1), arachidonic acid (0.35/100 g), and docosahexaenoic acid (0.35/100 g). If tolerant to the eHWF, children participated in a 7-day open food challenge with this eHWF. Anthropometrics and GI tolerability were assessed in an optional 16-weeks follow-up.

Results: Of the 34 children who started the DBPCFC with the eHWF, 25 subjects (19 boys, mean age: 61 weeks, 18 with IgE-mediated CMA) completed the DBPCFC and 7-day open challenge without major protocol deviations and tested negative at both challenges. One child experienced a late moderate eczematous allergic reaction in the optional follow-up period, indicating the need for close monitoring of subjects starting new formula. Weight and length gain followed the World Health Organization growth curves. Changes in frequency and consistency of stools upon test formula intake were transient.

Conclusions: The newly developed eHWF is a suitable option in CMA treatment as all subjects tolerated the product. This result is in line with the international criteria for hypoallergenicity (American Academy of Pediatrics) that state that more than 90% of CMA children must tolerate the formula. Use of the formula is also associated with normal growth curves and GI tolerability.

Trial registration: Trial NL3889, https://www.trialregister.nl/trial/3889.

Abbreviations: AAP, American Academy of Pediatrics; AE, adverse event; AST, all subjects treated; CM(P)A, cow's milk (protein) allergy; CMP, cow's milk protein; DBPCFC, double-blind, placebo-controlled food challenge; eHWF, extensively hydrolyzed whey-protein formula; ESPACI, European Society for Paediatric Allergology and Clinical Immunology; ESPGHAN, European Society for Paediatric Gastroenterology, Hepatology and Nutrition; GI, gastrointestinal; IgE, Immunoglobulin type E; PP, per protocol; WHO, World Health Organization.

KEYWORDS
clinical trial, infant formula, milk hypersensitivity

1 | INTRODUCTION

Over the last decades, food allergies have become an emerging healthcare issue not only in the western world but also in developing countries.[1] It is estimated that 6–8% of infants under 3 years of age have a food allergy, among which cow's milk allergy (CMA) is the most common.[2–4]

Clinical symptoms of CMA may start during the first weeks of life, shortly after the introduction of cow's milk proteins (CMP), characterized by cutaneous, gastrointestinal (GI), and/or respiratory symptoms and pain behavior.[2] CMA can be classified as immunoglobulin E (IgE)-mediated, usually characterized by the occurrence of symptoms immediately after antigen exposure, or non-IgE mediated, characterized by delayed allergic symptoms, or a combination of both. Its management in infants and young children requires total avoidance of CMP and, when breastfeeding is not possible, the use of "hypoallergenic" formulas. Hypoallergenic formulas based on extensively hydrolyzed proteins or amino acid mixtures are the only safe option.[2] These infant formula substitutes must be proven to be hypoallergenic and have good tolerability, safety, and nutritional adequacy. The nutritional quality is crucial since studies have shown that the growth of children with CMA may be compromised,[5,6] potentially because the dietary intake of macro- and micronutrients is below the recommendations compared to healthy children.[7]

The aim of this study was to investigate the hypoallergenicity and safety (growth and GI tolerability) of an extensively hydrolyzed whey-protein formula (eHWF), according to international guidelines (American Academy of Pediatrics [AAP], European Society for Paediatric Allergology and Clinical Immunology [ESPACI], and the European Society for Paediatric Gastroenterology, Hepatology and Nutrition [ESPGHAN]).[8–10]

Key Message

This study describes the safety and hypoallergenicity of a new extensively hydrolyzed whey-protein formula, derived by non-porcine enzyme hydrolysis, for infants with diagnosed cow's milk allergy (CMA). The use of this formula does not impact growth and is a suitable nutritional option for the dietary management of CMA in infants.

2 | METHODS

This prospective, randomized, controlled, multi-center study was conducted in the Netherlands, Italy, and Poland. Our primary objective was to assess the hypoallergenicity of a newly developed extensively hydrolyzed whey-protein infant formula in children with CMA according to international guidelines.[8–10] We also aimed to assess the effects on growth and tolerability of the formula in a 16-week follow-up. The study was registered in the Dutch Trial Register on 1 July 2013 (registration number NTR4051).

2.1 | Study population

We included infants and children with proven CMA from January 2013 till March 2016, with an age range from birth to 3 years. CMA had to be diagnosed within 2 months prior to the study start defined by a positive double-blind, placebo-controlled food challenge (DBPCFC) with cow's milk, *or* by a positive open or single-blind open food challenge with cow's milk, with clear immediate reactions and a positive test for specific IgE (in serum or skin prick test) carried out under the supervision of a pediatrician. We excluded infants and children with a confirmed history of an anaphylactic reaction to cow's milk, those with intolerance to lactose or any other component of the study formula, those with a previous allergic reaction to any existing eHWF, and those fed any amino acid-based formula (for more than 6 weeks preceding the first part of the DBPCFC) due to potentially severe CMA. Children with major congenital malformations and/or existing illnesses that could interfere with formula acceptance or identification of allergic reaction, children breastfed more than twice daily, participating in another clinical study, or whose parents were judged unable to comply with the protocol requirements were also excluded. The minimum expected consumption of the study formula during the open challenge phase had to be 250 ml per day.

2.2 | Study design

The AAP guidelines for clinical testing of hypoallergenic formulas, which have been supported by the ESPACI and ESPGHAN, suggest that the number of subjects must be sufficient to demonstrate with 95% confidence that 90% of the subjects will not react to the formula.[8–10] The number of subjects needed to project with 95% confidence (one-sided interval) that less than 10% of subjects will react to the product is 25 subjects if zero clinical reactions are observed, and 43 subjects if one clinical reaction is observed. These sample size estimates were derived based on binomial distribution techniques using Wilson's method for deriving confidence intervals for single proportions.

Prior to the DBPCFC, a blood sample was drawn from all participating children to determine total IgE and allergen-specific IgE levels. In addition, data on anthropometrics and recent GI symptoms were collected.

In the challenge, the new formula (manufactured by Nutricia Research, Cuijk, The Netherlands) was tested by a DBPCFC followed by an open food challenge for 1 week in case of a negative DBPCFC. The DBPCFC consisted of two separate hospital visits (visit 1 and visit 2) within a 1-week time frame; children were randomized to receive either: (1) the test formula on the first test day and the reference formula (amino acid formula) on the second test day or (2) the reference (amino acid formula) on the first test day and the test formula on the second test day. Randomization was computer-generated (using SAS Proc Plan) and stratified by the study site. Participating clinicians received the treatment order within sealed opaque envelopes.

The reference formula and the eHWF were reconstituted according to the manufacturer's instructions; for blinding, the latter was mixed with the placebo formula at a 1:1 ratio. The prescribed schedule was 1, 5, 10, 25, 50, 80 and 100 ml. The administered formulas were prepared by a staff member who was not involved in the patient's care. The investigator, the nursing staff and the family were therefore not informed of what formula the child was being fed.

Children were monitored in the hospital for acute allergic reactions for 2 h after the challenge. The children who did not show any allergic symptoms after the DBPCFC directly continued with openly consuming test formula for 1 week. The parents were asked to report by phone possible delayed allergic reactions during the week following the challenge. The investigator evaluated the plausibility of reported potentially allergic symptoms. The primary outcome was the incidence of immediate and/or delayed allergic reactions during the DBPCFC and/or subsequent open challenge with the test formula during this first phase of the study.

After participation in the DBPCFC and subsequent open challenge, an optional follow-up of 16 weeks was proposed. Children with consented to participation in the study continued consumption of the test formula and were weekly monitored for 16 weeks on anthropometrics and GI tolerability. The child was weighted on a calibrated weighing scale with a precision of 100 g. The length of the child was measured in full extension, using an infantometer or inflexible length board with a fixed headboard and moveable footboard with 0.1 cm precision. In case children were able to stand, general appropriate equipment with 0.1 cm precision was used. Parent-reported GI tolerability was assessed on a weekly basis on a four-point scale (absent/mild/moderate/severe) for colics/crying, vomiting, diarrhea/soft stools, constipation/hard stools, and any other symptoms. Parent-reported stool consistency and frequency were assessed on a weekly basis using for consistency a four-point scale (watery/soft/formed/hard) and for frequency the average number of stools per day in the last week.

2.3 | Formulae

The test formula was a nutritionally complete, powdered, newly designed; non-porcine enzymes derived, extensively hydrolyzed whey-based infant formula for infants/children with CMA (see Table 1).

TABLE 1 Product composition of the test eHWF formula

	Test eHWF (per 100 ml prepared product)
Energy	
kCal	66
Protein (g), of which	1.6
Whey protein	1.6
Carbohydrates (g), of which	7.2
Sugar, of which	4.6
Lactose	4.1
Polysaccharides	2.6
Fats (g), of which	3,3
Saturates	1.5
Fibre, soluble (g)	0.6

The formula was enriched with long-chain polyunsaturated fatty acids (arachidonic acid and docosahexaenoic acid) and a mixture of prebiotic short-chain galacto-oligosaccharides, and long-chain fructo-oligosaccharides (further characterization of the new extensively hydrolyzed protein will be described in *Knipping* et al. *manuscript in preparation*). The reference formula used in this study was a commercially available, amino acid-based, nutritionally complete, powdered, hypoallergenic infant formula for the management of (severe) CMA (Neocate, Nutricia Advanced Medical Nutrition).

2.4 | Ethics

The approval of the relevant ethics committees in the participating countries was obtained before the start of the study. The study was conducted according to ICH-GCP principles and in compliance with the principles of the "Declaration of Helsinki" (59th WMA General Assembly, Seoul, October 2008) and with the local laws and regulations of the country where the study was performed. Written informed consent was obtained from all parent(s)/guardian(s) before enrolment in the study.

2.5 | Statistical analysis

A challenge was considered positive when objective symptoms appeared and when there were severe, persisting (more than 40 min) subjective symptoms.[11] Based on the results of the DPBCFC and subsequent open challenge, the proportion of children tolerating the test formula was calculated with a one-sided 95% Wilson's score confidence interval (for a single proportion).

If the lower confidence limit of the one-sided 95% confidence interval for the population fraction of children with tolerance to the test formula is larger than or equal to 90%, we can claim with 95% confidence that 90% or more of the subjects will tolerate the formula.

For anthropometric data, length-for-age and weight-for-age z-scores were calculated according to the World Health Organization (WHO) reference values.[12]

Other data were described and/or summarized either by means and standard deviations or medians and interquartile ranges as appropriate for continuous data, or by number and percentages for categorical data.

3 | RESULTS

3.1 | Study subjects

Fifty children were screened, of which 14 failed screening and 36 were randomized. In the majority of the screening failures, CMA could not be confirmed according to the definition in the protocol. Of the 36 randomized children, 34 actually started test or reference formula intake (all subjects treated [AST]) of which 29 children completed the challenge phase of the study. Among the children who did not complete the challenge phase ($n = 5$), one subject reported aversion to the taste of the test formula. Of these 29 children, 25 children participated in the optional 16-week follow-up of the study. In total 25 children were considered eligible for the per-protocol (PP) population; they had no major protocol deviations that would affect the evaluability based on the international guidelines,[8–10,13] and 4 children were considered not evaluable. Reasons for exclusion from the PP population were erroneous randomization (not fulfilling the inclusion criterion of having a confirmed CMA; $n = 1$) and low test/reference formula intake during the DBPCFC or subsequent open challenge ($n = 3$) of the study. In total, 23 children completed the optional 16-week follow-up of the study. The subject flow chart is depicted in Figure 1.

3.2 | Demographics and baseline characteristics

The children in the PP population were recruited in Italy (72%) and the Netherlands (28%). A 76% of the recruited children were males and their age at baseline ranged from 15.1 to 142.7 weeks of age. Their gestational age, weight for age z-score, and length for age z-score at screening were all within the normal range.

In the PP population, 80% of the children reported skin symptoms and 68% GI symptoms when consuming CMP. At study entry, the majority of the children were fed an eHWF ($n = 14$), others reported consumption of rice hydrolysate ($n = 3$), soy ($n = 6$), and amino acid-based formulae consumption outside exclusion criterium of severe allergy ($n = 2$; Table 2). Ten children were mixed-fed with a combination of formula feeding and breastfeeding at the time of enrolment in the study. Data are depicted in Table 2.

The demographics and baseline characteristics for AST can be found in Table S1.

3.3 | Allergic reactions

None of the 25 children in the PP population showed allergic reactions to the test formula during the DBPCFC, nor in the open challenge (see Table 3). No reaction was reported among the 9 children that were excluded from the PP analysis. In accordance with the international guidelines,[8–10] this confirms that with 95% confidence, test formula was tolerated by at least 90% of infants or children with confirmed CMA.

One late allergic reaction was reported in the optional 16-week follow-up of the study. The reaction occurred in a boy (32 months of age), with high total IgE but negative specific IgE levels against CMP. The child passed the screening phase and showed no acute and/or delayed

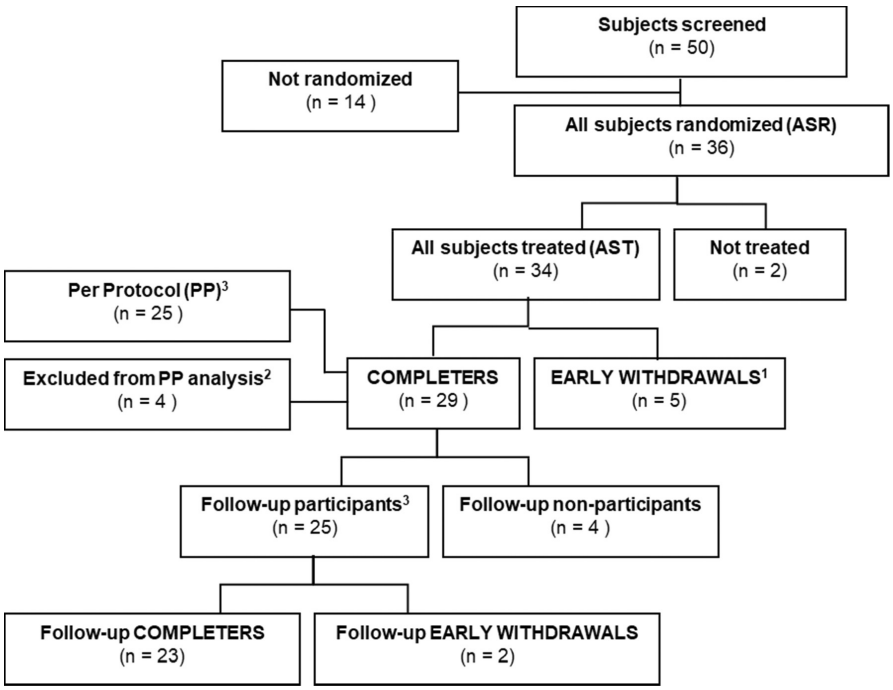

FIGURE 1 Subject flow chart. ASR, all subjects randomized; AST, all subjects treated; CMPA, cow's milk protein allergy; DBPCFC, double blind placebo-controlled food challenge; PP, per protocol. [1]Reasons for early withdrawal are non-compliance with the visit schedule ($n = 1$) and insufficient intake during the DBPCFC test ($n = 4$). [2]Exclusion from PP analysis are CMPA not confirmed ($n = 1$) and low study product intake ($n = 3$). [3]Inclusion in the PP analysis was not a criterion for participation in the follow-up. These numbers are incidentally the same

TABLE 2 Demographic data and subject characteristics at baseline for PP population

Characteristics	Statistics	Total ($n = 25$)
Age at baseline (weeks)	Mean (SD)	60.57 (32.06)
	Median (IQR)	52.6 (38.6–86.6)
	Min–Max	15.1–142.7
Country	Italy n (%)	18 (72.00%)
	Netherlands n (%)	7 (28.00%)
Ethnicity	Caucasian/White n (%)	21 (84.00%)
	Combination of ethnicity/other n (%)	4 (16.00%)
Gender	Male n (%)	19 (76.00%)
	Female n (%)	6 (24.00%)
Length-for-age z-score	Mean (SD)	−0.212 (1.210)
	Median (IQR)	−0.25 (−1.16 to 0.75)
	Min–Max	−2.53 to 2.18
Weight-for-age z-score	Mean (SD)	−0.238 (1.320)
	Median (IQR)	0.04 (−1.01 to 0.56)
	Min–Max	−3.45 to 1.63
CMA symptoms	Skin symptoms n (%)	20 (80%)
	Nasal symptoms n (%)	5 (20%)
	Ophthalmic symptoms n (%)	3 (12%)
	Respiratory symptoms n (%)	1 (4%)
	Gastric symptoms n (%)	17 (68%)
	Change in behavior n (%)	7 (28%)
IgE-mediated CMA[a]	Yes n (%)	18 (72%)
	No n (%)	6 (24%)
	Missing n (%)	1 (4%)
Other allergies[b]	None n (%)	19 (76%)
	House dust mite n (%)	1 (4%)
	Egg n (%)	5 (20%)
	Other n (%)	3 (12%)
Formula at enrolment[c]	Conventional cow's milk formula n (%)	0 (0.0%)
	Extensively hydrolyzed formula[d] n (%)	14 (56.0%)
	Amino acid-based formula n (%)	2 (8.0%)
	Soy formula n (%)	6 (24%)
	Rice formula n (%)	3 (12%)
	Missing n (%)	1

Abbreviations: CMA, cow's milk allergy; IQR, interquartile range; Max, maximum; Min, minimum; PP, per protocol; SD, standard deviation.

[a]IgE-mediated CMA is defined as clinical CMA together with positive levels of total IgE and/or cow's milk-specific IgE.

[b]Clinical allergies.

[c] One subject consumed 2 types of formula.

[d]The extensively hydrolyzed formula could be either whey or casein-based.

allergic symptoms during the DBPCFC with the test or the reference formula. Scattered single spots of eczema during the open challenge were not considered a positive allergic reaction by the investigator. However, during the follow-up phase repeated severe eczema with immediate relief after elimination of the test formula was interpreted by the investigator as proof of a delayed allergic reaction (type IV) to the test formula, and the intake of test formula was permanently stopped.

3.4 | Anthropometrics

Because of the wide variation in baseline age of the children individual length-for-age and weight-for-age growth data per gender were evaluated (Table S2). In the follow-up phase, weight and length gain followed the normal WHO growth curves, within the WHO ranges (The WHO Multicentre Growth Reference Study (MGRS)).

TABLE 3 Incidence of allergic reactions to the test formula during DBPCFC and subsequent open challenge in PP population

		Placebo ($N = 25$)	Verum ($N = 25$)	95% LCL
DBPCFC				
No	n (%)	25 (100.0%)	25 (100.0%)	
Yes	n (%)	0 (0.0%)	0 (0.0%)	
Open challenge				
No	n (%)		25 (100.0%)	
Yes	n (%)		0 (0.0%)	
DBPCFC + open challenge				
No	n (%)		25 (100.0%)	0.902[a]
Yes	n (%)		0 (0.0%)	

[a]95% lower confidence limit (of Wilson's score one-sided CI). DBPCFC = double-blind placebo-controlled food challenge.

3.5 | Safety outcomes

Safety was assessed on AST ($n = 34$). Overall, 94 adverse events (AE) occurred in 26 children (76.5%) from the start of test formula intake. Frequently seen AEs included GI disorders, infection and infestations, immune system disorders, and skin and subcutaneous tissue disorders (Table S3). Most of the AEs were mild and assessed by the investigator as not related to the test or reference formula. However, three AEs in two subjects were indicated to be possibly related to the test formula as indicated by the investigator. One subject developed mild choking and mild hunger, related to the physical properties and taste of the formula. No treatment was required, the event recovered in 6 days and the subject continued with the test formula. The other subject with a possibly related AE in the 16-week follow-up is described in more detail above.

Thirteen severe AEs were reported in 4 subjects. These were GI disorders, infections, investigations, nervous system disorders, and skin and subcutaneous tissue disorders. None of these events were indicated by the investigator to be related to the test formula intake. Most of them had a short duration and did not require medication. No serious adverse events were reported in this study.

3.6 | GI tolerability

By collecting parent-reported, non-allergy-related GI symptoms from the first day of the DBPCFC (hospital visit 2) up to and including the 16-week follow-up (Figure S1), the GI tolerability of the test formula was assessed. At baseline, the percentage of children with GI symptoms was low (<20%). At baseline, the majority of the children had stool consistency reported as "soft" or "formed." The use of an infant formula or changing to another infant formula can cause transient changes in stool patterns as also seen in other studies,[14,15] reflected in Figure 1 showing that the first couple of weeks of 16-week follow-up (weeks 3–8) the consistency slightly differed compared to baseline (predominantly softened). This observation seems to be transient as the consistency was back to baseline level by the end of the 16-week follow-up. Other GI symptoms were hardly reported during the first weeks of the follow-up period. Overall, almost all parent-reported symptoms were mild. Stool frequency was very consistent throughout the study (Table S4).

3.7 | Serum analysis

From the PP population, 18 children (72%) were classified as having an IgE-mediated allergy since they had positive IgE levels for cow's milk and/or specific cow's milk fractions. Six children (24%) were classified as non-IgE-mediated CMA and one child could not be classified since IgE was not determined. To characterize the children, a microarray chip technology (ISAC) was used to determine the IgE sensitization pattern to 112 allergens (Table S5). The children could roughly be divided into 3 groups: (1) no detectable IgE, (2) sensitization to cow's milk (Bos d 4, Bos d 5, Bos d 6, and Bos d 8) and/or hen's egg (Gal d 1, Gal d 2, and Gal d 3) with no or single co-sensitizations, or (3) polysensitized to a range of allergens beyond cow's milk and hen's egg only (Table S5).

4 | DISCUSSION

This study was designed according to international guidelines (AAP, ESPACI, and ESPGHAN), with the purpose of verifying the hypoallergenicity of a newly developed eHWF. The formula needs to be clinically tested, demonstrating hypoallergenicity in 90% of CMA infants with 95% confidence, by means of showing no defined symptoms to the formula under double-blind, placebo-controlled conditions.[8–10,13]

Twenty-five children were recorded as having no major protocol deviations. None of these children showed allergic reactions to the test formula during the DBPCFC, or during the subsequent open challenge, proving tolerability of 100% in this study cohort. In accordance with the international guidelines,[8–10,13] this proves hypoallergenicity of the tested eHWF.

Since nutritional adequacy in the management of CMA is crucial, continued use of the formula during a 16-week follow-up phase was proposed to enable measuring of the nutritional and anthropometric parameters. During this follow-up period, one late allergic reaction

was reported. Previous studies suggest that 2–10% of infants with uncomplicated CMA may be intolerant to eHWFs,[16,17] rising to 40% among infants with more complex forms of the disease.[18,19] Infants, who react to eHWFs, develop delayed non-IgE-mediated GI reactions to cow's milk and severe eczema more frequently than those who do not react to eHWFs.[20] The one late allergic reaction in the follow-up phase is in line with this information from the literature.

The IgE sensitization patterns of the subjects as determined by the ISAC method (see Table S5) could roughly be divided into 3 groups: (1) no detectable IgE, (2) sensitization to cow's milk and/or hen's egg with no or single co-sensitizations, or (3) polysensitized to a range of allergens beyond cow's milk and hen's egg only (method and result Table S5). However, this IgE sensitization pattern does not seem to be indicative of the tolerability of the test formula, as all subjects tolerated the eHWF investigated in this study.

Due to the general indication, children with a confirmed history of severe CMA with a high risk of anaphylaxis were excluded from participation. So, this study does not completely represent the total population of infants and children with CMA, but children who fit the criteria to be prescribed a cow's milk protein-based eHWF.[2] This however does represent the majority of CMA cases, as eHWF are considered the first line of treatment and are consequently widely used, constituting a source of nutrition of up to 100% of intake in the first 4–6 months of life. It is therefore recommended that studies aiming to demonstrate the hypoallergenic effects of these products also include an appropriate nutritional evaluation to determine their efficacy. This study showed that feeding with the test formula enabled growth in line with WHO standards, previously observed for cow's milk protein-based eHWF feeding.[21,22]

In addition to assessing growth, a clinical safety assessment was made. The frequency and types of reported AEs, as well as concomitant medication, were consistent with the studied population of young children with CMA and did not raise safety concerns. One subject experienced moderate eczema in the optional 16-week follow-up, which was indicated to be related to the test formula by the investigator as the symptoms resolved after withdrawal of the test formula. This is in line with the observation that CMA subjects on an eHWF can still mildly or moderately react to the hydrolysate. Subjects should therefore be closely monitored by their physician over a longer period.[2] Analyses of the GI tolerance data showed a transient shift toward softer stools, which is in line with the characteristic of hydrolysates to cause soft, liquid stools.[23]

It has long been known that there is a broad range of attributes among hydrolyzed formulas with significant differences, especially in their taste.[24] In this study only one parent reported aversion to the taste of the test product. This is in line with the observation of Maslin et al., who described that whey-based lactose-containing eHWFs are generally considered to be more palatable and accepted in comparison to other hydrolysates (non-lactose, casein-based).[25,26]

The main strength of the present study was that it has been designed according to the AAP guidelines for testing hypoallergenicity of a hydrolyzed formula,[8] which have also been adopted by the ESPACI and ESPGHAN. These criteria describe a formula to be hypoallergenic if at least 90% of infants with documented CMPA tolerated the hydrolysed formula under double-blind, placebo-controlled conditions, with a 95% confidence interval. In addition, we have included a voluntary 16-week follow-up period which allowed for assessment of growth and GI tolerance, strengthening the assessment of the overall tolerability of the hydrolyzed formula. Moreover, baseline analysis of total and cow's milk-specific serum IgE allowed us to conclude that the hydrolysate is tolerated in both IgE and non-IgE mediated cow's milk allergy.

A limitation of the study is the exclusion of infants that were on the amino acid formula for scientific and safety reasons. So, this study does not completely represent the total, global population of cow's milk allergic infants and children.

In conclusion, the study formula was tolerated by more than 90% of infants with a diagnosed CMA, with a 95% confidence interval, and is therefore in line with the criteria of hypoallergenicity and safety of the international guidelines (i.e., AAP) for eHWF. The formula also supported proper growth and GI tolerability in those infants. The palatability of the formula tested makes it a suitable option in the treatment of CMA in terms of efficacy, nutritional adequacy, acceptance, and tolerance.

AUTHOR CONTRIBUTIONS

Lamia Dahdah: Formal analysis (supporting); Investigation (equal); Writing—original draft (equal). **Mieke Roelofs:** Conceptualization (equal); Data curation (lead); Formal analysis (equal); Investigation (supporting); Methodology (lead); Project administration (equal); Visualization (equal); Writing—original draft (lead). **Karen Knipping:** Formal analysis (equal); Writing—original draft (equal). **Esther de Vries:** Investigation (equal); Writing—review & editing (equal). **Anneke Rijnierse:** Conceptualization (lead); Formal analysis (equal); Investigation (supporting); Project administration (equal); Writing—original draft (equal). **Johan Garssen:** Conceptualization (equal); Writing—review & editing (equal). **Paul L. P. Brand:** Investigation (equal); Writing—review & editing (equal). **Alessandro Fiocchi:** Conceptualization (equal); Formal analysis (equal); Investigation (lead); Supervision (equal); Writing—original draft (equal).

CONFLICT OF INTEREST

Johan Garssen is parttime employee of Danone Nutricia Research as well as Utrecht University.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/pai.13814.

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How to cite this article: Dahdah L, Roelofs M, Knipping K, et al. Hypoallergenicity assessment of an extensively hydrolyzed whey-protein formula in cow's milk allergic infants. *Pediatr Allergy Immunol*. 2022;33:e13814. doi:10.1111/pai.13814