UP International
ICC, Route de Pré-Bois, 20
CH 1215 Genève

PROPOSAL P1028 - CONSULTATION ON INFANT FORMULA PRODUCTS FOR SPECIAL DIETARY USE

UP INTERNATIONAL ANSWERS TO QUESTIONS RAISED BY FOOD STANDARDS AUSTRALIA NEW ZEALAND (FSANZ)

FOREWORD

The present document summarizes comments proposed by UP International on FSANZ's consultation paper (dated 3 August 2017), which compiles proposals for changing the food code related to Infant Formula Products for Special Dietary Uses (IFPSDU) and Infant Formula Products for Special Medical Purposes (IFPSMP). When relevant, propositions of answers to each specific question are displayed in the table below.

Part	Question	Page	Commentaries
DEFINITION	*Q1 Are there any other overseas regulations relevant to IFPSDU?*	10	
	Q2 What are the advantages and/or disadvantages of these options, in particular creating an 'infant formula product for special medical purposes' subcategory? If you support creation of a separate category for IFPSMP, should pre-term products be included?	15	Categorizing IFPSDU can be useful as specific conditions may require different nutritional requirements. The creation of a "transient gastrointestinal disorders" category is necessary as it allows the distinction between medical conditions requiring very specific adaptations (e.g. metabolic diseases such as phenylketonuria, etc.), and other conditions (colic, gastro-œsophageal reflux...), requiring functional adaptations, but which management may be improved with products having a specific composition. The latter products will be well captured by the proposed categorization. Establishing common characteristics for preterm and special medical purposes formulas seems difficult, owing to the particular needs of preterm and low-birth weight infants, and to the fact that prematurity is not a disease or medical condition per se. Therefore, the inclusion of preterm products to the IFPSMP category may not be preferable, and the creation of a separate IFPSDU category for such products may be sensible.
	Q3 Do you support inclusion of a category definition for IFPSDU in the Code? *Why or why not? Is the proposed definition of IFPSDU appropriate; if not, what should it say?*	18	
	Q4 If you support including a subcategory definition for IFPSMP in the Code, is the proposed definition of IFPSMP appropriate; if not, what should it say?	18	The establishment of a subcategory definition for IFPSMP may be useful, provided that the distinction between IFPSDU and IFPSMP is clear. Indeed, both products' categories share common features that make distinction difficult (*e.g.* the need for medical supervision). IFPSMP may also overlap with IFPSDU categories for specific products. The definition proposed by the FSANZ which states the following is adequate : "Infant formula product for special medical purposes means an infant formula product for special dietary use that is specifically formulated for infants: (a) who have (i) medically determined nutrient requirements, or (ii) limited or impaired capacity to take, digest, absorb, metabolise or excrete food including another type of infant formula product " The potential risk of consuming highly specialized products should be considered in IFPSMP definition. Therefore, adding the following bullet point to the definition may be useful: (iii) nutritional needs that cannot be covered by standard infant formula and/or for whom feeding with a standard infant formula can impair the health status

Part	Question	Page	Commentaries
	Q5 Are there any issues with the current definition for protein substitutes?	19	The current definition of protein substitute does not appear to raise any issues.
	Q6 Is there a benefit to defining one or more of the following in the Code: ▪ *Hypo-allergenic formula* ▪ *Partially hydrolysed formula* ▪ *Extensively hydrolysed formula* ▪ *Amino acid-based infant formula?* *If yes, what are the benefits of including these definitions? And what should be the key elements of each definition?*	19	This classification based on the degree of hydrolysis does not appear relevant because for some formulas this degree cannot be directly linked to the reduction of allergic reactions. For example rice-protein formulae are specially designed for infants with allergy to cow's milk proteins. These formulae are suitable for them because they do not contain milk, independently of the degree of hydrolysis of the protein. Nonetheless, if such definitions are to be included, then the proposed definition is not adapted. For example, sources of proteins should not be limited to whey, casein or soy proteins which are not the only protein sources used, and the term "extensively modified" is not precise enough. The definition of the above category has no benefits but only leads to misunderstanding.
	Q7 Are there any issues with the current definition for pre-term products?	21	
	Q8 What, if any, are the benefits of including age and weight parameters in the regulatory definition for pre-term products?	21	
	Q9 What is the general composition of human milk fortifiers for premature or low birthweight infants? ….and composition and uses for groups other than premature or low birthweight infants?	21	
	Q10 Is there a need to prescribe a name for IFPSDU – what are the implications for subcategories?	23	Prescribing a name for IFPSDU would clearly show to the consumer that the product is designed for a special dietary use and that it is different from a standard Infant formula or a follow-on formula.
	Q11 Is there a need to prescribe names for any the IFPSDU subcategories? If yes, what benefit would this provide?	23	It should be allowed to use prescribe names for IFPSDU subcategories that clearly state the indication of the product / the special dietary condition it is designed for. However, this prescribed names should not be fixed in the code to avoid imposing a name that is not completely adequate for the product.

Part	Question	Page	Commentaries
COMPOSITION	*Q12 Are any specific compositional requirements (energy/macronutrient etc) needed in the Code for formula intended for premature or low birthweight infants, or for those suffering metabolic etc. conditions? If so, what are they?*	26	Currently, medium chain triglycerides cannot be added to formulas intended for premature or low birthweight infant, or for those suffering of metabolic conditions. Added medium chain triglycerides are however authorized in protein substitutes. Maintaining this clause for protein substitutes and expanding this authorization to other formula categories would be relevant (infant formula, follow-on formula and all IFPSDU).
	Q13 Are any specific compositional changes needed in the Code for protein substitutes? If so, what are they and what is your justification for them?	26	Currently it is not clear if composition requirements of infant formula and follow-on formula about long chain fatty acids, vitamins, minerals (section 2.9.1-11 and 2.9.1-12) have to be followed for protein substitutes. Therefore, it should be considered to add the same provisions (1) and (2) which are already in sections 2.9.1-13 and 2.9.1-14, to the section 2.9.1-15.
	Q14 Are any specific compositional requirements (energy/macronutrient etc) needed in the Code if a new subcategory of formula for special medical purposes were created? If so, what are they?	26	If a new subcategory of formula for special medical purposes were created, then, provision (1) and (2) existing in sections 2.9.1-13 and 2.9.1-14 should be added.
	Q15 What benefit, if any, would the inclusion of a specific requirement for any IFPSDU to be demonstrated by generally accepted scientific data as: safe, beneficial and effective in meeting the specific nutritional requirements of intended infant subpopulation?	26	
	Q16 Are there any issues with the current requirements for micronutrients and nutritive substances in IFPSDU products?	28	Current requirements for micronutrients and nutritive substances in IFPSDU are compatible with Codex requirements. However, it is not the case with new European requirements (Delegated act 2016/128). For example, the range for vitamin D in Europe is 0.48 – 0.72 µg/100kJ. In standard 2.9.1, the current range is 0.25 – 0.63 µg/100kJ. It is in line with Codex, but it is not compatible with Europe because the common range is too small (0.48 – 0.63 µg/100kJ). A range of 0.25 – 0.72 µg/100kJ would become compatible with both Codex and European regulations. Noting the established Upper Level of Intake for vitamin D in Australia and New Zealand at 25 µg/d in infants aged 0-12 months, it is unlikely that such a maximum vitamin D dose in infant formulas raises health concerns in this population. Because of the same issue, the following range for choline in P1028 (amended 04/05/2016) appears adequate: 1.7 – 12 mg/100kJ.

Part	Question	Page	Commentaries
	Q17 Do you have any information to support the inclusion of a minimum and maximum amount of chromium in IFPSDU? If yes, should this be considered only in relation to certain categories of IFPSDU?	28	A maximum value for chromium is difficult to manage due to natural variation in raw materials. Currently, no upper level of intake was established by the FSANZ for chromium in infants aged 0-12 months. Similarly, such a value does not exist in Europe or the United States, due to the absence of adequate data reporting adverse effects. Therefore, setting a maximum value but keeping it large enough to avoid excessive technological constraints would be a valuable option. If the maximum is unable to be established, it should be kept open in order to align with Europe or the United States.
	Q18 Do you have any information to support the inclusion of a minimum and maximum amount of molybdenum in IFPSDU? If yes, should this be considered only in relation to certain categories of IFPSDU?	28	A maximum value for molybdenum is difficult to manage due to natural variation in raw materials. Currently, no upper level of intake was established by the FSANZ for molybdenum in infants aged 0-12 months. Similarly, such a value does not exist in Europe or the United States, due to the absence of adequate data reporting adverse effects. Therefore, setting a maximum value but keeping it large enough to avoid excessive technological constraints would be a valuable option. If the maximum is unable to be established, it should be kept open in order to align with Europe or the United States.
ADDITIVES	*Q19 Could one category of IFPSDU be used for all additional food additives, or should additional or modified subcategories be devised (noting the possible four subcategories in section 2.2).*	31	Keeping one category of IFPSDU for all additives appears as the most relevant approach. Defining additional food categories could be seen as confusing and would have an unnecessary impact on the potential of innovation and development of IFPSDU and IFPSMP. In addition, most additives will have properties that can be useful or beneficial for several categories, such as thickeners.
	Q20 Do you support the proposed amendments listed in Table 7 for IFPSDU at the amounts shown?	37	The approach of the FSANZ in the present proposal, which consists in aligning with the dispositions of the Codex and the European Commission regarding the regulation of infant formulas for special dietary uses / special medical purposes is sensible and may allow collecting more data and studies on the basis of a common regulation.

Part	Question	Page	Commentaries
	Q21 Can you provide information on suitable international safety assessment, a demonstrated history of safe use in the context of IFPSDU, and a technological justification for: ▪ *Calcium carbonates* ▪ *Calcium citrates* ▪ *Phosphoric acid* ▪ *Sodium alginate* ▪ *Xanthan gum* ▪ *Locust bean (carob bean) gum* ▪ *Pectins* ▪ *Sodium carboxymethylcellulose* ▪ *Sucrose esters of fatty acids* ▪ *Starch sodium octenylsuccinate*	37	Locust bean gum, pectins, xanthan gum have been added to infant formulas for special medical purposes for decades. These additives comply with the provisions laid down in European Regulation (EU) No 1333/2008 on food additives: ▪ Xanthan gum as a stabilizer ▪ Locust bean (carob bean) gum as a thickener ▪ Pectins as a thickener/gelling agent ▪ Starch sodium octenylsuccinate for its emulsifying and stabilizing properties Xanthan gum is authorized as a food additive in infant formula for special medical purposes in the European Union in accordance with Regulation (EC) No 1333/2008 on food additives, up to 1.2 g/l in Europe. The European Scientific Committee on Foods (SCF) first considered the use of xanthan gum in foods for special medical purposes for infants and young children as acceptable in 1999. Locust bean gum has a history of safe use in the European Union at a dose of 10 000 mg/kg in infant formula for special medical purposes. Locust bean gum thickened formulae are available in Europe for over 20 years. Its use in infant formula was approved by the European Scientific Committee on Foods (SCF) in 1994. A recent review of toxicological database and clinical evidence conclude that the consumption of locust bean gum is safe for use as thickener in infant formulas for treatment of uncomplicated but frequent troublesome regurgitation in infants (Meunier 2014). Regarding pectins, their safety and good tolerance was assessed and established in 5 recently published clinical trials, (listed hereafter), involving over 300 infants aged less than 18 months, two third of which were suffering from cow's milk protein allergy. In these studies, infants were fed formulas containing 0.5 g/100 mL of a fiber complex including pectins for up to 6 months. Results of these studies showed good acceptability, adequate growth against reference comparator, and significant benefits on a number of clinical outcomes. The fact that these products were well accepted, particularly in such a sensitive population as infants suffering from allergies, brings significant evidence of the safety of using this additive in infant formulas for special dietary uses. ▪ Vandenplas Y, De Greef E, Hauser B; Paradice Study Group. Safety and tolerance of a new extensively hydrolyzed rice protein-based formula in the management of infants with cow's milk protein allergy. Eur J Pediatr. 2014 Sep;173(9):1209-16. doi: 10.1007/s00431-014-2308-4. Epub 2014 Apr 12. PubMed PMID: 24723091; PubMed Central PMCID: PMC4134482.

Part	Question	Page	Commentaries
			▪ Meunier L, Garthoff JA, Schaafsma A, Krul L, Schrijver J, van Goudoever JB, Speijers G, Vandenplas Y. Locust bean gum safety in neonates and young infants: an integrated review of the toxicological database and clinical evidence. Regul Toxicol Pharmacol. 2014 Oct;70(1):155-69. doi: 10.1016/j.yrtph.2014.06.023. Epub 2014 Jul 2. PubMed PMID: 24997231. ▪ Dupont C, Kalach N, Soulaines P, Bradatan E, Lachaux A, Payot F, De Blay F, Guénard-Bilbault L, Hatahet R, Mulier S, Kapel N, Waligora-Dupriet AJ, Butel MJ. Safety of a New Amino Acid Formula in Infants Allergic to Cow's Milk and Intolerant to Hydrolysates. J Pediatr Gastroenterol Nutr. 2015 Oct;61(4):456-63. doi: 10.1097/MPG.0000000000000803. PubMed PMID: 25844709. ▪ Vandenplas Y, De Greef E, Xinias I, Vrani O, Mavroudi A, Hammoud M, Al Refai F, Khalife MC, Sayad A, Noun P, Farah A, Makhoul G, Orel R, Sokhn M, L'Homme A, Mohring MP, Merhi BA, Boulos J, El Masri H, Halut C; Allar Study Group. Safety of a thickened extensive casein hydrolysate formula. Nutrition. 2016 Feb;32(2):206-12. doi: 10.1016/j.nut.2015.08.008. Epub 2015 Sep 2. PubMed PMID: 26704966. ▪ Dupont C, Bradatan E, Soulaines P, Nocerino R, Berni-Canani R. Tolerance and growth in children with cow's milk allergy fed a thickened extensively hydrolyzed casein-based formula. BMC Pediatr. 2016a Jul 18;16:96. doi: 10.1186/s12887-016-0637-3. PubMed PMID: 27430981; PubMed Central PMCID: PMC4950604. ▪ Dupont C, Vandenplas Y; SONAR Study Group. Efficacy and Tolerance of a New Anti-Regurgitation Formula. Pediatr Gastroenterol Hepatol Nutr. 2016b Jun;19(2):104-9. doi: 10.5223/pghn.2016.19.2.104. Epub 2016b Jun 28. PubMed PMID: 27437186; PubMed Central PMCID: PMC4942307.
	Q22 Are there any technologically justified concerns with changing the permissions for citric and fatty acid esters of glycerol (472c) to: ▪ *MPL of 9000 mg/L for liquid products* ▪ *MPL of 7500 mg/L for powdered products?*	37	
	Q23 What is the technological justification for the use of diacyltartaric and fatty acid esters of glycerol (472e) in IFPSDU? Are there any technologically justified concerns with the removal of this permission?	37	
SAFETY	*Q24 Do you support retaining a maximum PRSL for any IFPSDU? Please provide your rationale.*	38	

Part	Question	Page	Commentaries
LABELLING	*Q25 To what extent is pre-term infant formula used following hospital discharge and how do caregivers access it (for example, by prescription)?*	48	
	Q26 Would you support the requirement for a statement that the product must be used under medical supervision, where the wording is not prescribed (an approach which harmonises with the overseas and international requirements)? Please describe your reasons why you do/do not support.	48	The requirement for such a statement for preterm infant formulas makes sense, because these products are very specific. Indeed, the name of the product and the distribution scheme would not be sufficient to inform consumers that the products must be used under medical supervision, especially if it is confirmed that these products are available for general sale. Not prescribing the wording, or letting the wording relatively open, would harmonize with EU and US provisions and ensure the 3 regions EU, US, & ANZ are aligned on wording for packaging, as stated by the FSANZ in its consultation paper.
	Q27 Are there any specific FSMP labelling requirements that you consider applicable to a particular type of IFPSDU	51	
	Q28 Are there any specific FSMP labelling requirements that should apply to all IFPSDU?	51	
	Q29 What specific labelling requirements for the safe preparation and use of IFPSDUs are being used that contradict the general requirements set out in subsection 2.9.1—19(3) of Standard 2.9.1?	54	
DISTRIBUTION AND ACCESS	*Q30 What evidence can you provide to support concerns regarding inappropriate access to any IFPSDU?*	60	

Part	Question	Page	Commentaries
ADDITIONAL COMMENT	Age of consumption of IFPSDU	NA	It is currently not clear if it is possible to place an IFPSDU on the market for use up to 3 years of age or older. However, some young children still have specific dietary requirements after one year of age (like children with cow's milk allergy for example) and would therefore need IFPSDU. A clarification on that point would be welcomed.

UP International
ICC, Route de Pré-Bois, 20
CH 1215 Genève

PROPOSAL P1028 - CONSULTATION ON INFANT FORMULA PRODUCTS FOR SPECIAL DIETARY USE

ANNEX:

PRESENTATION OF CLINICAL STUDIES

Annex related to Question n° 21 of FSANZ Consultation Paper on IFPSDU dated 3 August 2017

INTRODUCTION & OBJECTIVES

The present note aims at providing a rationale regarding the interest and safety of using pectin in infant formulas.

This document will first detail studies designed with Novalac formulas containing pectins and then, will present the regulatory context for several additives in the European Union and United States.

SUMMARY TABLE

1. SAFETY CONSIDERATIONS

1.1 NOVALAC STUDIES

Novalac has conducted a number of clinical studies with infant formulas containing pectins. Those studies have been performed in infants, aged from 1 to 18 months, fed with the study formula during up to 6 months.

5 clinical studies conducted by United Pharmaceuticals are presented below.

1.1.1 "ALLAR" study

Study ID

Two existing publications for this study:

Preliminary results:

Title: "Extensive protein hydrolysate formula effectively reduces regurgitation in infants with positive and negative challenge tests for cow's milk allergy"

Authors: Yvan Vandenplas M.D., Ph.D., Elisabeth De Greef M.D., ALLAR study group

Institution: UZ Brussel, Department of Pediatrics, Vrije Universiteit Brussel, Brussels, Belgium

Journal: Acta Pædiatrica, Volume 103, Issue 6, June 2014, Pages e243–e250

Link: here

Full reference: Vandenplas Y, De Greef E; ALLAR study group. Extensive protein hydrolysate formula effectively reduces regurgitation in infants with positive and negative challenge tests for cow's milk allergy. Acta Paediatr. 2014 Jun;103(6):e243-50. doi: 10.1111/apa.12615. Epub 2014 Mar 31. PubMed PMID: 24575806; PubMed Central PMCID: PMC4282102.

Final results:

Title: "Safety of a thickened extensive casein hydrolysate formula"

Authors: Yvan Vandenplas M.D., Ph.D., Elisabeth De Greef M.D. a, I. Xinias M.S. b, *et al.*, ALLAR study group

Institution: UZ Brussel, Department of Pediatrics, Vrije Universiteit Brussel, Brussels, Belgium

Journal: Nutrition, February 2016, Volume 32, Issue 2, Pages 206–212

Link: here

Full reference: Vandenplas Y, De Greef E, Xinias I, Vrani O, Mavroudi A, Hammoud M, Al Refai F, Khalife MC, Sayad A, Noun P, Farah A, Makhoul G, Orel R, Sokhn M, L'Homme A, Mohring MP, Merhi BA, Boulos J, El Masri H, Halut C; Allar Study Group. Safety of a thickened extensive casein hydrolysate formula. Nutrition. 2016 Feb;32(2):206-12. doi: 10.1016/j.nut.2015.08.008. Epub 2015 Sep 2. PubMed PMID: 26704966.

Study objectives

The aims of this study were to assess whether an extensively hydrolyzed casein formula (eCH) was well tolerated by at least 90% of the infants with cow's milk protein allergy (CMPA) and to compare the efficacy of a thickened (T-eCH) *versus* non-thickened (NT-eCH) version of this formula overall and for each symptom.

Methodology

Formula-fed infants less than 6 months of age with suspected cow's milk protein allergy (CMPA) and more than 5 episodes of regurgitation per day were given an extensively hydrolyzed casein formula in a randomized, controlled, double-blind manner. The formula contained extensively hydrolyzed casein, with a thickening fibre complex at a dose of 0.5 g/100 mL, including pectins. The comparator was a similar formula without the thickening complex. After one month feeding with the test formula, a food challenge was schedule to confirm the diagnosis of CMA. In case of negative challenge, the infant was fed with a standard formula, its participation to the study ended. If the challenge was positive, the parents were offered to feed their child with the study formula for an additional 5-month period.

Outcomes

The main outcomes were formula's hypoallergenicity, *i.e.* whether it was well tolerated by at least 90% of the infants with CMPA, with a 95% Confidence Interval, over a 1-month follow-up; and the number of regurgitations.

Other outcomes included anthropometric data, crying time, regurgitations' score, stool consistency, eczema, urticaria and respiratory symptoms. Anthropometric measures were weight, length, head circumference and body mass index (BMI); the z-scores were calculated. The cow's milk symptom score (CoMiSS) was used to assess the efficacy of each formula on symptoms of cow's milk allergy. It consists in a score calculated based on the presence of clinical manifestations (general comfort, dermatological, gastrointestinal and respiratory symptoms), ranging from zero to 33. The tool is based on an experts' consensus involving clinicians with expertise in managing children with gastrointestinal problems and/or atopic diseases (Vandenplas 2015)

CMPA symptoms were monitored at 0 and 1-month follow-up while anthropometrics were followed until 6 months.

Test formula composition

Compound	Dose per 100 g	Dose per 100 mL
Energy (kcal)	495.9	66.9
Protein (g)	12.1	1.6
Fat (g)	26.2	3.5
Carbohydrates (g)	52.7	7.1
Fibres (g)	3.6	0.5

Results

Population characteristics

77 infants were included in the study; 6 drop-out before the 1st month visit (1 in the thickened formula group, 6 in the control group). Among the 71 subjects available for follow-up at 1 month, 34 of which presented a cow's milk protein allergy confirmed by oral food challenge.

At inclusion, infants were aged 87.5 ± 46.2 days and weighed 5.4 ± 1.2 kg. Average birthweight and birth length were 3.2 ± 0.5 kg and 49.4 ± 2.4 cm respectively.

Hypoallergenicity

All subjects with confirmed CMPA (N=34) tolerated well the tested formula (thickened or not thickened) at 1 month (no drop-out).

CMPA symptoms

The CoMiSS decreased significantly during the first month in the study population (n=71; -7.5 ± 5.2, $p<0.001$) as well as in the subpopulation of infants having a proven CMA (-8.4 ± 5.2) with no significant differences between the two formulas.

Regurgitations

The daily number of regurgitations decreased significantly more in the thickened formula group (-4.36 ± 3.06) vs the control group (-2.91 ± 4.7), ($p=0.025$)

Growth parameters

Anthropometrics were evaluated at 6 months. While a slight growth faltering was observed at baseline in the whole population, normal growth *versus* WHO standards was observed at 6 months.

Drop-out & adverse events

No adverse events were related to the study formula. Six children dropped out before the end of the 1-month period. One was in the test group and was unable to accept the taste of the formula. The other five were in the control group (non-thickened formula) and the reasons were as follows: lost to follow-up (n=1), parents decision due to vomiting/liquid stools (n=2); successfully fed a AR formula (n=1), successfully fed an thickened extensively hydrolysed formula (n=1) None of the patients with proven CMPA dropped out during the 1-month intervention period.

Conclusion

This study show that in infants with regurgitations and a suspicion of CMA, an extensively hydrolysed formula thickened with pectin is well tolerated, allows the reduction of regurgitations and ensures a proper growth.

These results are in favor of the safety of using pectins in formulas for infants presenting persisting symptoms of cow's milk protein allergy.

1.1.2 "SYMPAL" study

Study ID

Title: "Safety of a New Amino Acid Formula in Infants Allergic to Cow's Milk and Intolerant to Hydrolysates"

Authors: Christophe Dupont, Nicolas Kalach, Pascale Soulaines *et al.*

Institution: Pediatric Gastroenterology, Hepatology and Nutrition Department, Necker Children's Hospital, Paris, France

Journal: Journal of Pediatric Gastroenterology & Nutrition: October 2015 - Volume 61 - Issue 4 - p 456–463

Link: here

Full reference: Dupont C, Kalach N, Soulaines P, Bradatan E, Lachaux A, Payot F, De Blay F, Guénard-Bilbault L, Hatahet R, Mulier S, Kapel N, Waligora-Dupriet AJ, Butel MJ. Safety of a New Amino Acid Formula in Infants Allergic to Cow's Milk and Intolerant to Hydrolysates. J Pediatr Gastroenterol Nutr. 2015 Oct;61(4):456-63. doi: 10.1097/MPG.0000000000000803. PubMed PMID: 25844709.

Study objectives

This trial aimed at comparing the efficacy of a new thickened amino-acid formula (TAAF, Novalac) containing a pectin-based thickener and a reference AAF (RAAF, Neocate) on allergy symptoms and safety parameters in infants below 18 months.

Methodology

This study was a randomized, double blind, controlled trial. Children who presented cow's milk protein allergy symptoms persisting after administration of an extensively-hydrolyzed casein formula were fed either with a thickened amino-acid formula containing fibers (including pectins) at a dose of 0.5 g/100 mL (Novalac; United Pharmaceuticals, Paris, France), or with a regular, non-thickened amino-acid fomula (Neocate; Nutricia, Erlangen, Germany) during 3 months. At the end of this period, all infants were fed with the test formula, for an additional duration of 3 months.

Primary outcome

Hypoallergenicity (*i.e.* the absence of allergic symptoms leading to study termination during the 1st month) of the formulas was assessed at 1-month follow-up.

Secondary outcomes

General symptoms potentially associated with CMPA such as irritability, crying frequency, crying time, sleep duration and quality (day and night) were recorded at 0, 3 and 6 months. Atopic dermatitis severity (SCORAD), growth parameters, stools' characteristics and biological parameters were also evaluated at the same time points.

Test formula composition

Compound	Dose per 100 g	Dose per 100 mL
Energy (kcal)	459.1	68.9

Compound	Dose per 100 g	Dose per 100 mL
Protein (g)	12.4	1.9
Fat (g)	21.5	3.2
Carbohydrates (g)	54	8.1
Fibres (g)	3.1	0.5

Results

Population characteristics

86 infants were included in the study. 11 infants were not included in the hypoallergenicity population for the following reasons: no confirmation of CMA (n=2); negative food challenge (n=8); no intolerance to eHF (n=1), so that the hypoallergenicity was evaluated on 75 infants. Out of the 75 infants, 54 had a CMPA confirmed by a gold standard test (double-blind, placebo-controlled oral food challenge), 20 by biological methods (skin prick test / specific IgE), and 1 by clinical history (anaphylactic risk).

At inclusion, infants were aged 6.2 ± 4.3 months and weighed 6.9 ± 1.9 kg.

Hypoallergenicity (primary outcome)

None of the 75 infants dropped out for intolerance at 1 month or at other time points.

CMPA symptoms

After 1 month, the main CMPA symptom resolved completely in 26 out of 42 and 17 out of 33 infants in the control and test groups respectively, and this symptom improved or resolved in 40 out of 42 and 32 out of 33 children in both groups respectively (no significant differences between formulas for both measurements).

Biological parameters

IgG, IgA, IgM, serum ferritin, and complete blood count were within normal range values at 3 months in both groups.

Plasma amino acid evaluation at 3 months in 47 children (25 in the test group and 22 in the control group) showed no significant differences between groups in amino acids concentrations, except for valine, lower and closer to those of breast fed babies (Picone 1989) in the test group (P=0.049). This difference was not clinically significant.

Atopic Dermatitis

An interesting finding in this study is the observed significant difference in reduction of the atopic dermatitis score (SCORAD) in the test group *versus* the control group (-27.3 ± 2.3 *versus* -20.8 ± 2.2 respectively, p=0.048).

Growth parameters

Weight, length, weight-for-length, BMI, and head circumference z scores were similar between groups at 3 months. While eight children presented baseline weight-for-age z-scores below – 2, growth at 3 and 6 months were within WHO growth standards in the thickened-feed group. In this group, length, weight-for-length, BMI, and head circumference z scores increased by 0.1 (±0.8), 0.1 (±0.8), 0.4 (±0.9), and 0.3 (±0.8), respectively, during the 6-month study.

Drop-out & adverse events

3 infants dropped out of the study during the 1st month, all in the control group and none in the hypoallergenicity population. The parents of 8 infants decided not to continue the study after 3 months (3 in the study formula and 5 in the control group).

A total of 7 serious adverse effects were recorded during the first 3 months: 5 in the test group (2 gastroenteritis, pneumonia, esophagitis and gastro-esophageal reflux) and 2 in the control group (gastroenteritis, esophagitis). 3 were recorded between 3 and 6 months (malaise, gastroenteritis, pneumonia). None of those SAE was related to the study formulas. Incidence of AE was not different between both groups.

Conclusion

These results were in favor of the safety of using pectins in an amino-acid formula for infants presenting persisting symptoms of cow's milk protein allergy.

1.1.3 "PARADICE" study

Study ID

Title: "Safety and tolerance of a new extensively hydrolyzed rice protein-based formula in the management of infants with cow's milk protein allergy"

Authors: Vandenplas Y, De Greef E, Hauser B; Paradice Study Group.

Institution: Department of Pediatrics, UZ Brussel, Vrije Universiteit Brussel, Brussels, Belgium, yvan.vandenplas@uzbrussel.be.

Journal: European Journal of Pediatrics September 2014, Volume 173, Issue 9, pp 1209–1216

Link: here

Full reference: Vandenplas Y, De Greef E, Hauser B; Paradice Study Group. Safety and tolerance of a new extensively hydrolyzed rice protein-based formula in the management of infants with cow's milk protein allergy. Eur J Pediatr. 2014 Sep;173(9):1209-16. doi: 10.1007/s00431-014-2308-4. Epub 2014 Apr 12. PubMed PMID: 24723091; PubMed Central PMCID: PMC4134482.

Study objectives

The study aimed at ascertaining the hypoallergenicity and safety of a rice-protein formula in infants with CMPA.

Methodology

Infants less than 6 months presenting symptoms of CMPA were selected for the study. They were included if CMPA was confirmed by an oral food challenge. The product consisted in a hydrolyzed rice-protein formula supplemented with lysine and tryptophan, containing a thickening complex with fibre at a dose of 0.5 g/100 mL including pectin (NovaRice, United Pharmaceuticals). Infants were fed with the product up to 6 months.

Primary outcome

Hypoallergenicity (no allergic reactions in 90 % of infants or children with confirmed cow's milk allergy, with 95% confidence) was evaluated at 1 month follow-up.

Secondary outcomes

Symptoms of CMPA were assessed at baseline, 1-, 3- and 6-month follow-up, using the cow's milk symptoms score (CoMiSS), which evaluates the occurrence of several clinical manifestations potentially related to CMPA (general comfort, dermatological, gastrointestinal and respiratory symptoms).

Growth (weight and length) was evaluated at baseline and 6-month follow-up using z-scores, as described by the WHO Child Growth Standards.

Test formula composition

Compound	Dose per 100 g	Dose per 100 mL
Energy (kcal)	480	64.8
Protein (g)	13.4	1.8
Fat (g)	25.5	3.4

Compound	Dose per 100 g	Dose per 100 mL
Carbohydrates (g)	49	6.6
Fibres (g)	4	0.5

Results

Population characteristics

42 patients were selected, 40 infants aged 3.4 ± 1.5 months had a CMPA confirmed by oral food challenge or by initial anaphylactic reaction, and were thus included in the study. The infants weighed 6.1 ± 1.1 kg and measured 61.9 ± 3.9 cm at inclusion. 2 patients dropped-out during the first month due to poor taste acceptance.

Hypoallergenicity (primary outcome)

Among the 38 children followed up at 1 month, none dropped out of the study for intolerance. In addition, only 2 subjects dropped out before 6 months, and the reasons were unrelated to safety (1 dislike odor and taste, 1 loss to follow-up).

CMPA symptoms

Each parameter composing the CoMiSS (called "SBS" for Symptom Based Score at the time of the study) had decreased after 1 month of dietary treatment with the study formula: 5.3% of infants had normal stools at baseline *versus* 52.6% at 1 month ($p<0.0001$), 57.9 % of the infants were crying more than 3 h/day at baseline *versus* none at 1 month ($p<0.0001$), and the regurgitation score decreased by 75 % over the same period ($p<0.0001$).

Growth parameters

Growth faltering was observed at inclusion, as evidenced by negative values (−0.7) of weight-for-age, weight-for-length, and BMI z-scores. From 1 month onwards, these parameters significantly increased and caught up the WHO Child Growth Standards by the end of the study period. Compared to baseline, the CoMiSS was significantly lower at each time point ($p<0.001$).

Drop-out & adverse events

No SAE were related to the study formula. One non-serious adverse event was reported as related to the study product, it was food refusal leading to the end of the study for this patient. Three parents decided to stop the trial because according to their opinion the infant did not like or accept the study formula and preferred the "initial" formula (which was given before the challenge). One patient did not show up for the visit after 1 month. 73% of the adverse events were related to ear-nose-throat infections.

Conclusion

These results provide evidence that a hydrolyzed rice-protein formula containing pectin is hypoallergenic and ensures proper growth in infants with cow's milk protein allergy. In addition, acceptability of the formula was good in this study.

1.1.4 "COMETE" study

Study ID

Title: "Tolerance and growth in children with cow's milk allergy fed a thickened extensively hydrolyzed casein-based formula"

Authors: Dupont C, Bradatan E, Soulaines P, Nocerino R, Berni-Canani R

Institution: Pediatric Gastroenterology, Hepatology and Nutrition Department, Necker Children's Hospital, 149, rue de Sèvres, 75015, Paris, France. christophe.dupont@nck.aphp.fr

Journal: BMC Pediatrics, BMC series open, inclusive and trusted – 201616:96

Link: here

Full reference: Dupont C, Bradatan E, Soulaines P, Nocerino R, Berni-Canani R. Tolerance and growth in children with cow's milk allergy fed a thickened extensively hydrolyzed casein-based formula. BMC Pediatr. 2016 Jul 18;16:96. doi:10.1186/s12887-016-0637-3. PubMed PMID: 27430981; PubMed Central PMCID: PMC4950604.

Study objectives

To evaluate the hypoallergenicity and tolerance of a thickened, extensively hydrolyzed casein formula in infants with CMPA.

Methodology

Infants aged between 1 and 12 months with CMPA confirmed either through a double-blind, placebo-controlled food challenge (DBPCFC) within 3 months prior to inclusion, or based on specific suggestive symptoms were eligible for inclusion in this trial. Children were fed an extensively hydrolyzed casein-based formula thickened with a complex containing fibre (including pectin) at 0.5 g/100 ml (Allernova AR®, Novalac, United Pharmaceuticals, France) for 4 months. If CMPA was not confirmed by a DBPCFC prior to inclusion, it had to be confirmed during the study.

Primary outcome

Formula's hypoallergenicity (defined as the absence, in infants with a proven CMPA, of any allergy symptoms that led to study discontinuation) was assessed during the first two weeks of study.

Secondary outcomes

Symptoms of CMPA were assessed at baseline and at each visit, using the cow's milk symptoms score (CoMiSS). Growth (weight and length) parameters were evaluated at baseline and 4-month follow-up. Eczema severity was assessed through the Scoring Atopic Dermatitis index (SCORAD). Stools characteristics were assessed using the Bristol Stool Scale.

Test formula composition

Compound	Dose per 100 g	Dose per 100 mL

Compound	Dose per 100 g	Dose per 100 mL
Energy (kcal)	495.9	74.4
Protein (g)	12.1	1.8
Fat (g)	26.2	3.9
Carbohydrates (g)	52.7	7.9
Fibres (g)	3.6	0.5

Results

Population characteristics

32 infants were included, CMPA was confirmed by a double-blind, placebo-controlled oral food challenge in 30 of them. Children were 4.8 ± 3.0 months at inclusion.

Hypoallergenicity (primary outcome)

None of the 30 infants dropped out of the study over the 4-month period, thus demonstrating hypoallergenicity.

Stools characteristics

90 % of infants had normal stools at 14 days compared to 66.7 % at inclusion ($p = 0.020$).

CMPA symptoms

The mean CoMiSS decreased significantly from 7.4 ± 4.4 at inclusion to 3.2 ± 2.3 at 14 days ($p<0.001$). More specifically, regurgitation and crying score also decreased significantly at follow-up compared to baseline ($p<0.001$).

Atopic dermatitis

In 9 patients presenting eczema, the SCORAD score decreased significantly by 15.5 (±6.7) and 21.1 (±11.2) after 14 and 45 days, respectively ($p < 0.001$).

Growth parameters

While impaired growth was obvious at inclusion, with negative mean (±SD) weight-for-age and length-for-age z-scores (−0.8 ± 0.8 and -0.7 ± 1.0, respectively), all growth indices showed significant improvements within the 4-month study, and were normalized *versus* WHO growth standards.

Drop-out & adverse events

No adverse events were related to the study formula nor led to feeding discontinuation. Two children dropped out: one infant tolerated a cow's milk-based formula introduced by his parents 5 days after study inclusion, excluding the CMPA diagnosis; another infant dropped out of the study before CMAP could be confirmed because of his parents' wish to withdraw.

Conclusion

The tested thickened extensively hydrolyzed formula was tolerated during 4 months by all infants with CMA proven by a double-blind, placebob-controlled, oral food challenge.

1.1.5 "SONAR" study

Study ID

Title: "Efficacy and Tolerance of a New Anti-Regurgitation Formula"

Authors: Dupont C, Vandenplas Y; SONAR Study Group.

Institution: Department of Paediatric Gastroenterology Hepatology and Nutrition, Hôpital Necker Enfants Malades, Paris, France.

Journal: Pediatric Gastroenterology, Hepatology & Nutrition, 19(02): 104-109

Link: here

Full reference: Dupont C, Vandenplas Y; SONAR Study Group. Efficacy and Tolerance of a New Anti-Regurgitation Formula. Pediatr Gastroenterol Hepatol Nutr. 2016 Jun;19(2):104-9. doi: 10.5223/pghn.2016.19.2.104. Epub 2016 Jun 28. PubMed PMID: 27437186; PubMed Central PMCID: PMC4942307.

Study objectives

The study aimed at evaluating the efficacy of a formula containing a new thickening agent on regurgitation and to assess its digestive tolerance.

Methodology

Children less than 5 months old and having at least 5 episodes of regurgitation per day were included in this study. They were fed an anti-regurgitation formula thickened with a complex of fibers (0.5g/100ml) including pectin and locust bean gum for 3 month.

Primary outcome

The daily number of regurgitations and the estimated regurgitated volume were recorded by the parents in a diary during two 3-day periods, after inclusion and just before day 14. Severity of regurgitation was evaluated at 14 days, using the Vandenplas score (Vandenplas 1994).

Secondary outcomes

Number of stools and stools consistency were evaluated by the parents at baseline and 14 days using the Bristol Stool Scale. Growth parameters were monitored at 3 months (weight, length, and head circumference).

Test formula composition

Compound	Dose per 100 g	Dose per 100 mL
Energy (kcal)	493.7	64.2
Protein (g)	1.6	12.1
Fat (g)	25.1	3.3
Carbohydrates (g)	53	6.9
Fibres (g)	3.7	0.5

Results

Population characteristics

100 children were included in the study. 10 dropped out before day 14; consequently, analyses were performed on 90 children aged on average 9.6 ± 5.8 weeks. 53.3% of the infants had been fed a thickened formula before inclusion (pre-thickened or standard formula + thickening agent).

Regurgitations (primary outcome)

At 14 days, the mean number of regurgitation had decreased significantly *versus* baseline (−6.3±3.3, $p < 0.001$).

Stools characteristics

The percentage of formed stools increased after 14 days from 36.7% to 51.1% ($p=0.053$)

Growth parameters

Parameters were within normal range during the study period. A significant increase was seen for weight-for-age and length-for-age z-scores from baseline to 3-month follow-up (−0.5 ± 1.0 to −0.1 ± 0.9 and −0.5 ± 1.2 to −0.0 ± 1.2 respectively, both $p<0.001$). During this period, BMI for age z-score increased non-significantly.

Drop-out & adverse events

The reason for drop-out between inclusion and day 14 were identified as follows; liquid stools (n=4), lost to follow up (n=1), withdrawal by parents for unknown reason (n=4), and one infant that was breastfed (n=1). No severe adverse events have been reported during the study.

Conclusion

In summary, the study above has shown reduced regurgitations severity within 14 days and adequate growth of infants within 3 months in infants fed a thickened formula containing pectin and locust bean gum at a total amount of 0.5 g/100 mL.

1.1.6 Conclusion on clinical trials

As highlighted, the safety of pectins used in Novalac formulas was confirmed by the fact that growth parameters were normal in all infants fed with the formulas, and was even improved in some infants suffering from allergies, as evidenced by a catch-up in growth. The occurrence of adverse events was rare, and mostly unrelated to study formulas, or with no differences between the two groups tested.

Therefore, no safety concerns were raised in these studies with the use of formulas containing a fibre complex (including pectin) at a dose of 0.5 g/100 mL, in a total of 304 infants aged less than 18 months, including 214 children with allergies over administration periods of up to 6 months.

1.2 REGULATORY STATUS WORLDWIDE

1.2.1 Europe

Locust bean gum, pectins, xanthan gum have been added to infant formulas for special medical purposes for decades. These additives comply with the provisions laid down in European Regulation (EC) No 1333/2008 on food additives:

- Xanthan gum as a stabilizer
- Locust bean (carob bean) gum as a thickener
- Pectins as a thickener/gelling agent

Xanthan gum is authorized as a food additive in infant formula for special medical purposes in the European Union in accordance with Regulation (EC) No 1333/2008 on food additives, up to 1.2 g/l in Europe. The European Scientific Committee on Foods (SCF) first considered the use of xanthan gum in foods for special medical purposes for infants and young children as acceptable in 1999 (SCF 1999).

Locust bean gum has a history of safe use in the European Union at a dose of 10 000 mg/kg in infant formula for special medical purposes. Locust bean gum thickened formulae are available in Europe for over 20 years. Its use in infant formula was approved by the European Scientific Committee on Foods (SCF) in 1994 (SCF 1994). A recent review of toxicological database and clinical evidence conclude that the consumption of locust bean gum is safe for use as thickener in infant formulas for treatment of uncomplicated but frequent troublesome regurgitation in infants (Meunier 2014).

Pectin is currently authorized in Europe for use as a food additive in Dietary foods for infants for special medical purposes and special formulae for infants. The maximum dose allowed is 10000 mg/kg. This additive can be used "From birth onwards in products used in case of gastro-intestinal disorders". Authorization of using pectin in dietary foods for special medical purposes for infants has been validated in 2003 by the European Scientific Committee on Foods (SCF 2003).

1.2.2 United States

Pectin has a Generally Recognized As Safe (GRAS) status in United States since 1977 (FDA 2017).

In its assessment, the FDA concluded: "There is no evidence in the available information on pectin and pectinates, including amidated pectins, that demonstrates or suggests reasonable grounds to suspect a hazard to the public when they are used at levels that are now current or that might reasonably be expected in the future." (SCOGS 1977).

Furthermore, the FDA states in part of the code of federal regulation related to pectin, that "The affirmation of these ingredients as generally recognized as safe (GRAS) as direct human food ingredients is based upon the following current good manufacturing practice conditions of use (SCOGS 1977):

(1) The ingredients are used as emulsifiers as defined in §170.3(o)(8) of this chapter and as stabilizers and thickeners as defined in §170.3(o)(28) of this chapter.

(2) The ingredients are used in food at levels not to exceed current good manufacturing practice."

2. CONCLUSIONS

Based on:

- the history of use of pectin, xanthan and locust bean gum in Europe and in the United States, notably in infant formula for special medical purposes,
- the evidence of safe use of pectin in infant formulas provided by the clinical studies conducted even in very sensitive populations,

we believe that pectin, xanthan and locust bean gum authorization in special infant formula with the same conditions as in Europe would not raise concerns in this population.

3. REFERENCES

Dupont C, Kalach N, Soulaines P, Bradatan E, Lachaux A, Payot F, De Blay F, Guénard-Bilbault L, Hatahet R, Mulier S, Kapel N, Waligora-Dupriet AJ, Butel MJ. Safety of a New Amino Acid Formula in Infants Allergic to Cow's Milk and Intolerant to Hydrolysates. J Pediatr Gastroenterol Nutr. 2015 Oct;61(4):456-63. doi: 10.1097/MPG.0000000000000803. PubMed PMID: 25844709.

Dupont C, Bradatan E, Soulaines P, Nocerino R, Berni-Canani R. Tolerance and growth in children with cow's milk allergy fed a thickened extensively hydrolyzed casein-based formula. BMC Pediatr. 2016a Jul 18;16:96. doi: 10.1186/s12887-016-0637-3. PubMed PMID: 27430981; PubMed Central PMCID: PMC4950604.

Dupont C, Vandenplas Y; SONAR Study Group. Efficacy and Tolerance of a New Anti-Regurgitation Formula. Pediatr Gastroenterol Hepatol Nutr. 2016b Jun;19(2):104-9. doi: 10.5223/pghn.2016.19.2.104. Epub 2016b Jun 28. PubMed PMID: 27437186; PubMed Central PMCID: PMC4942307.

Food & Drug Administration (FDA), CFR - Code of Federal Regulations Title 21, Chapter I, Subchapter B, Part 184, Subpart B, Sec. 184.1588 Pectins. Consulted September 2017, available online.

Meunier L, Garthoff JA, Schaafsma A, Krul L, Schrijver J, van Goudoever JB, Speijers G, Vandenplas Y. Locust bean gum safety in neonates and young infants: an integrated review of the toxicological database and clinical evidence. Regul Toxicol Pharmacol. 2014 Oct;70(1):155-69. doi: 10.1016/j.yrtph.2014.06.023. Epub 2014 Jul 2. PubMed PMID: 24997231.

Picone TA, Benson JD, Moro G, Minoli I, Fulconis F, Rassin DK, Raiha NC. Growth, serum biochemistries, and amino acids of term infants fed formulas with amino acid and protein concentrations similar to human milk. J Pediatr Gastroenterol Nutr. 1989 Oct;9(3):351-60. PubMed PMID: 2614623.

Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives (Text with EEA relevance) *Official Journal of the European Union*, L 354, 31.12.2008, available online

SCF (Scientific Committee on Food), 1994. Reports from the Scientific Committee for Food (32nd series). Opinion expressed 1992. Food science and techniques, 1994.

SCF (Scientific Committee for Food), 1999. Reports from the Scientific Committee for Food, Opinion expressed 1997, 43rd series. Food science and techniques, 1999.

SCF (Scientific Committee for Food), 2003. Report of the Scientific Committee on Food on the Revision of Essential Requirements of Infant Formulae and Follow-on Formulae (adopted on 4 April 2003).

Select Committee on GRAS Substances (SCOGS) Opinion: Pectin, amidated; Pectin, high ester; Pectin, low acid; Pectinates; Pectinic acid, SCOGS Report Number: 81, NTIS Accession Number: PB274477*, Year of Report: 1977, 21 CFR Regulation: 184.1588, available online

Vandenplas Y, Hachimi-Idrissi S, Casteels A, Mahler T, Loeb H. A clinical trial with an "anti-regurgitation" formula. Eur J Pediatr. 1994 Jun;153(6):419-23. PubMed PMID: 8088297.

Vandenplas Y, De Greef E, Hauser B; Paradice Study Group. Safety and tolerance of a new extensively hydrolyzed rice protein-based formula in the management of infants with cow's milk protein allergy. Eur J Pediatr. 2014 Sep;173(9):1209-16. doi: 10.1007/s00431-014-2308-4. Epub 2014 Apr 12. PubMed PMID: 24723091; PubMed Central PMCID: PMC4134482.

Vandenplas Y, De Greef E, Xinias I, Vrani O, Mavroudi A, Hammoud M, Al Refai F, Khalife MC, Sayad A, Noun P, Farah A, Makhoul G, Orel R, Sokhn M, L'Homme A, Mohring MP, Merhi BA, Boulos J, El Masri H, Halut C; Allar Study Group. Safety of a thickened extensive casein hydrolysate

formula. Nutrition. 2016 Feb;32(2):206-12. doi: 10.1016/j.nut.2015.08.008. Epub 2015 Sep 2. PubMed PMID: 26704966.

Vandenplas Y, Dupont C, Eigenmann P, Host A, Kuitunen M, Ribes-Koninckx C, Shah N, Shamir R, Staiano A, Szajewska H, Von Berg A. A workshop report on the development of the Cow's Milk-related Symptom Score awareness tool for young children. Acta Paediatr. 2015 Apr;104(4):334-9. doi: 10.1111/apa.12902. Epub 2015 Jan 29. PubMed PMID: 25557474.

Dupont et al. BMC Pediatrics (2016) 16:96
DOI 10.1186/s12887-016-0637-3

BMC Pediatrics

RESEARCH ARTICLE Open Access

Tolerance and growth in children with cow's milk allergy fed a thickened extensively hydrolyzed casein-based formula

Christophe Dupont[1*], Elena Bradatan[2], Pascale Soulaines[1], Rita Nocerino[3] and Roberto Berni-Canani[3]

Abstract

Background: In case of cow's milk allergy (CMA), pediatric guidelines recommend for children the use of extensively hydrolyzed formulas (eHFs) as elimination diet. According to the American Academy of Pediatrics, the hypoallergenicity of each specific eHF should be tested in subjects with CMA.

Methods: A prospective, multicenter trial was performed to assess the tolerance/hypoallergenicity of a thickened casein-based eHF (eHCF, Allernova AR®, United Pharmaceuticals, France) in infants aged <12 months with CMA proven by a double-blind placebo-controlled food challenge. Its efficacy, measured through allergy symptoms monitoring and Cow's Milk-related Symptom Score (CoMiSS) calculation, and safety were evaluated during a 4-month feeding period. Growth z-scores were computed based on WHO anthropometric data.

Results: Thirty infants (mean age: 4.8 ± 3.0 months) with CMA proven by a DBPCFC tolerated the eHCF during the 4-month study. The CoMiSS, crying and regurgitation scores significantly decreased by 4.2 ± 4.0, 0.9 ±1.2 and 0.7 ± 1.1 respectively, after 14 days of feeding ($p < 0.001$). The Scoring Atopic Dermatitis index, of 33.2 ± 14.8 at inclusion in 9 patients, significantly decreased by 15.5 ± 6.7 and 21.1 ± 11.2, after 14 and 45 days of feeding, respectively ($p < 0.001$). The percentage of infants having normal stool consistency (soft or formed stools) significantly improved from 66.7 % (20/30) at inclusion to 90.0 % (27/30) after 14 days of feeding ($p = 0.020$). The growth z-scores, negative at study inclusion, significantly improved over the 4-month study. No adverse event was related to the eHCF.

Conclusion: The thickened eHCF was tolerated by more than 90 % of included allergic infants with 95 % confidence interval and can therefore be considered as hypoallergenic in accordance with current guidelines. The improvement of growth indices and absence of related adverse events confirmed its safety. Results of this trial back the use of the tested thickened eHCF as an efficient and safe alternative in children with CMA.

Trial registration: ClinicalTrials.gov, number NCT02351531, registered on 27 January 2015

Keywords: Cow's milk allergy, Cow's Milk-related Symptom Score (CoMiSS), Hypoallergenic extensively hydrolyzed casein-based formula, Infant growth, Scoring Atopic Dermatitis (SCORAD)

* Correspondence: christophe.dupont@nck.aphp.fr
[1]Pediatric Gastroenterology, Hepatology and Nutrition Department, Necker Children's Hospital, 149, rue de Sèvres, 75015 Paris, France
Full list of author information is available at the end of the article

Background

Cow's milk allergy (CMA) is an immune-mediated reaction which can either be antibody-driven (IgE-mediated) or cell-mediated (non-IgE-mediated) or mixed, and elicits reactions which are reproducible upon re-exposure to cow's milk proteins (CMP) [1]. Estimates of CMA prevalence depend on the diagnosis procedure used; recently, a meta-analysis stated an overall pooled estimate for 0–1 year old infants of point prevalence of CMA reported by parents of 4.2 % (95 % confidence interval (CI): 3.2–5.4), decreasing to 2.0 % (1.5–2.5) when CMA was proven with a double-blind placebo-controlled food challenge (DBPCFC) [2]. CMA manifests through diverse and non-specific symptoms, rendering the CMA diagnosis very difficult [3–5]. CMA symptoms mainly concern the cutaneous area, the respiratory and gastrointestinal tracts but can also be general [3–6]. The DBPCFC is therefore considered as the gold standard for CMA diagnosis [4, 6, 7]. CMA treatment consists in the elimination of any source of non-hydrolyzed CMP from the diet, which is mainly achieved in children by using extensively hydrolyzed formulae (eHFs) based on cow's milk [4–6, 8]. As the molecular weight profile of a given hydrolysate cannot predict potential reaction in a given child [9], the American Academy of Pediatrics recommended that tolerance/hypoallergenicity of any formula intended for allergic children should be clinically tested in that specific population [10]. eHFs should also be tested for their growth adequacy in allergic children [6, 8, 11] as CMA may result in growth retardation [12]. Regurgitations, which are the most typical presentation of infantile gastro-esophageal reflux, are common complaints in infancy [13]. Although they may be a symptom of CMA, they may also occur in allergic infants independently of their allergic disease. To effectively manage both conditions in infants, the new eHF based on casein (eHCF) tested in this trial has been thickened. Therefore, this trial was aimed at evaluating the tolerance/hypoallergenicity of the thickened eHCF in infants with CMA proven through DBPCFC, as well as its efficacy on allergy symptoms and its impact on growth during a 4-month feeding period.

Methods

Study population

Infants aged between 1 and 12 months with CMA, either confirmed through a DBPCFC within 3 months prior to inclusion, or highly suspected based on specific suggestive symptoms, were included in this prospective, multicenter study. The main exclusion criteria were: infants mainly or exclusively breastfed with mother's willingness to continue breastfeeding, infants who would need an amino acid-based formula (AAF) according to pediatric recommendations [3, 4], infants fed an eHF with no improvement of their allergy symptoms, infants who refused to drink an eHF any time prior to inclusion and infants fed the non-thickened version of the tested formula. At study enrolment, if CMA was not already diagnosed by a DBPCFC, such a challenge had to be performed within the 3 months following inclusion. In case of negative challenge, subject's participation in the trial ended and the patient was included in the Safety population (defined in *Study outcomes*) only. The challenge was performed according to guidelines [3]: in short, the child was fed on two different days with volumes being increased every 20 min under medical supervision of either an AAF (Neocate®, Nutricia, Germany) as placebo or a formula which blended two thirds of a standard CMP-based infant formula with one third of Neocate® to ensure double-blinding. The child was observed for 2 additional hours after the last dosage administration to monitor immediate reactions. After completion of both challenge days, in the absence of immediate reaction to CMP, the child had to drink at least 250 ml per day of a standard CMP-based formula for up to one week [3]. At home, parents monitored the appearance of delayed allergy reactions and reported them to the physician. In case of delayed allergy reaction, the exclusive bottle-feeding of the tested formula was immediately reinitiated. If no reaction occurred either during both challenge test days or during one-week feeding with the standard CMP-based formula, cow's milk challenge was considered negative and CMA diagnosis was excluded.

Study formula feeding

Infants were exclusively bottle-fed the tested formula (Allernova AR®, Novalac, United Pharmaceuticals, France) for 4 months. The tested formula contains an extensively casein-based hydrolysate as protein source and is thickened with a patented complex containing fibers (0.5 g/100 ml), mainly composed of pectin, to reduce regurgitation but also to help intestinal transit regulation. Its nutritional composition complied with the applicable European regulation, particularly regarding the amino acid profile.

Study outcomes

The primary outcome was the tolerance/hypoallergenicity of the tested formula, defined as the absence, in infants with a proven CMA, of any allergy symptoms that led to study discontinuation during the first two weeks. It was evaluated on patients in the Tolerance/Hypoallergenicity population, i.e. all patients fed the tested formula at least once and for whom the CMA was proven. Patients fed the tested formula at least once formed the Safety population. The secondary outcomes were the efficacy of the studied formula on allergy symptoms (mainly including the evolution of the Cow's Milk-

related Symptom Score [14] and the main CMA symptom), its impact on growth parameters and on parents and investigators satisfaction. These outcomes were assessed on the Full Analysis Set (FAS) population comprising patients from the Safety population with evaluation of the main efficacy criterion at baseline and at 2 weeks. Adverse events (AEs) were registered in patients in the Safety population.

Study interventions

Visits were planned 14, 45, 90 and 120 days after inclusion. Other CMA diagnosis tests, dosage of serum IgE specific to cow's milk (sIgE), skin prick test (SPT) and atopy patch test (APT), were performed if deemed necessary by the physician according to his usual practice; when carried out before study inclusion, the results of these tests were also collected. From serum, sIgE were analyzed with enzymatic immunoassay (Phadia 100 ThermoFisher Scientific CAP system), the limit of detection being 0.1 kU/l. For SPT, commercial UHT milk, and histamine dihydrochloride (10 mg/ml) and isotonic saline solution (NaCl 0.9 %) as positive and negative control, respectively, were applied to the patients' volar forearm. SPT were performed using a 1-mm single peak lancet (ALK, Copenhagen, Denmark) in Italy and Stallerpoint® (Stallergenes SA, France) in France and Belgium. Reactions were recorded on the basis of the largest diameter (in millimeters) of the wheal and flare at 15 min. The SPT result was considered "positive" if the wheal diameter induced by cow's milk minus that induced by negative control was larger than 3 mm. For APT, 1–2 drops of commercial UHT milk was placed on filter paper and applied with adhesive tape to the unaffected skin of the child's back, using 12-mm aluminum cups (Finn Chambers® on Scan pore). Isotonic saline solution was the negative control. The occlusion time was 48 h and results were read 20 min and 24 h after removal of the cups. The test result was considered positive if at least a significant erythema was present. IgE-mediated was defined as having either positive sIgE or positive SPT to cow's milk.

Parents were instructed to eliminate any milk or dairy products from the diet throughout the entire study and to not introduce hen's egg, soy protein, peanut or any new food in their infant's diet in the first two weeks of the study. Patient selection was performed in hospital outpatient clinics and private practices in France, Belgium and Italy.

Study measurements

During 3 days before each visit, parents were asked to record data on formula intake, number of regurgitations, stool patterns and duration of crying. At inclusion and each follow-up visit, the presence and severity of CMA symptoms were registered by the same investigator, based on clinical examination and parents report. CMA symptoms were itemized for each concerned area: cutaneous (urticaria, angioedema and eczema, the severity of the latter being assessed as mild, moderate or severe, on head, neck and trunk and on arms, hands, legs and feet), respiratory symptoms (such as wheezing, rhinitis, bronchitis, bronchospasm, their severity being assessed as slight, mild or severe), digestive (regurgitations assessed through the regurgitation scale defined by Vandenplas et al. [15], vomiting, bloody stools, stool consistency assessed through the Bristol stool scale [16]), and digestive discomfort as general symptom (mild, moderate or severe intensity and reflected by abdominal pain, gas, bloating and irritability). Daily unexplained crying time was registered through a scale with the following points: less than one hour/day, 1–1.5 h/d, 1.5–2 h/d, 2–3 h/d, 3–4 h/d, 4–5 h/d and more than 5 h/d. During a workshop held in 2014, a Cow's Milk-related Symptom Score (CoMiSS) was defined [14]. It comprises five items (crying, regurgitations, stool consistency, skin and respiratory symptoms), which were all assessed during the study, enabling the calculation of the CoMiSS for each infant at each visit. Eczema severity was assessed using the Scoring Atopic Dermatitis index (SCORAD) [17], as this score is a valid tool, commonly and easily used by hospital physicians. Because of the diversity of CMA symptoms in general [3–5], the pediatrician had to determine the main CMA symptom for each subject at baseline, and assess its evolution at each follow-up visit. At each visit, the pediatrician measured weight, length and head circumference and registered stool frequency, sleep quality (either agitated, i.e. excessive waking with no clear cause, or quiet, i.e. absence of or few awakenings) and adverse events (AEs).

Statistical analysis

In order to be considered hypoallergenic, a formula must demonstrate that with 95 % CI, it does not provoke allergic reactions in 90 % of subjects with confirmed CMA [10]. In case of no reaction, a sample size of 29 participants is sufficient.

For quantitative parameters, intra-group changes were analysed using the Student's test or Wilcoxon's test (non-normal data). For qualitative parameters, changes from baseline within treatment group were analysed by symmetry test, or by McNemar test for binary variables. Statistical analyses were conducted using SAS version 9.2 (SAS Institute Inc., United States). Significance was set at $p < 0.05$. Weight-for-age (WFA), length-for-age (LFA), weight-for-length (WFL), body mass index (BMI)-for-age and head circumference-for-age (HCA) z-scores were computed based on WHO anthropometric data [18]. The CoMiSS was calculated for each patient and at each visit [14].

The study design was approved by independent ethic committees: Ile-de-France III (Paris, France), Medical Ethics Committee of the Regional Hospital of Namur (Belgium) and Ethics Committee of the University of Naples, Federico II. This study was conducted in accordance with ethical standards laid down by the Declaration of Helsinki. Parents, or others legally responsible for the infants, provided written consent regarding their acceptance to participate and the study procedures.

Results

Thirty two infants were included in 3 centers from November 2013 to July 2014. CMA was confirmed in 30 of them through a DBPCFC and therefore constituted the hypoallergenicity population (Fig. 1). One infant tolerated a cow's milk-based formula introduced by his parents 5 days after study inclusion, excluding the CMA diagnosis. Another infant dropped out of the study before CMA could be confirmed because of his parents' wish to withdraw. According to the investigator, it was not due to any medical reason, and the patient could have continued to participate in the study. All those 30 infants completed the 4-month study.

Main baseline characteristics of included subjects are described in Table 1 and Additional file 1 [19, 20]. 70.0 % (21/30) of infants had IgE-mediated CMA. At

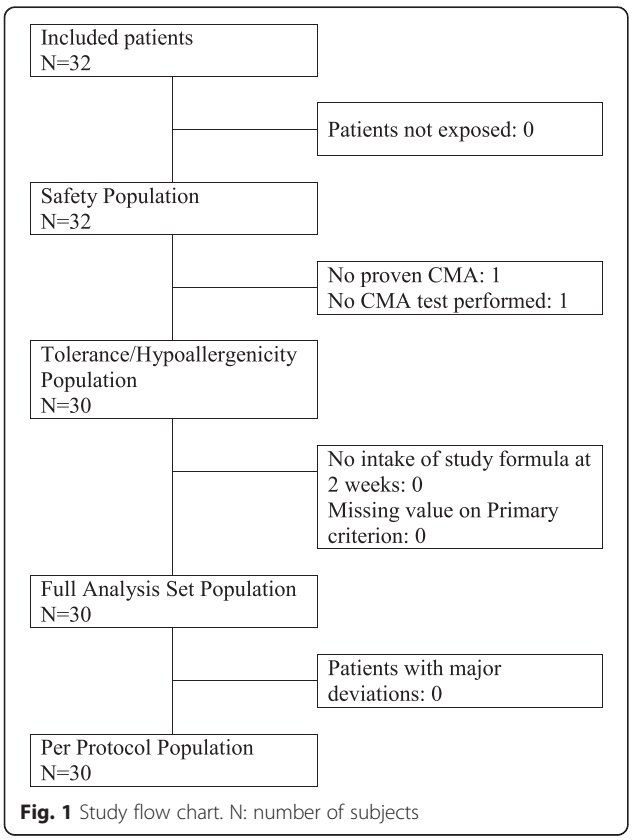

Fig. 1 Study flow chart. N: number of subjects

Table 1 Main demographic and clinical characteristics of FAS population ($N = 30$) at inclusion

Characteristics	
Boys, N (%)	18 (60.0)
Age, mean (± SD), months	4.8 (3.0)
Gestational age, mean (± SD), weeks	38.7 (1.0)
WFA z-score at birth, mean (± SD)	−0.1 (1.1)
LFA z-score at birth, mean (± SD)	0.0 (1.3)
Feeding history	
Ever breastfed, N (%)	25 (83.3)
Duration of exclusive breastfeeding, mean (± SD), weeks	11.5 (7.7)
Duration of partial breastfeeding, mean (± SD), weeks	8.5 (6.1)
Type of feeding at study entry, N (%)	
Exclusively formula-fed	29 (96.7)
Partially breast-fed[a]	1 (3.3)
Type of formula used before study inclusion, N (duration of use, mean ± SD, weeks)	
Non-hydrolyzed CMP-based formula	6 (8.5 ± 5.0)
Extensively hydrolyzed formula based on CMP	13 (6.2 ± 8.8)
Amino acid-based formula	6 (2.7 ± 1.1)
Vegetable-based formula	5 (2.6 ± 2.1)
Anthropometric data	
WFA z-score, mean (± SD)	−0.8 (0.8)
LFA z-score, mean (± SD)	−0.7 (1.0)
WFL z-score, mean (± SD)	−0.4 (1.1)
BMI-for-age z-score, mean (± SD)	−0.6 (1.0)
HCA z-score, mean (± SD)	−0.3 (1.2)
Allergy characteristics	
Family history of allergy‡, N (%)	10 (33.3)
Age at onset of allergy symptoms, mean (± SD), months	2.5 (2.3)
Time since the start of the exclusion diet, median [min – max; IQR], weeks	2.7 [0.0–36.0; 1.1–6.3]
Delay between onset of allergy symptoms and start of exclusion diet, median [min – max; IQR], weeks	1.3 [0.0–34.9; 0.6–4.1]
Types of first allergy symptoms, N (%)	
Exclusively digestive	17 (56.7)
Exclusively cutaneous	10 (33.3)
At least two concerned areas	3 (10.0)
CMA diagnosis tests, number of subjects with positive reactions/number of subjects with test performed (%)	
Atopy patch test to CMP	8/18 (44.4)
Skin prick test to CMP	20/30 (66.7)
Serum IgE specific to CMP	3/6 (50.0)

N number of subjects, *min* minimum, *max* maximum, *IQR* interquartile range
[a]the mother excluded CMP from her regimen; ‡at least one parent or sibling with confirmed allergy

inclusion, 80.0 % of infants (24/30) were on elimination diet. DBPCFC was performed for 16.7 % of patients at a median of 3.9 [range: 0.1-8.7] weeks before study inclusion, and during study course for 83.3 % of infants, at a median of 0.4 [0.1-11.3] weeks after study inclusion. 22 patients had immediate reactions to CMP during the DBPCFC (Table 2).

No infant from the Tolerance/Hypoallergenicity population dropped out of the study and all of them tolerated the tested formula.

The main CMA symptom was digestive for 63.3 % (19/30) of infants, cutaneous for 33.3 % (10/30) of infants and general for one infant. It was resolved or improved as of day 14 for 83.3 % of the patients ($p < 0.001$, proportion test) and for 100 % of patients within 45 days. The mean (± standard deviation (SD)) CoMiSS, regurgitation and crying scores significantly decreased by 4.2 (±4.0), 0.7 (±1.1) and 0.9 (±1.2) respectively after 14 days of feeding (Table 3). At inclusion, 90.0 % (27/30) of infants cried ≥1.5 h per day, significantly decreasing to 66.7 and 46.7 % after 14 and 45 days respectively ($p = 0.020$; $p < 0.001$, McNemar test). At inclusion, 3 patients had angioedema, this symptom disappearing after 14 days. 9 patients had eczema at inclusion with a mean (±SD) SCORAD index of 33.2 (±14.8) which significantly decreased by 15.5 (±6.7) and 21.1 (±11.2) after 14 and 45 days, respectively ($p < 0.001$, Student's test). At inclusion, 22 infants experienced vomiting; at 14 days, this number was significantly reduced by half ($p = 0.002$, McNemar test). 6 patients had bloody stools at inclusion, decreasing to 3 after 14 days, and to none after 45. Normal stool consistency (formed or soft stools), present in 66.7 % (20/30) of infants at inclusion, significantly increased to 90.0 % (27/30) after 14 days ($p = 0.020$, McNemar test).

Digestive discomfort, present in 25 patients at inclusion, of which 12 patients had symptoms of moderate/severe intensity, decreased to 17 patients after 14 days ($p = 0.011$, McNemar test), of which only one patient had symptoms of moderate/severe intensity. Stool frequency did not significantly change after 14 and 45 days. 73.3 % (22/30) of infants had 1–3 stools/day on day 14. Agitated sleep significantly decreased from 83.3 % (25/30) of infants at baseline to 43.3 % (13/30) after 14 days ($p = 0.001$, McNemar test).

Table 2 Characteristics related to DBPCFC of the FAS population ($N = 30$)

Characteristics	
Immediate reactions to CMP, N (%)	22 (73.3)
Types of immediate reactions to CMP, N (%)	
Digestive signs	19 (86.4)
Local cutaneous signs	7 (31.8)
General cutaneous signs	6 (27.3)
Laryngeal edema	2 (9.1)
Bronchospasm	1 (4.5)
Delayed reactions to CMP, N (%)	10 (33.3)
Types of delayed reactions to CMP, N (%)	
Digestive	8 (80.0)
Cutaneous	5 (50.0)
Cumulative dose of non-hydrolyzed CMP-based formula eliciting immediate reactions, median [minimum-maximum], ml	15 [5–95]
Time for eliciting immediate reactions, mean (± SD), minutes	83.8 (16.1)

N number of subjects

Table 3 Change from baseline of CoMiSS and parameters contributing to the CoMiSS at 14 days

	Inclusion ($N = 30$)	D14 ($N = 30$)
CoMiSS, mean (± SD)	7.4 (4.4)	3.2 (2.3)*
Regurgitation score[a], mean (± SD)	1.6 (1.6)	0.9 (1.0)*
Crying score[a], mean (± SD)	1.7 (1.1)	0.8 (0.6)*
Stool consistency, N (%)		
Type I/II (hard)	6 (20.0)	2 (6.7)
Type III/IV (formed)	16 (53.3)	20 (66.7)
Type V (soft)	4 (13.3)	7 (23.3)
Type VI (mushy)	3 (10.0)	1 (3.3)
Type VII (watery)	1 (3.3)	0 (0.0)
Urticaria, N (%)		
Presence	7 (23.3)	0 (0.0)
Absence	23 (76.7)	30 (100.0)
Eczema, N (%)		
Head, neck, trunk		
Absence	21 (70.0)	24 (80.0)
Mild	3 (10.0)	4 (13.3)
Moderate	5 (16.7)	2 (6.7)
Severe	1 (3.3)	0 (0.0)
Arms, hands, legs, feet		
Absence	23 (76.7)	24 (80.0)
Mild	3 (10.0)	3 (10.0)
Moderate	3 (10.0)	3 (10.0)
Severe	1 (3.3)	0 (0.0)
Respiratory symptoms, N (%)		
Absence	25 (83.3)	28 (93.3)
Mild	4 (13.3)	1 (3.3)
Moderate	1 (3.3)	1 (3.3)

D day, *N* number of subjects
**P*-values vs. inclusion < 0.001 (Wilcoxon's test)
[a]Sub-scores included in the calculation of the CoMiSS

The mean (±SD) feeding duration was 113.6 (±27.8) days and the mean daily intake of study formula was higher than 600 ml/day during the entire study course. 33 AEs were reported in 24 patients: 48.5 % (16/33) were respiratory infections and one third gastroenteritis. None were related to the tested formula nor led to feeding discontinuation of the tested formula. No serious AEs were reported. Between birth and inclusion, the mean (±SD) WFA and LFA z-scores had significantly decreased by 0.7 (±1.0) and 0.6 (±1.1), respectively ($p < 0.001$; $p = 0.003$, Student's test). All growth indices, negative at study inclusion, showed significant improvements within the 4-month study (Table 4). As of 14 days of feeding, 73.3 % (22/30) of the investigators and 71.4 % (20/28) of the parents were globally satisfied with the formula, 75.8 % (22/29) of parents being satisfied or very satisfied in particular with their child's acceptance of the formula's taste.

Discussion

This study demonstrates the hypoallergenicity, efficacy and positive effect on growth catch-up of the studied eHCF in infants with CMA. As all infants with CMA, confirmed by a DBPCFC, tolerated the tested formula, this formula meets the hypoallergenicity criteria of the American Academy of Pediatrics [10].

In this study, CMA was proven in all subjects by a DBPCFC, the gold standard for CMA diagnosis [3, 4, 7]. In addition, in the absence of a reference group, which allows controlling for the natural evolution of the disease, the symptom evolution was first evaluated 2 weeks after study enrollment, which is close enough to the time of diagnosis to exclude the possibility of a natural evolution of symptoms [3, 4].

The efficacy of the studied eHCF was thoroughly documented in this trial, by assessing all parameters contributing to an existing Symptoms-Based Score (SBS) [21–23]. A working group recently considered the SBS as a valuable tool for evaluating and quantifying the evolution of CMA symptoms during therapeutic interventions and renamed it Cow's Milk-related Symptom Score (CoMiSS) [14]. Here, this score was significantly reduced as early as 14 days after eHCF feeding initiation. A similar evolution was reported in previous studies following young infants with proven CMA and under elimination diet by using this score. In 37 and 34 infants fed respectively an eHF based on rice proteins and an eHCF, the mean SBS (±SD) significantly decreased after one month-feeding from 13.0 (±5.2) to 3.5 (±2.3) and from 14.3 (±3.3) to 5.7 (±3.7) [22, 23]. In another study, 59 infants fed an eHCF or an eHF based on whey proteins (eHWF) showed a mean SBS of 13.6 (±1.7) at inclusion that decreased to 5.1 (±3.4) after one month-feeding [21]. Compared with these previous results, the mean CoMiSS value at inclusion reported here was relatively small and lower than the value (≥12) which could have an 80 % positive predictive value for CMA diagnosis at the start of an elimination diet followed by a decrease to ≤6 under an elimination diet with eHF. This can be

Table 4 Growth indices at inclusion and follow-up visits (D45, D90 and D120)

	Inclusion	D45	D90	D120
Age, mean (± SD), months	4.8 (3.0)	6.3 (3.1)	7.8 (3.0)	8.7 (3.0)
Weight-for-age z-score, mean (± SD)	−0.8 (0.8)	−0.2 (0.7)	0.1 (0.7)	0.4 (0.8)
N		29	29	29
P-values vs. baseline		<0.001[a]	<0.001[a]	<0.001[a]
Length-for-age z-score, mean (± SD)	−0.7 (1.0)	−0.3 (1.2)	0.0 (1.2)	0.4 (1.1)
N		29	29	28
P-values vs. baseline		0.008[a]	<0.001[a]	<0.001[a]
Weight-for-length z-score, mean (± SD)	−0.4 (1.1)	0.0 (0.8)	0.2 (0.6)	0.3 (0.7)
N		29	29	28
P-values vs. baseline		0.002[a]	<0.001[a]	<0.001[a]
Body mass index-for-age z-score, mean (± SD)	−0.6 (1.0)	−0.1 (0.8)	0.2 (0.7)	0.3 (0.8)
N		29	29	28
P-values vs. baseline		0.001[a]	<0.001[a]	<0.001[a]
Head circumference-for-age z-score, mean (± SD)	−0.3 (1.2)	0.2 (1.0)	0.7 (0.8)	1.1 (0.9)
N		27	29	29
P-values vs. baseline		<0.001[b]	<0.001[b]	<0.001[b]

D day, *N* number of subjects
[a]Student's test
[b]Wilcoxon's test

explained by the fact that 80.0 % of enrolled infants were on an elimination diet, more than half with eHF based on CMP (54.2 %), one quarter with AAF and 20.8 % with vegetable-based formulas.

In the absence of a validated CMA severity score [14], previous similar studies frequently focused on the SCORAD index evolution, a validated tool for assessment of eczema severity [17], especially since some eHFs, but not all [24], based on casein [25, 26] or whey proteins [25, 27] efficiently induced a decrease in this score in CMA patients. In this present study, less than one third of patients had eczema at inclusion, and their SCORAD index significantly decreased 14 and 45 days after eHCF feeding initiation.

CMA treatment relies on dietary elimination of intact CMP [3, 4, 8] which may induce nutritional deficiencies in children in case of an inadequate elimination diet. As shown by negative growth indices in children with CMA at study enrollment [26], CMA is frequently associated with a growth deficit [28, 29]. The mechanisms for impaired growth are not entirely clear but may rise from a sustained inflammation and subsequent reduced bioavailability or loss of nutrients in the gastrointestinal tract, while metabolic requirements may be increased by skin inflammation and disrupted sleep [12]. A delayed diagnosis and thus a delay in initiation of an appropriate dietary management is a risk factor for impaired growth in children with a food allergy [30]. Here, CMA symptoms appeared during the first months of life, as previously reported [5, 6], and the median [range] delay between their appearance and implementation of an elimination diet was 1.3 [0.0–34.9] weeks. As shown before [26, 31, 32], WFA and LFA z-scores significantly decreased between birth and study inclusion. Feeding with the study eHCF enabled growth normalization in line with WHO standards, as already observed for eHCF feeding [23, 26, 32].

In this study, whatever their CMA type, IgE-mediated or not, all infants tolerated the eHCF during 4 months, and notably with consumptions of high volumes. Parents sometimes ask for an eHF feeding change for various reasons, for example because of a poor taste acceptability—eHFs are known for their bitterness [3, 4, 9, 33, 34]—or for poor digestive comfort including regurgitations [35]. All infants who were already on an elimination diet for various time periods and with different types of formulas devoid of non-hydrolyzed CMP tolerated the studied eHCF.

Conclusions

The tested thickened eHCF was tolerated during 4 months by all infants with CMA proven by a DBPCFC, either IgE or non-IgE mediated and whether already fed or not an elimination diet. The formula feeding efficiently reduced the SCORAD index in patients with eczema and the CoMiSS, a recently developed tool to follow allergy symptoms, in all subjects. This study was adequately powered to demonstrate the hypoallergenicity of the studied formula, but the results observed on allergy symptoms and growth indices deserve confirmation in a larger sample.

The CONSORT guidelines [36], when applicable, were followed for reporting data of this study.

Additional file

Additional file 1: Table S1. Supplementary baseline characteristics of FAS population ($N = 30$) at inclusion. (DOCX 16 kb)

Abbreviations

AAF, amino acid-based formula; AEs, adverse events; APT, atopy patch test; BMI, body mass index; CI, confidence interval; CMA, cow's milk allergy; CMP, cow's milk proteins; CoMiSS, Cow's Milk-related Symptom Score; DBPCFC, double-blind placebo-controlled food challenge; eHCF, extensively hydrolyzed casein-based formula; eHFs, extensively hydrolyzed formulae; eHWF, extensively hydrolyzed whey-based formula; FAS, full analysis set; HCA, head circumference-for-age; LFA, length-for-age; SBS, Symptoms-Based Score; SCORAD, scoring atopic dermatitis; SD, standard deviation; SPT, skin prick test; WFA, weight-for-age; WFL, weight-for-length

Funding

The authors (or their institutions) received honoraria from United Pharmaceuticals, Paris, France for their work in this study. This study was funded by United Pharmaceuticals, Paris, France.

Availability of data and materials

The data will not be made available in order to protect the participants' identity.

Authors' contributions

CD and RBC conceptualized and designed the study. CD, EB, PS, RN and RBC participated in the recruitment and follow-up of patients and were involved in the data collection. All authors critically reviewed and approved the final manuscript.

Competing interests

C. Dupont received honoraria (personal and institutional) for this trial as well as conferences fees from United Pharmaceuticals (Paris, France), honoraria for scientific board membership from Nestlé, Nutricia, and Sodilac, and conference fees from Wyeth Nutrition. The other authors report no other conflict of interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

The study design was approved by independent ethic committees: Ile-de-France III (Paris, France), Medical Ethics Committee of the Regional Hospital of Namur (Belgium) and Ethics Committee of the University of Naples, Federico II. This study was conducted in accordance with ethical standards laid down by the Declaration of Helsinki. Parents, or others legally responsible for the infants, provided written consent regarding their acceptance to participate and the study procedures.

Author details

[1]Pediatric Gastroenterology, Hepatology and Nutrition Department, Necker Children's Hospital, 149, rue de Sèvres, 75015 Paris, France. [2]Department of Pediatrics, Regional Hospital, Namur, Belgium. [3]Department of Translational Medical Science and European Laboratory for the Investigation of Food Induced Diseases and CEINGE Advanced Biotechnologies, University of Naples "Federico II", Naples, Italy.

Received: 16 September 2015 Accepted: 12 July 2016
Published online: 18 July 2016

References

1. Johansson Bieber T, Dahl R, Friedmann PS, Lanier BQ, Lockey RF, et al. Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. J Allergy Clin Immunol. 2004;113:832–6.
2. Nwaru BI, Hickstein L, Panesar SS, Roberts G, Muraro A, Sheikh A, et al. Prevalence of common food allergies in Europe: a systematic review and meta-analysis. Allergy. 2014;69:992–1007.
3. Vandenplas Y, Koletzko S, Isolauri E, Hill D, Oranje AP, Brueton M, et al. Guidelines for the diagnosis and management of cow's milk protein allergy in infants. Arch Dis Child. 2007;92:902–8.
4. Koletzko S, Niggemann B, Arato A, Dias JA, Heuschkel R, Husby S, et al. European Society of Pediatric Gastroenterology, Hepatology, and Nutrition. 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.
5. Luyt D, Ball H, Makwana N, Green MR, Bravin K, Nasser SM, et al. BSACI guideline for the diagnosis and management of cow's milk allergy. Clin Exp Allergy. 2014;44:642–72.
6. Fiocchi A, Brozek J, Schünemann H, Bahna SL, von Berg A, Beyer K, et al. World Allergy Organization (WAO) Diagnosis and Rationale for Action against Cow's Milk Allergy (DRACMA) Guidelines. Pediatr Allergy Immunol. 2010;21:1–125.
7. Dupont C. Diagnosis of cow's milk allergy in children: determining the gold standard? Expert Rev Clin Immunol. 2014;10:257–67.
8. Dupont C, Chouraqui JP, de Boissieu D, Bocquet A, Bresson JL, Briend A, et al. Dietary treatment of cows' milk protein allergy in childhood: a commentary by the Committee on Nutrition of the French Society of Paediatrics. Br J Nutr. 2012;107:325–38.
9. Høst A, Koletzko B, Dreborg S, Muraro A, Wahn U, Aggett P, et al. Dietary products used in infants for treatment and prevention of food allergy. Joint Statement of the European Society for Paediatric Allergology and Clinical Immunology (ESPACI) Committee on Hypoallergenic Formulas and the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Committee on Nutrition. Arch Dis Child. 1999;81:80–4.
10. American Academy of Pediatrics. Committee on Nutrition. Hypoallergenic infant formulas. Pediatrics. 2000;106:346–9.
11. Giovannini M, D'Auria E, Caffarelli C, Verduci E, Barberi S, Indinnimeo L, et al. Nutritional management and follow up of infants and children with food allergy: Italian Society of Pediatric Nutrition/Italian Society of Pediatric Allergy and Immunology Task Force Position Statement. Ital J Pediatr. 2014;40:1.
12. Meyer R, Venter C, Fox AT, Shah N. Practical dietary management of protein energy malnutrition in young children with cow's milk protein allergy. Pediatr Allergy Immunol. 2012;23:307–14.
13. Nelson SP, Chen EH, Syniar GM, Christoffel KK. Prevalence of symptoms of gastroesophageal reflux during infancy. A pediatric practice-based survey. Pediatric Practice Research Group. Arch Pediatr Adolesc Med. 1997;151(6):569–72.
14. Vandenplas Y, Dupont C, Eigenmann P, Host A, Kuitunen M, Ribes-Koninckx C. A workshop report on the development of the Cow's Milk-related Symptom Score awareness tool for young children. Acta Paediatr. 2015;104:334–9.
15. Vandenplas Y, Hachimi-Idrissi S, Casteels A, Mahler T, Loeb H. A clinical trial with an "anti-regurgitation" formula. Eur J Pediatr. 1994;153:419–23.
16. Lewis SJ, Heaton KW. Stool form scale as a useful guide to intestinal transit time. Scand J Gastroenterol. 1997;32:920–4.
17. Consensus Report of the European Task Force on Atopic Dermatitis. Severity scoring of atopic dermatitis: the SCORAD index. Dermatology. 1993;186:23–31.
18. WHO Multicentre Growth Reference Study Group. WHO Child Growth Standards: Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: Methods and development. Geneva: World Health Organization; 2006.
19. Aggett P, Agostoni C, Axelsson I, Goulet O, Hernell O, Koletzko B, et al. Core data for nutrition trials in infants: a discussion document–a commentary by the ESPGHAN Committee on Nutrition. J Pediatr Gastroenterol Nutr. 2003;36:338–42.
20. Koletzko B, Fewtrell M, Gibson R, van Goudoever JB, Hernell O, Shamir R, et al. Core data necessary for reporting clinical trials on nutrition in infancy. Ann Nutr Metab. 2015;66:31–5.
21. Vandenplas Y, Althera Study Group, Steenhout P, Grathwohl D. A pilot study on the application of a symptom-based score for the diagnosis of cow's milk protein allergy. SAGE Open Medicine. 2014;2:2050312114523423.
22. Vandenplas Y, De Greef E, Hauser B, Paradice Study Group. An extensively hydrolysed rice protein-based formula in the management of infants with cow's milk protein allergy: preliminary results after 1 month. Arch Dis Child. 2014;99:933–6.
23. Vandenplas Y, De Greef E, ALLAR study group. Extensive protein hydrolysate formula effectively reduces regurgitation in infants with positive and negative challenge tests for cow's milk allergy. Acta Paediatr. 2014;103:e243–50.
24. 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.
25. Niggemann B, von Berg A, Bollrath C, Berdel D, Schauer U, Rieger C, et al. Safety and efficacy of a new extensively hydrolyzed formula for infants with cow's milk protein allergy. Pediatr Allergy Immunol. 2008;19:348–54.
26. Dupont C, Hol J, Nieuwenhuis EE. Cow's Milk Allergy Modified by Elimination and Lactobacilli study group. An extensively hydrolysed casein-based formula for infants with cows' milk protein allergy: tolerance/hypo-allergenicity and growth catch-up. Br J Nutr. 2015;113:1102–12.
27. 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.
28. Isolauri E, 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;132:1004–9.
29. Agostoni C, Grandi F, Scaglioni S, Giannì ML, Torcoletti M, Radaelli G, et al. Growth pattern of breastfed and nonbreastfed infants with atopic dermatitis in the first year of life. Pediatrics. 2000;106:E73.
30. Venter C, Laitinen K, Vlieg-Boerstra B. Nutritional aspects in diagnosis and management of food hypersensitivity-the dietitians role. J Allergy (Cairo). 2012;2012:269376.
31. Savino F, Castagno E, Monti G, Serraino P, Peltran A, Oggero R, et al. Z-score of weight for age of infants with atopic dermatitis and cow's milk allergy fed with a rice-hydrolysate formula during the first two years of life. Acta Paediatr Suppl. 2005;94:115–9.
32. Agostoni C, Fiocchi A, Riva E, Terracciano L, Sarratud T, Martelli A, et al. Growth of infants with IgE-mediated cow's milk allergy fed different formulas in the complementary feeding period. Pediatr Allergy Immunol. 2007;18:599–606.
33. de Jong NW, Sprikkelman AB, Oude Elberink HN, Arends NJ, Vlieg-Boerstra BJ. Blinded sensory evaluation of extensively hydrolyzed formulas and amino acid formulas. Ann Allergy Asthma Immunol. 2014;113:235–6.
34. Miraglia Del Giudice M, D'Auria E, Peroni D, Palazzo S, Radaelli G, Comberiati P, et al. Flavor, relative palatability and components of cow's milk hydrolysed formulas and amino acid-based formula. Ital J Pediatr. 2015;41:42.
35. Medjad-Guillou N, Henocq A, Arnaud-Battandier F. [Does the hydrolysis of proteins change the acceptability and the digestive tolerance of milk for infants? The results of a comparative and randomized prospective study]. Ann Pediatr (Paris). 1992;39:202–6.
36. Schulz KF, Altman DG, Moher D, CONSORT Group. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. BMC Med. 2010;8:18.

Nutrition 32 (2016) 206–212

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Applied nutritional investigation

Safety of a thickened extensive casein hydrolysate formula

Yvan Vandenplas M.D., Ph.D. [a,*], Elisabeth De Greef M.D. [a], I. Xinias M.S. [b], O. Vrani M.D. [b], A. Mavroudi M.D. [b], M. Hammoud M.D. [c], F. Al Refai M.D. [c], M.C. Khalife M.D. [d], A. Sayad M.D. [d], P. Noun M.D. [d], A. Farah M.D. [d], G. Makhoul M.D. [e], R. Orel M.D., Ph.D. [f], M. Sokhn M.D. [g], A. L'Homme M.D. [h], M.P. Mohring M.D. [h], B. Abou Merhi M.D. [i], J. Boulos M.D. [j], H. El Masri M.D. [k], C. Halut M.D. [l], the Allar Study Group

[a] UZ Brussel, Department of Pediatrics, Vrije Universiteit Brussel, Brussels, Belgium
[b] Department of Pediatrics, Hippocration Hospital, Thessaloniki, Greece
[c] Faculty of Medicine, Department of Pediatrics, Kuwait University, Kuwait City, Kuwait
[d] Holy Spirit University of Kaslik, Kaslikand UH-NDS, Beyruth, Lebanon
[e] Tripoli, Lebanon
[f] University Children's Hospital, Ljubljana, Slovenia
[g] University of Balamand, Beirut, Lebanon
[h] Department of Pediatrics, La Citadelle Regional Hospital, Liège, Belgium
[i] Beirut, Lebanon
[j] Mount Lebanon Hospital, Beyruth, Lebanon
[k] Bahman Hospital, Medical Director Dar Hawraa Center, Beyruth, Lebanon
[l] Department of Pediatrics, Regional Hospital, Namur, Belgium

ARTICLE INFO

Article history:
Received 6 April 2015
Accepted 8 August 2015

Keywords:
Antiregurgitation formula
Cow's milk allergy
(Extensive) hydrolysate
Infant nutrition
Thickened formula

ABSTRACT

Objectives: Cow's milk allergy (CMA) is treated in formula-fed infants with an extensive protein hydrolysate. This study aimed to evaluate the nutritional safety of a non-thickened and thickened extensively casein hydrolyzed protein formula (NT- and T-eCHF) in infants with CMA.
Methods: Infants younger than 6 mo old with a positive cow milk challenge test, positive IgE, or skin prick test for cow milk were selected. Weight and length were followed during the 6 mo intervention with the NT-eCHF and T-eCHF.
Results: A challenge was performed in 50/71 infants with suspected CMA and was positive in 34/50. All children with confirmed CMA tolerated the eCHF. The T-eCHF leads to a significant improvement of the stool consistency in the whole population and in the subpopulation of infants with proven CMA. Height and weight evolution was satisfactory throughout the 6 mo study.
Conclusions: The eCHF fulfills the criteria of a hypoallergenic formula and the NT- and T-eCHF reduced CMA symptoms. Growth was within normal range.

Introduction

Cow's milk protein is a major food allergen in infants [1–4]. A food allergy is defined as an adverse health effect arising from a specific immune response that occurs after exposure to the responsible food allergen [5]. This immune reaction may be IgE or non-IgE mediated. Symptoms of cow's milk allergy (CMA) are not specific and most frequently involve the skin (e.g. atopic dermatitis), the gastrointestinal (GI) tract (regurgitation, vomiting, diarrhea, and constipation), the respiratory tract (wheezing or sneezing) or are more general (colic or anaphylaxis) [1]. To date, the diagnosis of CMA requires an elimination diet followed by a food challenge, which sometimes causes concern to (and is often refused) by the parents [6].

Correct diagnosis enables appropriate feeding of affected infants to sustain normal growth and development. Guidelines define a therapeutic hypoallergenic formula as one tolerated by

Y. Vandenplas is a consultant for United Pharmaceuticals and Biocodex. United Pharmaceuticals provided the tested formulas and an unrestricted grant to support the research.

* Corresponding author. Tel.: +32 2477 5780; fax: +32 2477 5784.
E-mail address: yvan.vandenplas@uzbrussel.be (Y. Vandenplas).

http://dx.doi.org/10.1016/j.nut.2015.08.008
0899-9007/

Table 1
Formula composition (/100 g of powder)

For 100 g of powder	Unit	T-eCHF	NT-eCHF
Protein (casein) (n x 6.25)	g	12.1	12.0
Lipid	g	26.2	27.1
Carbohydrates	g	52.7	55.0
Starch	g	1.0	–
Fibres	g	3.6	–
Energy	kcal	510	512

NT-eCHF, non-thickened extensive casein hydrolysate formula; T-eCHF, thickened extensive casein hydrolysate formula; n, number of subjects

at least 90% of CMA infants with a 95% confidence interval [1,2,7]. These criteria are met by several extensively hydrolyzed protein formulas, based on whey or casein. The hypoallergenicity of this extensively hydrolyzed casein formula (eCHF) was published before [8]. This paper reports the anthropometric evolution over 6 mo feeding with the test formulas.

Materials and methods

Formula-fed infants were eligible for inclusion in this prospective, randomized, double-blind trial if they were less than 6 mo old with symptoms suggesting CMA, including frequent, troublesome regurgitation and/or vomiting at a frequency of more than 5 episodes a day [8]. Two formulas were compared: a non-thickened and a thickened casein extensive hydorlysate formula (NT- and a T-eCHF); the composition of the tested formulas is listed in Table 1. Infants already fed with an extensively hydrolyzed protein formula, or having experienced previous anaphylactic reactions, were not eligible for inclusion [8]. The trial was registered at ClinicalTrials.gov under Identifier NCT01985607, and the 1 mo results in 72 infants were published prior [8]. Criteria used to suspect CMA, inclusion, and exclusion criteria can be found in the first report (Supplement 1) [8].

The primary goal of this paper is present anthropometric data over a period of 6 mo in infants fed both versions of the eCHF. Anthropometric data (weight, length, and head-circumference), were collected at 1, 3, and 6 mo and the corresponding z-score were calculated according to the World Health Organization Child Growth Standards [9].

Secondary aims were to confirm the hypoallergenicity and the efficacy of two NT- and T-eCHF. The cow milk symptom score (CoMiSS) was used to assess the efficacy of each formula at the end of the 1 mo feeding period with the formula [10].

Before any statistical analyses, the normality of the quantitative variables were tested using the Shapiro-Wilk's test. In case of normality ($P > 0.05$) or number of patients >30 per group, continuous variables were tested using a Student *t* test. In case of non-normality and number of patients ≤30 per group, the non-parametric Mann-Whitney-Wilcoxon test were used instead. The categorical variables were tested using Chi^2 test (expected frequency >5), otherwise using Fisher exact test.

The main criterion (changes in score of regurgitation between D30 and D0) was compared between groups using an analysis of covariance (ANCOVA), including the baseline value as covariate if the conditions of normality were respected, otherwise using a Wilcoxon test or an ANCOVA based on ranks. This criterion was also analyzed within each group with a paired *t* test if the conditions of normality were respected, otherwise using a Wilcoxon matched-pairs signed ranks test. The secondary criteria were analyzed in the same way. Results are presented as mean +/− standard deviation and/or median (quartile 1–quartile 3).

Full–analysis set (FAS) population was defined as all infants from the safety population having an evaluation of the main criteria.

Moreover, "CMA+" population was defined as all infants from the FAS having a CMA confirmed by either a positive food challenge or positive skin prick test (i.e., a papula to cow's milk at least 3 mm bigger than the negative control) or positive specific IgE (i.e., >0.35 kU/l). Infants with a negative food challenge and infants who did not undergo the food challenge constituted the "CMA?" group.

The study was approved by the Ethical Committee of the UZ Brussels as the primary center and by each participating hospital. Physicians from nine centers in five different countries were selected because of their qualifications and interest in participating in this trial. Informed consent was obtained from parents before randomization.

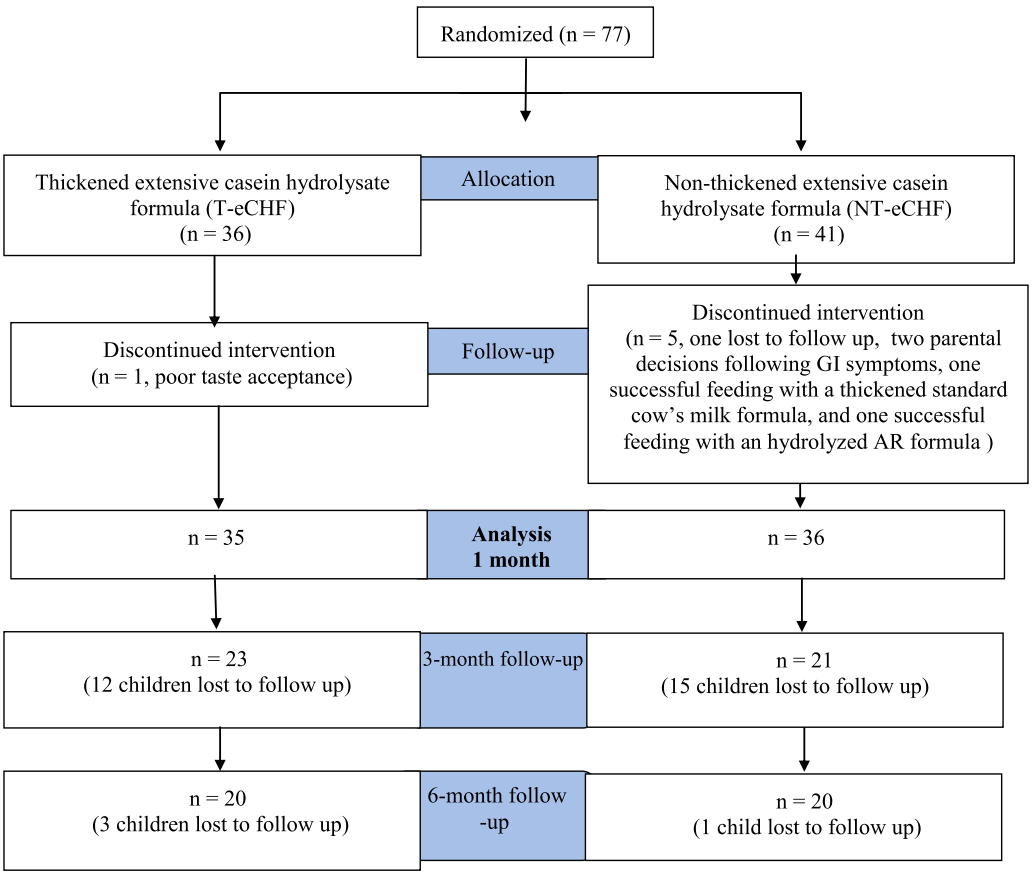

Fig. 1. Flow diagram. n, number of subjects; PPR, per protocol data set for regurgitations; PPA, per protocol data set for allergy; GI, gastrointestinal; AR, antiregurgitation.

Table 2
Patient's characteristics

Patient characteristics	Total	T-eCHF	NT-eCHF	*P*-values	CMA+
n	71	35	36		37
Male/Female	34/37	13/22	21/15	0.074*	17/20
Birth weight-for-age z-score (mean ± SD)	−0.31 ± 1.05	−0.40 ± 1.2	−0.22 ± 0.89		−0.38 ± 0.95
Birth Length-for-age Z score (mean ± SD)	1.1 ± 1.28	−0.25 ± 1.41	0.26 ± 1.11		−0.04 ± 1.17
GA (weeks), mean ± SD	38.38 ± 1.59	38.32 ± 1.89	38.43 ± 1.27	0.786†	38.41 ± 1.62
Fam hist +, mean ± SD	1.80 ± 2.0	2.09 ± 2.28	1.53 ± 1.66	0.242†	1.73 ± 2.22
At inclusion					
Age (days), mean ± SD	90.51 ± 49.02	80.77 ± 43.17	99.97 ± 43.17	0.038†	90.49 ± 43.78
Weight –for –age Z score at inclusion (mean ± SD)	−0.64 ± 1.18	−0.67 ± 1.11	−0.61 ± 1.27	0.835†	−0.68 ± 1.37

BW, birth weight; BL, birth length; CMA+, cow's milk allergy positive; Fam hist +, positive family history for atopy (this score was calculated as follows: a score of 1 was attributed to each member of the family [mother, father, or sibling] having a suspected allergic disease; this score was 2 for each member having a medically diagnosed allergic disease, the family score was the sum of each member score); GA, gestational age; n, number of subjects; NT-eCHF, non-thickened extensive casein hydrolysate formula; SD, standard deviation; T-eCHF, thickened extensive casein hydrolysate formula

* Chi-2.

† Student's *t* test.

Results

Eighteen pediatricians included 77 infants with clinical symptoms suggesting CMA. Six children dropped out before the end of the 1 mo period. One was in the T-eCH group and was unable to accept the taste of the formula. The other five were in the NT-eCH group. One of these was lost to follow up, two families decided to stop because of vomiting/liquid stools (one of those has been later fed Neocate with no improvement), one infant was switched and successfully fed with a non-hydrolyzed protein antiregurgitation formula, and parents of the last one successfully switched to a commercialized extensively hydrolyzed antiregurgitation formula (Allernova AR) (Fig. 1). The CMA diagnosis was not confirmed in any of these six cases. None of the patients with proven CMA dropped out during the 1 mo intervention period.

The patients' characteristics of the full analysis set are listed in Table 2. There were no significant differences between both groups for weight-for-age z-scores at inclusion, gestational age, and family score for atopy. Considering the FAS population, a milk challenge was performed in 50/71 (70.4%) infants. Indeed, despite initial agreement to perform a challenge at recruitment (as part of the informed consent), parents of 21 (29.6%) infants changed their minds and refused the challenge procedure (Table 3). The challenge was positive in 15/36 (41.6%) and in 19/35 (54.3%) children in the NT-eCHF and T-eCHF group, respectively (NS). Additionally, in the population which did not undergo the oral food challenge, one had a positive SPT, one had positive specific IgE, and one had both specific IgE and SPT. Therefore the CMA+ population was made of 37 children, among whom 34 (91.8%) had a positive food challenge.

There was no difference in CoMiSS at inclusion, neither between the groups receiving the NT-eCHF and the T-eCHF, nor between the groups in which CMA was later confirmed or not (Table 4). The CoMiSS decreased significantly after the first month of dietary intervention by -7.5 (±5.2; $P < 0.001$) in the entire group, by −8.4 (±5.2; $P < 0.001$) in the group in which CMA was confirmed and by −6.5+/−4.5 in the group "CMA?", the score remaining above 6 (7.3+/−4) after 1 mo in this group.

The CoMiSS decrease did not differ between both versions of the eCHF (−7.6 ± 5.2 versus −7.4 ± 5.3 in the T and NT group respectively) regardless of the result of the challenge test.

Crying time was significantly reduced in the study population. 42.3% of all infants were crying more than 3 h/d at inclusion, but only 9.9% of them still cried more than 3 h/d at the end of the first month observation period ($P < 0.0001$), without a significant difference between groups (Table 5).

A significant reduction in the number of regurgitation was observed after 1 mo for both versions of the eCHF (−5 [−6; −3]; median [Q1; Q3]; $P < 0.001$ for the T-eCHF and −2 [−5; 0]; $P < 0.001$ for the NT-eCHF), this decrease being significantly more important with the T eCHF ($P = 0.025$) (Table 6). When the CMA was not confirmed ("CMA?" population), the T-eCHF seems to reduce regurgitations more than the NT-eCHF (-5 [−6; −3] versus −3 [−5; 0]; NS) (Table 6). After 3 mo, the number of regurgitations was even more reduced in both groups (data not shown). There was also a significant improvement of the "Vandenplas regurgitation score" for all infants and for all populations (Table 5).

In the total study population, a 1 mo dietary intervention led to a normalization of the stool consistency (12.7% of normal/soft stools at inclusion versus 31% after 1 mo, $P = 0.009$). This normalization was significant in infants fed the T-eCHF in the total population (8.6% to 34.3%, $P = 0.013$) and in the subpopulation of infants with proven CMA (T-eCHF 9.5% to 42.5%, $P = 0.020$) but was not significant with the NT-eCHF formula (total population: 16.7% to 27.8%; CMA+ population: 12.5% to 37.5%) (Table 5).

Cutaneous symptoms' score significantly decreased in the whole population after 1 mo (-1.3 ± 1.6, $P < 0.001$). Similarly, the respiratory symptoms score decreased significantly in the total population (-0.48 ± 0.69, $P < 0.001$) with no difference between both formulas.

In the whole study population, the weight-for-age and BMI-for-age z-scores increased significantly from the first month and during the total intervention period. At inclusion, weight-for-age, weight-for-length, and BMI-for-age z-scores were negative (around −0.5) with no differences between the groups nor according to the diagnosis, indicating a slight growth faltering (Table 7). Weight and length-for-age z-scores increased significantly during the 6 mo study, with no difference between

Table 3
Challenge test results on the FAS population

Patient characteristics	Formulas		
	T eCHF	NT eCHF	Total
	n = 35	n = 36	n = 71
Negative	6	10	16 (32.0%)
Positive	19	15	34 (68.0%)
Refused	10	11	21

NT-eCHF, non-thickened extensive casein hydrolysate formula; T-eCHF, thickened extensive casein hydrolysate formula; n, number of subjects

Table 4
Evolution of the cow's milk related symptom score between inclusion and 1 mo of dietary treatment. Results are expressed as mean ± standard deviation

Patient characteristics	Total	T-eCHF	NT-eCHF	*P*-values between groups	CMA+	CMA?	*P*-values between groups	CMA+			CMA?		
								T-eCHF	NT-eCHF	*P*-values between groups	T-eCHF	NT-eCHF	*P*-values between groups
Baseline	14.1 ± 3.5	14 ± 3.6	14.1 ± 3.4	0.842*	14.1 ± 3.4	13.8 ± 3.0	0.805†	13.8 ± 2.6	14.6 ± 4.3	0.975†	13.9 ± 3.5	13.8 ± 2.8	1.000†
1 mo	6.6 ± 3.8	6.4 ± 4.1	6.7 ± 3.6	0.747*	5.7 ± 3.7	7.3 ± 4	0.153†	5.6 ± 4	5.9 ± 3.3	0.710†	7.9 ± 4.7	7 ± 3.6	0.685†
Evolution	−7.5 ± 5.2	−7.6 ± 5.2	−7.4 ± 5.3	0.919*	−8.4 ± 5.2	−6.5 ± 4.5	0.244†	−8.2 ± 4.5	−8.7 ± 6.2	0.988†	−6.0 ± 4.6	−6.8 ± 4.6	0.820†
P-values vs. baseline	<0.001*	<0.001*	<0.001*		<0.001†	<0.001†		<0.001†	<0.001†		<0.002†	<0.002†	

CMA+, cow's milk allergy positive; CMA?, cow's milk allergy negative or not known; NT-eCHF, non-thickened extensive casein hydrolysate formula; SD, standard deviation; T-eCHF, thickened extensive casein hydrolysate formula

* Student's *t* test.

† Wilcoxon's test.

the T-eCHF and NT-eCHF groups (Figs. 2–5). Growth was normal for all children during the 6 mo trial.

Discussion

Unfortunately, 21/71 (29.6%) parents refused the challenge test despite their initial agreement when signing the informed consent. Three of these children were included in the CMA group because of a positive skin prick test (n:2) and/or a positive specific IgE (n:2). According to literature, both parameters have a specificity, which was 100% in a previous report [6]. However, it is likely that the challenge test would have been positive in some of the 15 infants in whom the test was refused. Therefore, it is likely that some infants included in the CMA-negative group were in fact allergic. The results observed in this study demonstrate that the tested eCHF meets the criteria of the American Academy of Pediatrics (AAP) for hypoallergenic formula, since the formula was tolerated by more than 90% of infants with proven CMA, with a 95% confidence interval [7].

The study provides evidence that the eCHF was well tolerated by infants with confirmed CMA. All the growth parameters improved within 6 mo for the whole population in the study. The development of anthropometric parameters was normal [11,12].

Table 5
Evolution after 1 mo of secondary outcomes contributing to the cow's milk related symptom score

Patient characteristics	Total	T-eCHF	NT-eCHF	*P*-values between groups	CMA+		
					T-eCHF	NT-eCHF	*P*-values between groups
Regurgitations score evolution, (Vandenplas score)							
Mean ± SD	−2.2 ± 1.4	−2.3 ± 1.4	−2.1 ± 1.5		−2.3 ± 1.3	−2.2 ± 1.8	
Median – [Q1; Q3]	−2 [−3; −1]	−2 [−3; −1]	−2 [−3; −1]	0.538*	−2 [−3; −1]	−2 [−3; −1]	0.837†
P-values vs. baseline	<0.001*	<0.001*	<0.001*		<0.001†	<0.001†	
Crying score evolution							
Mean ± SD	−2.1 ± 2.8	−2.1 ± 2.3	−2.2 ± 2		−2.8 ± 2.4	−1.9 ± 2.0	
Median – [Q1; Q3]	−2 [−4; −1]	−2 [−4; 0]	−2 [−3; −1]	0.964*	−3 [−5; −1]	−1 [−3; −1]	0.290†
P-values vs. baseline	<0.001*	<0.001*	<0.001*		<0.001†	<0.001†	
Proportions of patients (%) crying ≥3 h/d							
Baseline	42.3	54.3	30.6	0.043‡	61.9	31.0	0.065‡
1 mo	9.9	17.1	2.8	0.055§	9.5	6.3	1.000§
P-values vs. baseline	<0.0001‖	0.0008‖	0.0016‖		0.0009‖	0.045‖	
Proportions of patients (%) with normal stools (type C, D, and E)							
Baseline	12.7	8.6	16.7	0.478§	9.5	12.5	1.000§
1 mo	31.0	34.3	27.8	0.553‡	42.9	37.5	0.742‡
P-values vs. baseline	0.009‖	0.013‖	0.248‖		0.020‖	0.157‖	
Respiratory symptom score evolution							
Mean ± SD	−0.5 ± 0.7	−0.5 ± 0.7	−0.4 ± 0.7		−0.6 ± 0.7	−0.6 ± 0.7	
Median – [Q1; Q3]	0 [−1; 0]	0 [−1; 0]	0 [−1; 0]	0.448*	0 [−1; 0]	−1 [−1; 0]	0.959†
P-values vs. baseline	<0.001*	<0.001*	<0.001*		0.002†	0.002†	
Cutaneous symptoms score evolution (eczema at both body sites)							
Mean ± SD	−1.3 ± 1.6	−1.0 ± 1.3	−1.6 ± 1.7		−0.8 ± 1.3	−1.9 ± 1.6	
Median – [Q1; Q3]	−1 [−2; 0]	−1 [−2; 0]	−1 [−3; 0]	0.5161‖	0 [−2; 0]	−2 [−3; −1]	0.6913‖
P-values vs. baseline	<0.001†	<0.001†	<0.001*		<0.011¶	<0.01*	

CMA+, cow's milk allergy positive; NT-eCHF, non-thickened extensive casein hydrolysate formula; SD, standard deviation; T-eCHF, thickened extensive casein hydrolysate formula

* Student test.

† Wilcoxon test.

‡ Chi-2 test.

§ Fisher's test.

‖ MacNemar test.

¶ Ancova.

Table 6
Evolution of the daily number of regurgitations during the first month

Patient characteristics	Total	T-eCHF	NT-eCHF	*P*-values between groups*	CMA+			CMA?		
					T-eCHF	NT-eCHF	*P*-values between groups*	T-eCHF	NT-eCHF	*P*-values between groups*
Mean ± SD	−3.65 ± 3.98	−4.36 ± 3.06	−2.91 ± 4.70	0.025	−4.82 ± 2.55	−4.14 ± 5.22	0.185	−3.50 ± 3.87	−2.22 ± 3.47	0.144
Median [Q1; Q3]	−4 [−6; −2]	−5 [−6; −3]	−2 [−5; 0]		−5 [−7; −3]	−3 [−5; −2]		−5 [−6; −3]	−3 [−5; 0]	
P-values vs. baseline*	<0.001	<0.001	<0.001		<0.001	<0.001		0.0074	0.019	

CMA+, cow's milk allergy positive; CMA?, cow's milk allergy negative and not known; NT-eCHF, non-thickened extensive casein hydrolysate formula; SD, standard deviation; T-eCHF, thickened extensive casein hydrolysate formula
* Wilcoxon's test.

We analyzed the efficacy and growth data in the CMA-positive and CMA-negative or unknown ("CMA? population") groups, as this represents daily clinical reality in primary health care. Before the diagnosis of CMA can be established, infants are put on an elimination diet as part of the diagnostic procedure. Subsequently, many parents refuse a challenge, which is mandatory to confirm the diagnosis, because the symptoms decreased significantly. Therefore, it is relevant to have efficacy, but even more safety data on growth for these infants, who may be inappropriate for long term consumption of an eHF.

An oral challenge test is considered the gold standard to diagnose CMA [1]. However, many parents refuse a challenge [6]. In this study, 29.5% of the parents refused despite an initial agreement, since the challenge was part of the informed consent, a percentage which is similar to a previously reported incidence in a comparable study design and study population [6]. The CoMiSS was specifically developed as an awareness tool to select infants with a high risk of symptoms related to ingestion of cow's milk and to assess the evolution of symptoms during dietary intervention [10]. A challenge to confirm the diagnosis of CMA remains imperative.

Regurgitation was significantly decreased in all groups but the T-eCHF was more effective for all infants during the first month. In infants with confirmed CMA, the NT-eCHF decreased

Table 7
Evolution of anthropometric parameters during the study period. Results are expressed as mean ± standard deviation

Patient characteristics	Total	T-eCHF	NT-eCHF	*P*-values between groups	CMA+	CMA+		*P*-values between groups
						T-eCHF	NT-eCHF	
Weight-for-age z-score								
Baseline	−0.64 ± 1.18	−0.67 ± 1.11	−0.61 ± 1.27	0.835*	−0.70 ± 1.39	−0.52 ± 1.14	−0.93 ± 1.67	0.510†
1 mo	−0.31 ± 1.09	−0.37 ± 1.01	−0.25 ± 1.17	0.660*	−0.30 ± 1.29	−0.24 ± 1.10	−0.38 ± 1.53	0.890†
P (D30–D0)	<0.001*	0.004*	0.001*	0.691*	<0.001†	0.052†	0.005	0.415†
3 mo	−0.00 ± 1.00	0.04 ± 1.01	−0.05 ± 1.01	0.991†	0.16 ± 1.00	0.23 ± 0.93	0.07 ± 1.11	1.000†
P (D90–D0)†	<0.001	0.002	0.003	0.937†	<0.001†	0.002	0.005	0.123†
6 mo	0.33 ± 0.98	0.52 ± 0.86	0.13 ± 1.08	0.348†	0.59 ± 0.85	0.73 ± 0.58	0.42 ± 0.98	0.482†
P (D180–D0)†	<0.001	<0.001	0.001	0.481†	<0.001†	<0.001	0.003	0.186†
Length-for-age z-score								
Baseline	−0.51 ± 1.34	−0.66 ± 1.32	−0.36 ± 1.36	0.343*	−0.61 ± 1.55	−0.71 ± 1.51	−0.47 ± 1.66	0.425†
1 mo	−0.31 ± 1.27	−0.46 ± 1.31	−0.17 ± 1.33	0.332*	−0.50 ± 1.44	−0.63 ± 1.28	−0.33 ± 1.69	0.319†
P (D30–D0)	0.965*	0.222*	0.141*	0.965*	0.447†	0.720†	0.354†	0.818†
3 mo	−0.07 ± 1.40	−0.21 ± 1.04	0.08 ± 1.73	0.200†	−0.19 ± 1.38	−0.23 ± 1.01	−0.14 ± 1.78	0.340†
P (D90–D0)†	0.021	0.124	0.091	1.000	0.111	0.236	0.303	0.742
6 mo	0.29 ± 1.35	0.31 ± 0.89	0.27 ± 1.71	0.473†	0.17 ± 1.48	0.28 ± 0.97	0.03 ± 1.97	0.984†
P (D180–D0)†	<0.001	0.005	0.026	0.285	0.005	0.017	0.152	0.275
Weight-for- length z-score								
Baseline	−0.25 ± 1.47	−0.08 ± 1.42	−0.42 ± 1.53	0.345*	−0.23 ± 1.78	0.20 ± 1.60	−0.79 ± 1.89	0.133†
1 mo	−0.01 ± 1.30	0.07 ± 1.19	−0.09 ± 1.42	0.601*	0.18 ± 1.49	0.43 ± 1.21	−0.15 ± 1.77	0.319†
P (D30–D0)	0.048*	0.206*	0.075*	0.486*	0.035†	0.187†	0.034†	0.319†
3 mo	0.21 ± 1.29	0.38 ± 1.28	0.02 ± 1.31	0.254†	0.53 ± 0.99	0.67 ± 0.96	0.37 ± 1.04	0.290†
P (D90–D0)†	0.054	0.510	0.051	0.398	0.015	0.283	0.023	0.314
6 mo	0.33 ± 1.26	0.57 ± 0.98	0.08 ± 1.47	0.330†	0.77 ± 0.87	0.88 ± 0.65	0.64 ± 1.10	0.620†
P (D180–D0)†	0.075	0.357	0.137	0.473	0.024	0.258	0.054	0.361
BMI for age z-score								
Baseline	−0.49 ± 1.33	−0.40 ± 1.24	−0.56 ± 1.43	0.614*	−0.48 ± 1.61	−0.15 ± 1.35	−0.92 ± 1.85	0.312†
1 mo	−0.17 ± 1.24	−0.14 ± 1.12	−0.21 ± 1.36	0.816*	−0.02 ± 1.43	0.17 ± 1.14	−0.26 ± 1.74	0.571†
P (D30–D0)	0.003*	0.044*	0.031*	0.656*	<0.001†	0.120†	0.025†	0.319†
3 mo	0.09 ± 1.39	0.27 ± 1.30	−0.12 ± 1.36	0.259†	0.42 ± 1.03	0.56 ± 0.99	0.23 ± 1.09	0.331†
P (D90–D0)†	0.007	0.057	0.053	0.851	<0.001	0.024	0.023	0.555
6 mo	0.24 ± 1.31	0.49 ± 1.00	−0.02 ± 1.55	0.304†	0.70 ± 0.94	0.81 ± 0.66	0.56 ± 1.21	0.606†
P (D180–D0)†	0.010	0.019	0.110	0.925	0.004	0.023	0.054	0.512

BMI, body mass index; CMA+, cow's milk allergy positive; NT-eCHF, non-thickened extensive casein hydrolysate formula; SD, standard deviation; T-eCHF, thickened extensive casein hydrolysate formula
* Student's *t* test.
† Wilcoxon's test.

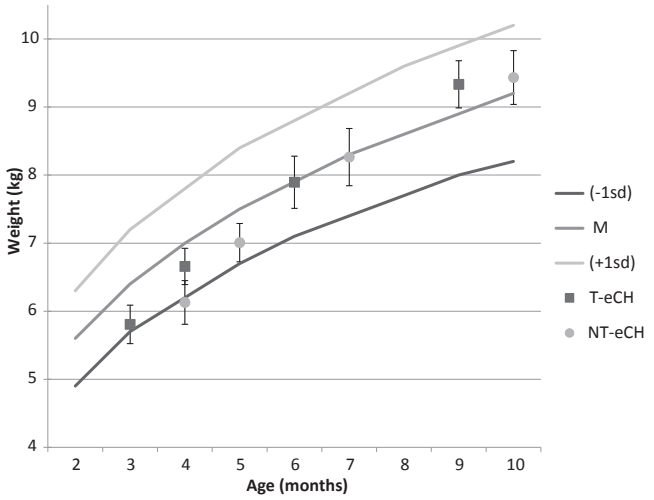

Fig. 2. Weight evolution in boys for both formulas compared to World Health Organization standard chart. NT-eCHF, non-thickened extensive casein hydrolysate formula; T-eCHF, thickened extensive casein hydrolysate formula; M, mean; SD, standard deviation.

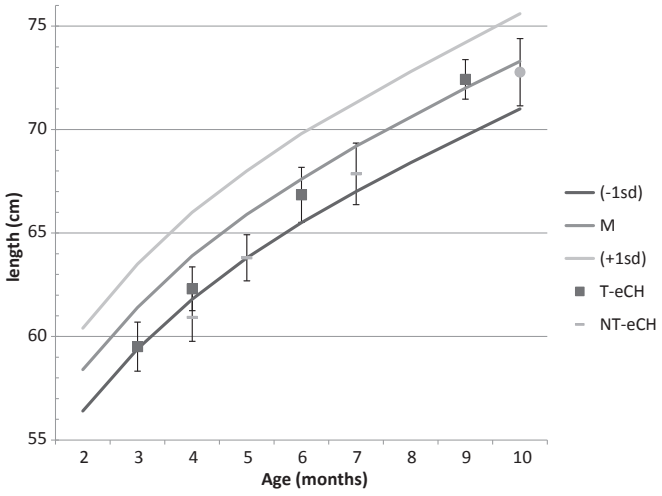

Fig. 4. Height evolution in boys for both formulas compared to WHO standard chart. NT-eCHF, non-thickened extensive casein hydrolysate formula; T-eCHF, thickened extensive casein hydrolysate formula; M, mean; SD, standard deviation.

regurgitation. The normalization of the stool consistency observed only in the subgroup fed the T-eCHF is an interesting characteristic since hydrolysates are known to cause soft, liquid stools [3]. Indeed, the patented thickening complex present in the T-eCHF contains specific fibers selected for their ability to regulate the transit, i.e., to induce neither liquid nor hard stools.

Conclusion

The therapeutic efficacy of the tested eCHF fulfills the requirements to be designated as a hypo-allergenic formula. A thickened extensive hydrolysate is a new development. CMA management should reflect not only basic research but also a newer and better appraisal of the literature in light of the values and preferences shared by patients and their caregivers [13]. Overall, the T and NT-eCHF are effective to alleviate symptoms of CMA. However, in case of CMA suspicion, the thickened hydrolysate is more efficient to reduce regurgitations and also improves the stool consistency. The evolution of the anthropometric parameters was excellent with both variants of the eCHF.

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.nut.2015.08.008

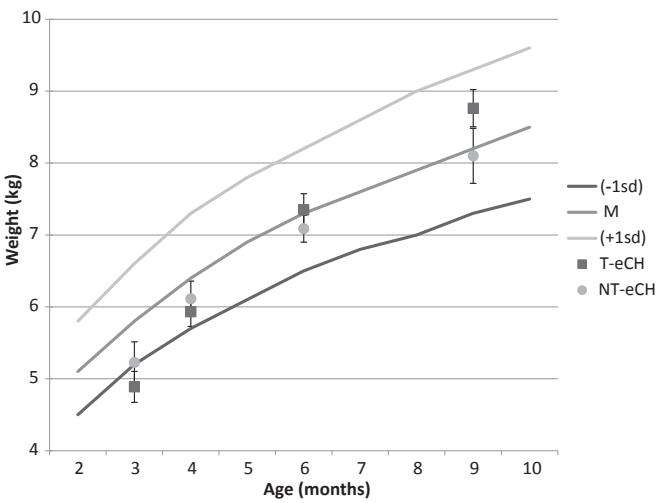

Fig. 3. Weight evolution in girls for both formulas. Compared to WHO standard chart. NT-eCHF, non-thickened extensive casein hydrolysate formula; T-eCHF, thickened extensive casein hydrolysate formula; M, mean; SD, standard deviation.

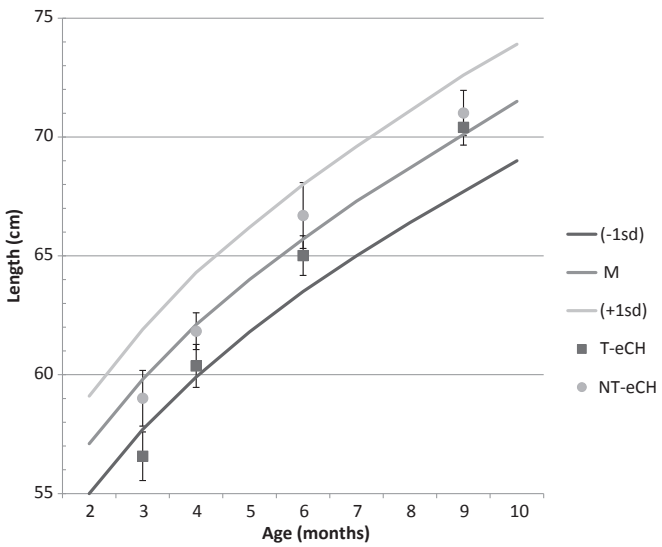

Fig. 5. Height evolution in girls for both formulas compared to WHO standard. NT-eCHF, non-thickened extensive casein hydrolysate formula; T-eCHF, thickened extensive casein hydrolysate formula; M, mean; SD, standard deviation.

References

[1] Koletzko S, Niggemann B, Arato A, Dias JA, Heuschkel R, Husby S, et al. Diagnostic approach and management of cow's milk protein allergy in infants and children: A practical guideline of the GI-committee of ESPGHAN. J Pediatr Gastroenterol Nutr 2012;55:221–9.

[2] Muraro A, Dreborg S, Halken S, Host A, Niggeman B, Aalberse R, et al. Dietary prevention of allergic diseases in infants and small children. Part I: Immunologic background and criteria for hypoallergenicity. Pediatr Allergy Immunol 2004;15:103–11.

[3] Niggemann B, von Berg A, Bollrath C, Berdel D, Schauer U, Rieger C, et al. Safety and efficacy of a new extensively hydrolyzed formula for infants with cow's milk protein allergy. Pediatr Allergy Immunol 2008;19:348–54.

[4] Rona RJ, Keil T, Summers C, Gislason D, Zuidmeer L, Sodergren E, et al. The prevalence of food allergy: A meta-analysis. J Allergy Clin Immunol 2007;120:638–46.

[5] Boyce JA, Assa'ad A, Burks AW, Jones SM, Sampson HA, Wood RA, et al. Guidelines for the diagnosis and management of food allergy in the United States: Report of the NIAID-sponsored expert panel. J Allergy Clin Immunol 2010;126:S1–58.

[6] Vandenplas Y, Steenhout P, Planoudis Y, Grathwohl D, Althera Study Group. Treating cow's milk protein allergy: A double-blind randomised trial comparing two extensively hydrolyzed formulas with probiotics. Acta Paediatr 2013;102:990–8.

[7] American Academy of Pediatrics. Committee on Nutrition. Hypoallergenic infant formula. Pediatrics 2000;106:346–9.

[8] Vandenplas Y, De Greef E, ALLAR study group. Extensive protein hydrolysate formula effectively reduces regurgitation in infants with positive and negative challenge tests for cow's milk allergy. Acta Paediatr 2014;103:e243–50.

[9] WHO Child Growth Standards. The WHO Multicentre Growth Reference Study (MGRS). Available at: http://www.who.int/childgrowth/standards/en12.http://www.fda.gov/downloads/Drugs/.../Guidances/ucm201790.pdf. Accessed May 5, 2015.

[10] Vandenplas Y, Dupont C, Eigenmann P, Host A, Kuitunen M, Ribes-Koninckx C, et al. A workshop report on the development of the Cow's Milk-related Symptom Score awareness tool for young children. Acta Paediatr 2015;104:334–9.

[11] Hernell O, Lönnerdal B. Nutritional evaluation of protein hydrolysate formulas in healthy term infants: plasma amino acids, hematology, and trace elements. Am J Clin Nutr 2003;78:296–301.

[12] Scalabrin DM, Johsnton WH, Hoffman DR, P'Pool VL, Harris CL, Mitmesser SH. Growth and tolerance of healthy term infants receiving hydrolyzed infant formulas supplemented with Lactobacillus rhamnosus GG: randomized, double-blind, controlled trial. Clin Pediatr 2009;48:734–44.

[13] Fiocchi A, Schünemann HJ, Brozek J, Restani P, Beyer K, Troncone R, et al. Diagnosis and rationale for action against cow's milk allergy (DRACMA): a summary report. J Allergy Clin Immunol 2010;126:1119–28.

Eur J Pediatr
DOI 10.1007/s00431-014-2308-4

ORIGINAL ARTICLE

Safety and tolerance of a new extensively hydrolyzed rice protein-based formula in the management of infants with cow's milk protein allergy

Yvan Vandenplas · Elisabeth De Greef · Bruno Hauser · Paradice Study Group

Received: 26 December 2013 / Revised: 3 March 2014 / Accepted: 24 March 2014

Abstract Guidelines recommend the use of extensively hydrolyzed cow's milk protein-based formulas (eHF) in the treatment of infants with cow's milk protein allergy (CMPA). Extensively hydrolyzed rice protein infant formula (eRHF) has recently become available and could offer a valid alternative. A prospective trial was performed to evaluate the hypo-allergenicity and safety of a new eRHF in infants with a confirmed CMPA. Patients were fed the study formula for 6 months. Clinical tolerance of the eRHF was evaluated with a symptom-based score (SBS) and growth (weight and length) was monitored. Forty infants (mean age, 3.4 months; range, 1–6 months) with CMPA confirmed by a food challenge were enrolled. All infants tolerated the eRHF and the SBS significantly decreased as of the first month of intervention. Moreover, the eRHF allowed a catch-up to normal weight gain as of the first month as well as a normalization of the weight-for-age, weight-for length, and BMI *z*-scores within the 6-month study period. *Conclusion*: In accordance with current guidelines, this eRHF was tolerated by more than 90 % of children with proven CMPA with a 95 % confidence interval. This eRHF is an adequate and safe alternative to cow milk-based eHF.

Keywords Cow's milk protein allergy · Extensive hydrolysate · Extensively hydrolyzed rice protein formula

Abbreviations

AAP	American Academy of Pediatrics
CI	Confidence interval
CMP(A)	Cow's milk protein (allergy)
E(R)HF	Extensive (rice) hydrolysate formulaESPGHAN European Society of Paedaitric Gastroenterology, Hepatology and Nutrition
IgE	Immunoglobulin E
pRHF	Partial rice hydrolysate formula
SBS	Symptom-based score
SPT	Skin-prick test
SIF	Soy infant formula
WHO	World Health Organization

Introduction

Guidelines for the dietary management of infants diagnosed with cow's milk protein allergy (CMPA) recommend the substitution of cow's milk with extensively hydrolyzed casein or whey protein formulas (eHF) [3, 4, 6, 13]. Up to 14 % of infants with CMPA will also react to soy infant formula (SIF) [1, 4], even though tolerance of soy is better in immunoglobulin E (IgE) compared with non-IgE-mediated CMPA [27]. ESPGHAN and an Australian expert panel recommend not using SIF before the age of 6 months [12, 13]. In addition, the American Academy of Pediatrics (AAP) recommends an eHF as a preferred therapeutic option

Communicated by David Nadal

Paradice Study Group: C. Halut, MN. Robberecht, N. Balduck, A. l'Homme, MP. Mohring, T. Carvelli, E. Defontaine, JF. Questiau, J. Christens, F. Henckens, R. Lemmens, L. Vercammen

Y. Vandenplas (✉) · E. De Greef · B. Hauser
Department of Pediatrics, UZ Brussel, Vrije Universiteit Brussel, Brussels, Belgium
e-mail: yvan.vandenplas@uzbrussel.be

E. De Greef
e-mail: elisabeth.degreef@uzbrussel.be

B. Hauser
e-mail: bruno.hauser@uzbrussel.be

Published online: 12 April 2014

with SIF as a second choice [4]. However, eHFs are substantially more expensive than standard or soy infant formulae and generally have a bitter taste, which often hampers their acceptability [4]. Moreover, some parents may look for vegetable alternatives due to various opinion or convictions. Some infants may still be intolerant or allergic to these eHFs [3, 6, 13]. In those cases, amino acid formulae (AAF) are an effective dietary treatment [4, 6, 13] but are even substantially more expensive and have also a bitter taste.

As a result, affordable and better-tasting dietary options in the treatment of CMPA would be welcomed as an alternative. Hydrolyzed formulas based on rice protein may offer such an option [7, 9, 10, 19, 20]. Therefore, the efficacy of such a new extensively hydrolyzed rice protein infant formula (eRHF) was evaluated in infants with CMPA.

Materials and methods

This study was conducted between April 2011 and March 2013. Infants who initially presented with symptoms suggesting CMPA were selected. Diagnostic criteria to suspect CMPA were based on the presence of a combination of the following symptoms: general discomfort (persistent distress or colic, ≥3 h/day and wailing/irritability at least 3 days/week since at least 1 week), gastrointestinal signs and symptoms (frequent regurgitation, vomiting, diarrhoea, constipation with or without perianal rash, and blood in the stools), respiratory symptoms (runny nose, otitis media, chronic cough, and wheezing unrelated to infection), and dermatological manifestations (atopic dermatitis, angio-oedema, urticaria unrelated to acute infections, drug intake, etc.) [13, 23, 25]. A symptom-based

Table 1 Symptom-based clinical score (adapted from refs. [20, 23, 24])

Symptom	Score			
Crying[a]	0 to 6	0	1 h/day	
		1	1–1.5 h/day	
		2	1.5–2 h/day	
		3	2 to 3 h/day	
		4	3 to 4 h/day	
		5	4 to 5 h/day	
		6	>5 h/day	
Regurgitation [22]	0 to 6	0	0–2 episodes/day	
		1	≥3 to ≤5 of small volume	
		2	>5 episodes of >1 coffee spoon	
		3	>5 episodes of±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 volume after each feeding	
Stools (according to Bristol stool scale [15])	0 to 6	4	Types 1 and 2 (hard stools)	
		0	Types 3 and 4 (normal stools)	
		2	Type 5 (soft stool)	
		4	Type 6 (mushy/liquid stool, if unrelated to infection)	
		6	Type 7 (watery stools)	
Dermatological symptoms	0 to 6	Atopic eczema		
			Head–neck–trunk	Arms–hands–legs–feet
		Absent	0	0
		Mild	1	1
		Moderate	2	2
		Severe	3	3
	0 to 6	Urticaria (0 no/6 yes)		
Respiratory symptoms	0 to 3	0	No respiratory symptoms	
		1	Mild symptoms	
		2	Moderate symptoms	
		3	Severe symptoms	

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

score (SBS) considering the vast majority of the symptoms of CMPA reported in literature was developed and the severity of each presenting symptom was scored (Table 1) [21, 24, 25].

Infants were included after the diagnosis of CMPA was confirmed by a positive challenge, except if the challenge was contra-indicated, in accordance to recent guidelines [13]. The challenge was performed with standard infant formula, following a standardised challenge test procedure [13]. The challenge procedure lasted one week, of which the first half day consisted of gradual introduction of cow's milk protein (CMP). If no reaction occurred during this half day, parents administered at least 250 ml/day of standard infant formula per day during 1 week. During that week, on a daily basis, parents had to fill in a diary with information on regurgitation, stools, and duration of crying. Parents had to report any change/reaction they noticed. If any, the child was presented at the outpatient clinic and the physician evaluated the evolution of the SBS. The paediatricians evaluated the SBS before and during the food challenge, as well as 1, 3, and 6 months after initiation of the dietary treatment with the eRHF. Baseline score was defined as the score reached when a positive reaction occurred during the challenge, both for immediate and late reactions. It was up to the physician to decide to perform a skin-prick test (SPT) and measure-specific IgE. The SPT was evaluated according to the standard criteria, i.e., a papula of 3 mm induration compared with a negative control with saline solution [8].

A positive challenge was the inclusion criterion for this study; included infants were fed with the new eRHF during 6 months. Infant formulas are the only recommended food for infants below 6 months. Weaning foods were introduced following paediatricians' advice, with specific recommendation to avoid cow's milk containing products.

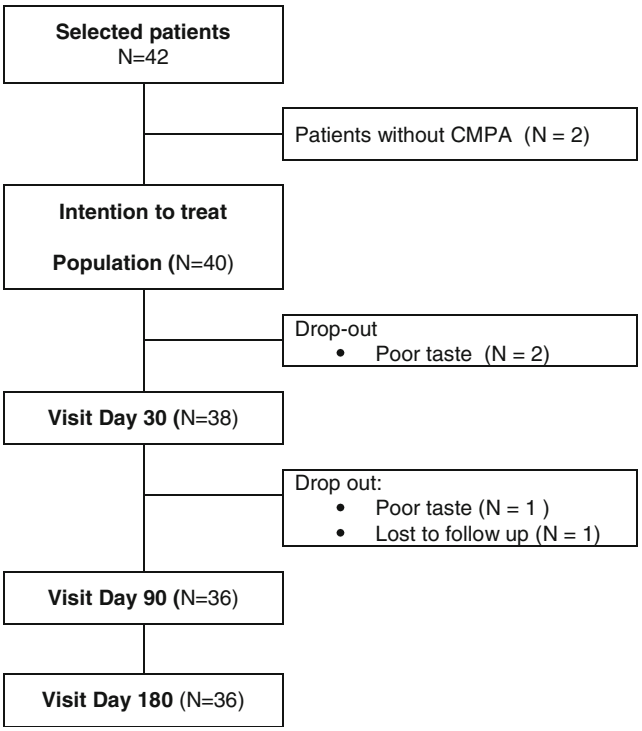

Fig. 1 Flow chart

The SBS was evaluated 1, 3, and 6 months after initiation of the dietary treatment with the eRHF. Growth (weight and length) was monitored and evaluated as *z*-scores according to the WHO Child Growth Standards [26]. Feeding tolerance and adverse events were registered throughout the 6 months study period.

The test formula (NovaRice, United Pharmaceuticals) contains extensively hydrolyzed-rice protein supplemented with lysine and tryptophan to improve the nutritional quality by providing an amino-acid profile similar to that of mother's

Table 2 Average nutritional composition of the study formula

	Unit	/100 g	/100 ml
Proteins	g	13.4	1.8
Fats	g	25.5	3.4
Saturated fatty acids	g	9.9	1.3
Monounsaturated fatty acids	g	9.2	1.2
Polyunsaturated fatty acids	g	5.1	0.7
Linoleic acid	g	4.5	0.6
Alpha-linolenic acid	mg	425	57.4
Medium-chain triglycerides	g	2.3	0.3
Carbohydrates	g	49	6.6
Maltodextrins	g	46	6.2
Starch	g	1	0.1
Fibers	g	4	0.5
Fibers	g	4	0.5
Energy	kcal	487	65.7

The composition of the formula may be adjusted for compliance to various regulations, without any impact on the hypoallergenicity of the formula, and its nutritional value

Table 3 Description of the included population

Boy/girl	21/19
Age at inclusion (months) mean±SD	3.4+±1.5
Median (range)	3 (0–6)
Time since the first apparition of the symptoms (months), mean±SD	1.9±1.2
Median (range)	1.8 (0.2–5.4)
Infants never breast fed (*n* (%))	9 (23.1)
Duration of exclusive breast feeding (weeks), mean±SD	5.2±5.0
Median (range)	4 (0–18)
Duration of partial breast feeding (weeks; mean±SD)	2.3±4.0
Median (range)	1 (0–16)
Infants with at least one parent or sibling having a proven or suspected allergic disease (*n* (%))	36 (90.0)

Table 4 Evolution of the global symptom-based score (SBS)

	Before challenge (n=38) (A)	Inclusion (n=38) (B)	1 month (n=38) (C)	3 months (n=36) (D)	6 months (n=36) (E)
Mean± SD	8.6±5.6	13.5±5.2	3.5±2.3	2.4±1.9	1.5±2.0
p		A–B, <0.0001 [b]	B–C, <0.001[b]	B–D, <0.001[a]	B–E, <0.001[a]

[a] Paired Student's t test

milk, in compliance with the recommendation of the EU Directive on infant formulas (composition of the formula, Table 2). More than 95 % of the peptides in the eRHF have a molecular weight of less than 3 kDa, and most of these are under 1.5 kDa. It also contains a thickening complex using pectin, as extensive hydrolysates are particularly liquid. The formula is lactose free and complies with EU regulation.

The study was approved by the Ethical Committee of the UZ Brussel, acting as the leading center, and of each participating center; 14 investigators from 11 centers participated in

Table 5 Evolution of the different components of the symptom-based score (SBS)

	Before challenge (n=38)	Inclusion (n=38) (A)	1 month (n=38) (B)	3 months (n=36) (C)	6 months (n=36) (D)
Crying (n (%))					
<3 h/day	26 (68.4)	16 (42.1)	38 (100)	36 (100)	36 (100)
≥3 h/day	12 (31.6)	22 (57.9)	0 (0)	0 (0)	0 (0)
p			A–B, 0.0001[c]	A–C, <0.0001[c]	A–D, <0.0001[c]
Crying score					
Mean±SD	2.2±1.8	3.8±2.0	0.5±0.8	0.2±0.4	0.1±0.4
p			A–B, <0.001[d]	A–C, <0.001[d]	A–D, <0.001[d]
Regurgitation score [22]					
Mean±SD	1.5±1.9	2.4±2.2	0.6±0.9	0.5±0.9	0.1±0.3
p			A–B, 0.001[d]	A–C, <0.001[d]	A–D, <0.001[d]
Stools (n (%))					
Normal stools (type III or IV)	5 (13.2)	2 (5.3)	20 (52.6)	21 (58.3)	28 (77.8)
Abnormal stools (type I, II, V, VI, or VII)	33 (86.8)	36 (94.7)	18 (47.4)	15 (41.7)	8 (22)
p			<0.0001[c]	<0.0001[c]	<0.0001[c]
Urticaria (n (%))					
	2 (5.3)	6 (15.8)	0 (0.0)	0 (0.0)	0 (0.0)
p			<0.02[c]	<0.02[c]	<0.02[c]
Eczema (n (%)), head, neck, trunk (n (%))					
Absent	23 (60.5)	18 (47.4)	30 (78.9)	31 (86.1)	31 (86.1)
Mild	7 (18.4)	6 (15.8)	7 (18.4)	4 (11.1)	4 (11.1)
Moderate	7 (18.4)	10 (26.3)	1 (2.6)	1 (2.8)	1 (2.8)
Severe	1 (2.6)	4 (10.5)	0	0	0
p			<0.05[a]	<0.05[a]	<0.05[a]
Arms, hands, legs, and feet (n (%))					
Absent	27 (71.1)	23 (60.5)	33 (86.8)	33 (91.7)	32 (88.9)
Mild	2 (5.3)	3 (7.9)	5 (13.2)	3 (8.3)	4 (11.1)
Moderate	7 (18.4)	8 (21.1)	0	0	0
Severe	2 (5.3)	4 (10.5)	0	0	0
p			0.055[a]	0.02[a]	0.058[a]
Respiratory symptoms (n (%))					
Absent	31 (81.6)	29 (76.3)	31 (81.6)	27 (75 %)	29 (80.6 %)
Light	5 (13.2)	6 (15.8)	5 (13.2)	8 (22.2)	5 (13.9
Mild	1 (2.6)	2 (5.3)	2 (5.3)	1 (2.8)	1 (2.8)
Severe	1 (2.6)	1 (2.6)	0 (0.0)	0 (0.0)	1 (2.8)
p			NS	NS	NS

NS not significant

[a] Symmetry test

[b] Paired Student's t test

[c] McNemar's test

[d] Wilcoxon's test

the trial. A written informed consent was obtained from all parents. United Pharmaceuticals provided free formula for the study period. The study was registered at clinicaltrials.gov NCT number NCT01998074

To be considered hypoallergenic, a therapeutic formula must demonstrate in a clinical study that with 95 % confidence it does not provoke allergic reactions in 90 % of infants or children with confirmed cow's milk allergy [3]. In case of no reaction, the lower 95 % confidence interval (CI) for the proportion of patients with no reaction should be greater than 90 %; a sample size of 29 participants is sufficient to show hypo-allergenicity. Considering possible dropouts or deviation to inclusion criteria, the target was to recruit 36 patients. Statistical analysis was carried out using SAS 9.2 software. For qualitative parameters classified in two categories, McNemar's test was used and in case of more than 2 categories, symmetry test was used. Paired Student's *t* test was used for quantitative parameters. The normality of distribution was systematically checked using Shapiro–Wilk's test and the Wilcoxon's test was used in case of non-normality.

Results

Forty-two patients were selected for the study. Forty infants were included (21 boys, 19 girls; age, 3.4±1.5 months (mean±SD); range, 0–6 months) (Fig. 1; Table 3). Thirty-eight infants had a positive challenge confirming CMPA and two patients were not challenged because of an initial anaphylactic reaction. This was the intention to treat population, used to assess the hypo-allergenicity and growth parameters evolution. Fourteen out of 38 infants had an immediate type of reaction. A SPT was performed in 17 infants and was positive in 15 (mean wheal, 11 mm (range, 3–25 mm)).

Four patients dropped out before the end of the study (Fig. 1). Three parents decided to stop the trial because according to their opinion the infant did not like or accept the study formula and preferred the "initial" formula (which was given before the challenge). One patient did not show up for the visit after 1 month.

The tolerance was evaluated on the intention-to-treat population of 40 patients, consisting of all patients with a confirmed CMPA. None of them dropped out for intolerance.

Seventy-nine adverse events have been reported during the 6 months observation period. Among them, five were serious adverse events all unrelated to the study formula (two bronchiolitis, one pneumonia, and two pyelonephritis). One non-serious adverse event was reported as related to the study product, it was food refusal leading to the end of the study for this patient. Other adverse events were mainly related mainly to ear-nose-throat (73 %), gastro-intestinal tract infections (14.9 %), or varicella (4.1 %), the remaining (8 %) being various such as fever, conjunctivitis.

The SBS change was evaluated on the 38 allergic infants who were presented after one month eRHF feeding. Thirty-six out of 38 were fed the study formula for 6 months.

The SBS was significantly lower at each time point (1, 3, or 6 months) than at baseline (Table 4, $p<0.001$).

All parameters composing the SBS score had decreased after 1 month of dietary treatment with the study formula (Table 5), and this evolution was confirmed after 3 and 6 months. At baseline, 5.3 % of the infants had "normal" stools while after only one month feeding with the eHRF

Table 6 Anthropometric data at inclusion and after 1, 3, and 6 months feeding with the extensive rice hydrolysate

	Inclusion	1 month	3 months	6 months
Age (months)				
No. of subjects (N)	40	38	36	36
Mean±SD	3.4±1.5	4.4±1.5	6.4±1.6	9.6±1.7
Range	1–6	2–7	4–10	7–13
Weight (kg)				
N	38	38	36	36
Mean±SD	6.1±1.1	6.7±1.1	7.6±1.1	8.8±1
Weight-for-age *z*-score				
Mean±SD	−0.7±1.0	−0.5±0.9	−0.3±1.0	−0.1±0.9
p (visit inclusion)		<0.001[b]	<0.001[a]	<0.001[a]
Length (cm)				
N	37	38	36	36
Mean±SD	61.9±3.9	64.3±3.7	67.8±3.5	72.1±3.3
Length-for age *z*-score				
Mean±SD	−0.1±1.0	−0.1±1.1	−0.1±1.1	−0.1±1.1
p (visit inclusion)		NS[a]	NS[a]	NS[a]
Weight-for-length *z*-score				
Mean±SD	−0.7±0.9	−0.5±0.8	−0.3±0.9	0±0.8
p (visit inclusion)		0.018[a]	<0.001[a]	<0.001[a]
BMI (kg/m^2)				
N	37	38	36	36
Mean±SD	15.7±1.6	16.2±1.4	16.5±1.3	16.8±1.2
BMI-for-age *z*-score				
Mean±SD	−0.7±0.9	−0.6±0.8	−0.4±0.9	0.0±0.8
p (visit inclusion)		0.012[a]	<0.001[a]	<0.001[a]
Head circumference (cm)				
N	37	38	36	36
Mean±SD	40.8±1.9	42.1±1.6	43.6±1.8	45.5±1.6
Head circumference *z*-score				
Mean±SD	0.1±1.1	0.3±0.9	0.3±1.2	0.5±1.0
p (visit inclusion)		0.020[b]	NS[a]	<0.001[a]

p values are related to *z*-score variation between inclusion and each visit

[a] Student's *t* test

[b] Wilcoxon's test

52.6 % had normal stools ($p<0.0001$). At the end of the 6-month period, 77.8 % of the infants had normal stools. At baseline, 57.9 % of the infants were crying more than 3 h/day, whereas, after 1 month, none of the infants were crying more than 3 h/day ($p<0.0001$), and 65.8 % were crying less than 1 h/day. At three months, 86.1 % of the infants were crying less than 1 hour a day. The regurgitation score [24] decreased by 75 % over 1 month (from 2.4±2.2 to 0.6±0.9, $p<0.0001$), and this decrease persisted at days 90 (0.5±0.9) and 180 (0.1±0.3).

Thirty-six infants were fed with the study formula for at least 6 months. Growth parameters were evaluated as *z*-scores according to the WHO Child Growth Standards [21] and are shown in Table 6 and Fig. 2. At inclusion, weight-for-

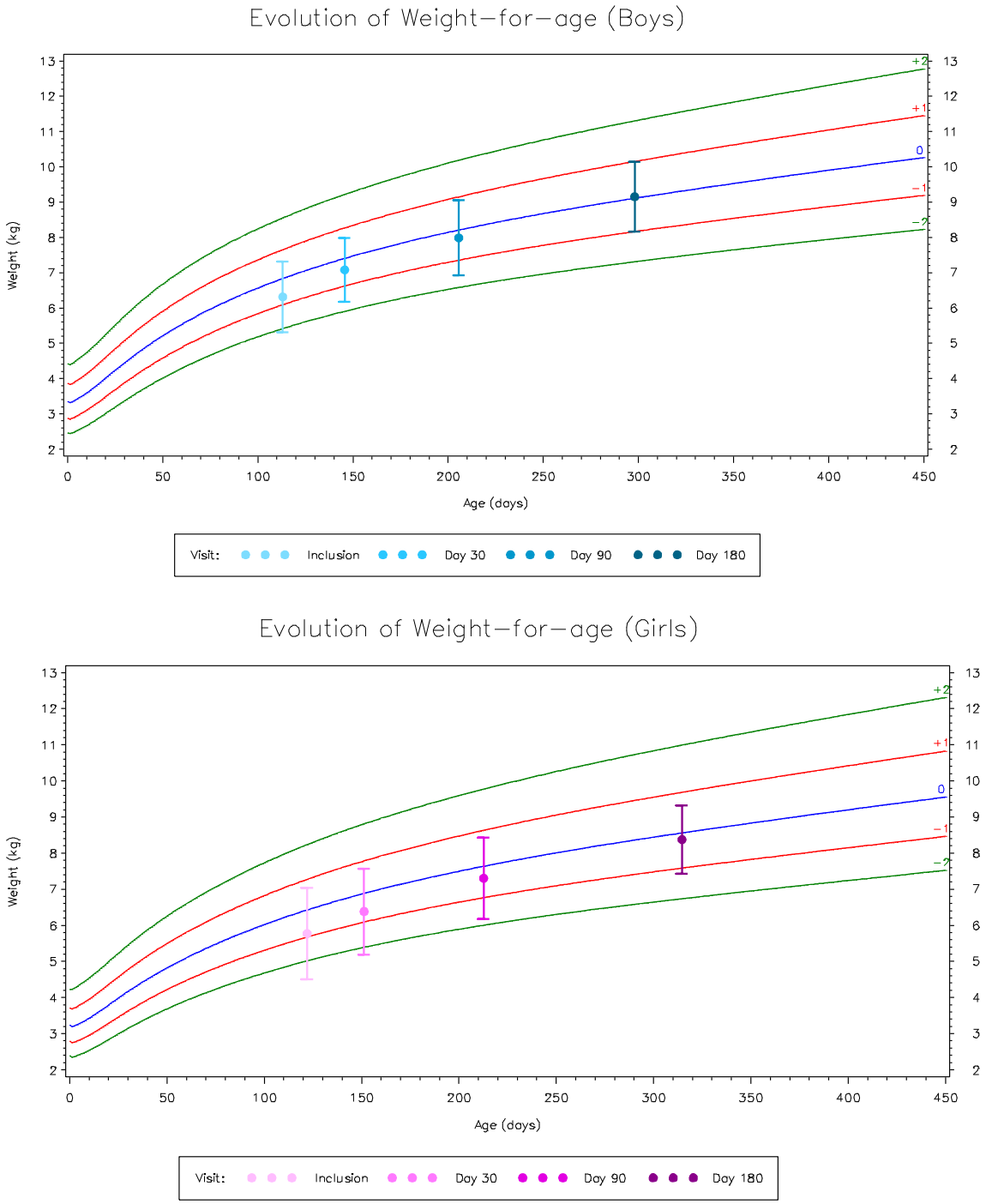

Fig. 2 Evolution of weight-for-age *z*-score for boys and girls

age, weight-for-length, and BMI *z*-scores were all negative (−0.7) indicating a slight growth faltering. As of the 1st month of feeding with the study formula, the weight-for-age, weight-for-length, and BMI *z*-scores significantly increased and were normalized with a catching up of the WHO Child Growth Standards by the end of the study period.

Discussion

This extensively hydrolyzed rice protein formula was tolerated by infants with a proven CMPA and contributed to catch-up growth. To date, all studies with hydrolyzed rice protein formulas (RHF) were performed with a partial rice protein hydrolysate (pRHF). Nevertheless, these studies also focused on their tolerance in infants with CMPA [9, 10, 19]. Two studies by Fiocchi et al have shown that infants with CMPA and other food allergies tolerated pRHF [9, 10]. Reche et al. demonstrated a 95 % efficacy rate with a pRHF in infants with CMPA [19]. We demonstrated a 100 % efficacy rate with this eRHF.

Despite the doubts raised in an article [20] regarding the nutritional adequacy of pRHF, growth was shown to be adequate in this trial as well as in other studies carried out using a pRHF in infants with CMPA [2, 14]. A normalization of the weight-for-age, weight-for-length, and BMI was observed in those infants presenting on average a faltering growth at inclusion (mean weight-for-age, weight-for-length, and BMI *z*-scores of −0.7).

Rice has also recently been criticized regarding its possible arsenic content. However, this concerned mainly organic brown rice syrup and was not related to infant formula based on extensively hydrolyzed rice protein. There is no EU regulation fixing limits to arsenic in infant formulas. In particular, this study formula contains less than 10 μg/L of arsenic, which is the maximum content allowed in drinking water according to EU regulation [5] (drinking water being the only food in which arsenic content is regulated) and infant formulas are reconstituted with approximately 86 to 87 % of water. In this study, the rice-protein based formula was generally well tolerated, with parents of three patients ending the study formula with the argument that their infant did not like the taste of the formula. In general, one of the main complaints of parents is that infants refuse hydrolyzed formulas because of their unpleasant bitter taste. A double-blind study evaluating the palatability of different formulas used to feed infants with CMPA showed that soy and rice-based formulas had better taste scores than CMP hydrolyzed formulas [18]. Good acceptability because of its pleasant odor, taste, and flavor was confirmed for rice formulas in healthy infants [9, 19]. In this study, while acceptance was not unanimous, 81.2 % of the parents reported that infants liked the taste of the formula.

Moreover, in this study, a normalization of the stool's consistency was observed as of the first month of feeding with the thickened eRHF whereas frequent and/or liquid stools are often associated with feeding children with hydrolyzed protein formula [17] (before the challenge, only 13.2 % of the infants had normal stools; Table 7).

Hydrolyzed formulas are very liquid. Although they have been reported in literature to not increase regurgitation [11], there are conflicting data suggest they increase the frequency of regurgitation by 18 % [16]. In this study, regurgitation decreased significantly during the first month of feeding with the thickened eRHF. The same thickening complex was added to en extensive hydrolysed CMP (casein) based formula and had similar beneficial effects on normalization of stool consistency as well as a decrease of regurgitation in infants with CMPA [21]. Besides efficacy, nutritional value and acceptability, the cost of infant formula is also of importance as affordability may promote compliance. While cost of infant formulas differ from country to another, overall it can be said that the cost of eRHF is significantly less than one of an extensive cow milk hydrolysate.

In conclusion, the study formula was tolerated by more than 90 % of infants with a demonstrated CMPA, with a 95 % CI. The formula also ensured a proper growth of those infants. The excellent acceptability of the eRHF tested makes this kind of formula an interesting option in the treatment of CMPA in terms of efficacy, nutritional value, affordability, acceptance, and tolerance. However, more studies with a greater number of subjects targeting safety, anthropometric growth and development with these new formulas are needed.

Table 7 Evolution of stool consistency according to the Bristol stool scale

	Before challenge	At inclusion	1 month	3 months	6 months
Type 1 or 2: separate hard lumps, like nuts (hard to pass), or sausage-shaped, but lumpy	11 (28.9 %)	9 (23.7 %)	3 (7.9 %)	2 (5.6 %)	0 (0 %)
Type 3 or 4: like a sausage or snake smooth and soft	5 (13.2 %)	2 (5.3 %)	20 (52.6 %)	21 (58.3 %)	28 (77.8 %)
Type 5: soft blobs with clear cut edges (passes easily)	9 (23.7 %)	4 (10.5 %)	4 (10.5 %)	8 (22.2 %)	5 (13.9 %)
Type 6: fluffy pieces with ragged edges, a mushy stool	9 (23.7 %)	11 (28.9 %)	10 (26.3 %)	5 (13.9 %)	2 (5.6 %)
Type 7: watery, no solid pieces, or entirely liquid	4 (10.5 %)	12 (31.6 %)	1 (2.6 %)	0 (0 %)	1 (2.8 %)

Funding United Pharmaceuticals provided free formula and financial support for the research.

Conflict of interest Y. Vandenplas is a consultant for United Pharmaceuticals and Biocodex. The other authors did not report a COI.

References

1. Agostoni C, Axelsson I, Goulet O, ESPGHAN committee of Nutrition (2006) Soy protein infant formulae and follow on formulae a commentary by the ESPGHAN. J Pediatr Gastroenterol Nutr 42(4):352–361
2. Agostoni C, Fiocchi A, Riva E, Terracciano L, Sarratud T, Martelli A, Lodi F, D'Auria E, Zuccotti G, Giovannini M (2007) Growth of infants with IgE-mediated cow's milk allergy fed different formulas in the complementary feeding period. Pediatr Allergy Immunol 18(7):599–606
3. American Academy of Pediatrics (2000) Committee on Nutrition. Hypoallergenic infant formula. Pediatrics 106(2Pt1):346–349
4. Bhatia J, Greer F, American Academy of Pediatrics Committee on Nutrition (2008) Use of soy protein-based formulas in infant feeding. Pediatrics 121(5):1062–1068. doi: 10.1542/peds.2008-0564
5. Commission Directive 98/83/EC on the quality of water intended for human consumption
6. Committee on Nutrition of the French Pediatrics Society (2000) Use of infant formulas with reduced antigenic content. Arch Pediatr 7(3): 302–306
7. D'Auria E, Sala M, Lodi F, Radaelli G, Riva E, Giovannini M (2003) Nutritional value of a rice-hydrolysate formula in infants with cows' milk protein allergy: a randomized pilot study. J Int Med Res 31(3): 215–222
8. Dreborg S, Frew A (1993) EAACI Position paper: allergen standardization and skin tests. Allergy 48(suppl 14):48–82
9. Fiocchi A, Restani P, Bernardini R, Lucarelli S, Lombardi G, Magazzù G, Marseglia GL, Pittschieler K, Tripodi S, Troncone R, Ranzini C (2006) A hydrolyzed rice-based formula is tolerated by children with cow's milk allergy: a multi-centre study. Clin Exp Allergy 36(3):311–316
10. Fiocchi A, Travaini M, D'Auria E, Banderali G, Bernardo L, Riva E (2003) Tolerance to a rice hydrolysate formula in children allergic to cow's milk and soy. Clin Exp Allergy 33(11):1576–1580
11. Hauser B, Keymolen K, Blecker U, Suys B, Vandenplas Y (1993) A comparative evaluation of whey hydrolysate and whey-predominant formulas. How well do infants accept and tolerate them? Clin Pediatr (Phila) 32(7):433–437
12. Kemp AS, Hill DJ, Allen KJ, Anderson K, Davidson GP, Day AS, Heine RG, Peake JE, Prescott SL, Shugg AW, Sinn JK, Australian consensus panel (2008) Guidelines for the use of infant formulas to treat. CMP allergy: an Australian consensus panel opinion. Med J Austr 188(2):109–112
13. Koletzko S, Niggemann B, Arato A, Dias JA, Heuschkel R, Husby S, Mearin ML, Papadopoulou A, Ruemmele FM, Staiano A, Schäppi MG, Vandenplas Y, European Society of Pediatric Gastroenterology, Hepatology, and Nutrition (2012) Diagnostic approach and management of cow's-milk protein allergy in infants and children: ESPGHAN GI Committee practical guidelines. J Pediatr Gastroenterol Nutr 55(2):221–229. doi: 10.1097/MPG.0b013e31825c9482
14. Lasekan JB, Koo WK, Walters J, Neylan M, Luebbers S (2006) Growth, tolerance and biochemical measures in healthy infants fed a partially hydrolyzed rice protein-based formula: a randomised, blinded prospective trial. J Am Coll Nutr 25(1): 12–19
15. Lewis SJ, Heaton KW (1997) Stool form scale as a useful guide to intestinal transit time. Scand J Gastroenterol 32(9):920–924
16. Medjad-guillou N, Henocq A, Arnaud-Battandier F (1992) Does the hydrolysis of proteins change the acceptability and the digestive tolerance of milk for infants? The results of a comparative and randomized prospective study. Ann Pediatr 39(3):202–206
17. Niggemann B, von Berg A, Bollrath C, Berdel D, Schauer U, Rieger C, Haschke-Becher E, Wahn U (2008) Safety and efficacy of a new extensively hydrolyzed formula for infants with cow's milk protein allergy. Pediatr Allergy Immunol 19(4):348–354. doi: 10.1111/j.1399-3038.2007.00653
18. Pedrosa M, Pascual CY, Larco JI, Esteban MM (2006) Palatability of hydrolysates and other substitution formulas for cow's milk-allergic children: a comparative study of taste, smell, and texture evaluated by healthy volunteers. J Investig Allergol Clin Immunol 16(6):351–356
19. Reche M, Pascual C, Fiandor A, Polanco I, Rivero-Urgell M, Chifre R, Johnston S, Martín-Esteban M (2010) The effect of a partially hydrolyzed formula based on rice protein in the treatment of infants with cow's milk protein allergy. Pediatr Allergy Immunol 21(4Pt1): 577–585. doi: 10.1111/j.1399-3038.2010.00991
20. Savino F, Castagno E, Monti G, Serraino P, Peltran A, Oggero R, Fanaro S, Vigi V, Silvestro L (2005) Z-score of weight for age of infants with atopic dermatitis and cow's milk allergy fed with a rice-hydrolysate formula during the first two years of life. Acta Paediatr Suppl 94(449):115–119
21. Vandenplas Y, De Greef E, ALLAR study group (2014) Extensive protein hydrolysate formula effectively reduces regurgitation in infants with positive and negative challenge tests for cow's milk allergy. Acta Paediatr (in press)
22. Vandenplas Y, Hachimi-Idrissi S, Casteels A, Mahler T, Loeb H (1994) A clinical trial with an anti-regurgitation formula. Eur J Pediatr 153(6):419–423
23. Vandenplas Y, Koletzko S, Isolauri E, Hill D, Oranje AP, Brueton M, Staiano A, Dupont C (2007) Guidelines for the diagnosis and management of cow's milk protein allergy in infants. Arch Dis Child 92(10):902–908
24. Vandenplas Y, Steenhout P, Grathwohl D, Althera Study Group (2014) A pilot study on the application of a symptom-based score for the diagnosis of cow's milk protein allergy. SAGE Open Med 2: 2050312114523423
25. Vandenplas Y, Steenhout P, Planoudis Y, Grathwohl D, Althera Study Group (2013) A double-blind randomized trial comparing two extensively hydrolyzed formulaswith probiotics for the treatment of cow's milk protein allergy. Acta Paediatr 102(10):990–998. doi: 10.1111/apa.12349
26. WHO Child Growth Standards The WHO Multicentre Growth Reference Study (MGRS)
27. Zeiger RS, Sampson HA, Bock SA, Burks AW Jr, Harden K, Noone S, Martin D, Leung S, Wilson G (1999) Soy allergy in infants and children with IgE-associated cow's milk allergy. J Pediatr 134(5): 614–622

pISSN: 2234-8646 eISSN: 2234-8840
http://dx.doi.org/10.5223/pghn.2016.19.2.104
Pediatr Gastroenterol Hepatol Nutr 2016 June 19(2):104-109

Original Article

PGHN

Efficacy and Tolerance of a New Anti-Regurgitation Formula

Christophe Dupont, Yvan Vandenplas*, and SONAR Study Group†

Department of Paediatric Gastroenterology Hepatology and Nutrition, Hôpital Necker Enfants Malades, Paris, France,
**Department of Paediatrics, UZ Brussel, Vrije Universiteit Brussel, Brussels, Belgium*

Purpose: Regurgitation is a common physiological phenomenon in infants. The aim of the present study was to evaluate the efficacy of a new anti-regurgitation (AR) formula (Novalac), thickened with an innovative complex including fibres, on the daily number of regurgitations and to assess its impact on stool consistency and frequency.
Methods: Infants younger than five months, presenting at least 5 regurgitations per day were recruited in this trial. The efficacy of the new formula on regurgitation (daily number and Vandenplas score), stool frequency and consistency were assessed at day 14 and 90. Growth data were recorded at each study visit.
Results: Ninety babies (mean age 9.6±5.8 weeks) were included in the full analysis data set. The mean number of regurgitation episodes at inclusion was 7.3±3.4. In all infants, regurgitations improved after 2 weeks. The daily number of regurgitations decreased significantly (−6.3±3.3, $p<0.001$) including in those previously fed a thickened formula (−6.2±3.0, $p<0.001$). There was no significant change in stool consistency at day 14. After 3 months, 97.5% of infants had formed or soft stools. Growth was appropriate with a slight increase of weight-for-age z-score (from −0.5±1.0 to −0.1±0.9) and no change of weight-for length z-score (−0.1±1.1 to −0.1±−1.1).
Conclusion: The new AR formula thickened with an innovative complex is very effective in reducing the daily number of regurgitations without having a negative impact on stools consistency.

Key Words: Gastroesophageal reflux, Infant formula

INTRODUCTION

Regurgitation is a common physiologic phenomenon in infants with a peak prevalence at three to four months of age and occurs during this period in 50% to 70% of infants [1-3]. According to a thorough review of the literature, about 25% of the parents seek medical help because of infant regurgitation [4].

According to the NASPGHAN-ESPGHAN guidelines on the management of reflux, the recommended course of action in case of regurgitation is

Received : January 20, 2016, Revised : February 7, 2016, Accepted : February 29, 2016

Corresponding author: Yvan Vandenplas, Department of Paediatrics, UZ Brussel, Laarbeeklaan 101, 1090 Brussels, Belgium. Tel: +32-2-4775780, Fax: +32-2-4775784, E-mail: yvan.vandenplas@uzbrussel.be
†SONAR Study Group: Amrein J, Attal P, Balduck N, Batard C, Bouttens-Marchand S, Bradatan E, Defontaine E, Goulamhoussen S, Lannoo P, Orbegozo J, Robberecht MN, Rondeleux E, Tran M, Verboven M, Vulser F, Zouari M.

"parental education, reassurance and anticipatory guidance, and in formula fed infants, a thickened formula (or anti-regurgitation [AR] formula if available) to reduce the frequency of overt regurgitation and vomiting" [1].

Currently, several pre-thickened AR infant formulas using different types of thickening agents are available for the management of regurgitation [5].

The clinical efficacy on regurgitation of AR formulas thickened with locust bean has been demonstrated in several clinical trials [6-8]. However, infants fed with such formulas may have softer and more frequent stools than those fed with starch-thickened formulas [9].

The aim of this trial was to evaluate the efficacy of a formula containing a new thickening agent on regurgitation and to assess its digestive tolerance.

MATERIALS AND METHODS

This prospective international open pilot multicentre clinical trial was conducted to evaluate the efficacy of a new formula (Novalac, Paris, France) on regurgitation and defecation. The formula contains an innovative thickening complex made of fibres including specially selected pectin. Fully formula fed infants less than 5 months old with at least 5 episodes of regurgitation per day and who had not yet started solids were eligible for inclusion.

After informed consent was obtained, the parents were given the appropriate number of tins of formula needed to cover their infant's needs for two weeks (±3 days). Parents were asked to report information on regurgitation and stools consistency in a diary during two 3-day periods: just after inclusion and just before day 14, when the infant was re-examined by the investigator.

After this visit, if an AR formula was still indicated and if parents wished to continue with the same formula, the investigator could provide more tins of the new AR formula for up to three months after inclusion or until the child reached the age of six months whichever occurred last. A third visit was planned 90 days after inclusion to further assess growth.

The efficacy of this new AR formula on regurgitation was assessed through the daily number of regurgitations and the estimated regurgitated volume (adapted Vandenplas score [8]; Table 1). The daily number of stools and stools consistency according to the Bristol scale were also recorded [10]. For analysis, four categories were defined regarding stool consistency: hard (Bristol scale 1, 2), formed (Bristol scale 3, 4), soft/mushy (Bristol scale 5, 6) and loose/ watery (Bristol scale 7).

The growth parameters (weight, length, and head circumference) were collected at baseline, day 14, and day 90. Anthropometric parameters, including the body mass index (BMI) were expressed as z-score according to the World Health Organization Child Growth Standards [11].

The safety population was defined as all infants who consumed at least once the product. The full analysis set population is composed of all infants having an evaluation of the main outcome, i.e., the daily number of episodes of regurgitation at day 14. A posteriori, two subgroups were analysed according to whether the infants had or had not been fed with a thickened formula prior to inclusion.

The SAS software for Windows (version 9.2; SAS Institute, Cary, NC, USA) was used to perform the statistical procedures. Statistical analyses were performed in accordance with ICH-E9 guideline.

The study was approved by independent ethics committees: CPP Ile-de-France III, Paris, France,

Table 1. Adapted Vandenplas Score on Regurgitation

Score	Regurgitation
0	0-2 episodes/day
1	≥3-≤5 of small volume
2	>5 episodes of >1 coffee spoon
3	>5 episodes of ±half of the feedings in <half of the feedings
4	Continuous regurgitations of small volumes >30 minutes 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

and VUB Ethics Committee, Brussels, Belgium. It was registered at ClinicalTrials.gov under the identifier NCT02425423. Parents or others legally responsible for the infants provided written consent.

RESULTS

Sixteen paediatricians (five in Belgium and eleven in France) included 100 infants. After 14 days of treatment, 90 infants (mean age at inclusion 9.6±5.8 weeks) were included in the full analysis data set (Fig. 1). The reason for drop-out between inclusion and day 14 were identified as follows; liquid stools (n=4), lost to follow up (n=1), withdrawal by parents for unknown reason (n=4), and one infant that was breastfed (n=1).

About half of the infants (48/90; 53.3%) had been fed a thickened formula before inclusion (pre-thickened or standard formula+thickening agent), but had still at least 5 episodes of regurgitation per day.

The mean number of regurgitation episodes was 7.3±3.4 at inclusion. After 14 days, the mean number of regurgitation had decreased significantly with −6.3±3.3 ($p<0.001$) (Table 2 and Fig. 2). In the subgroup of infants who were fed a thickened formula at inclusion, regurgitation also significantly decreased from 7.1±2.9 to 0.9± −1.1 after 14 days. There was no difference between both sub-populations regarding the effect on regurgitation. After two weeks, regurgitations decreased in all infants and 85.6% had no more than two regurgitations per day. Parents reported a significant decrease of the daily number of regurgitation already after three days: −6.1±3.7, $p<0.001$ with improvement in 94.5% (52/55). The regurgitation score had also decreased significantly after 14 days (Table 2).

There was no significant effect of the formula on

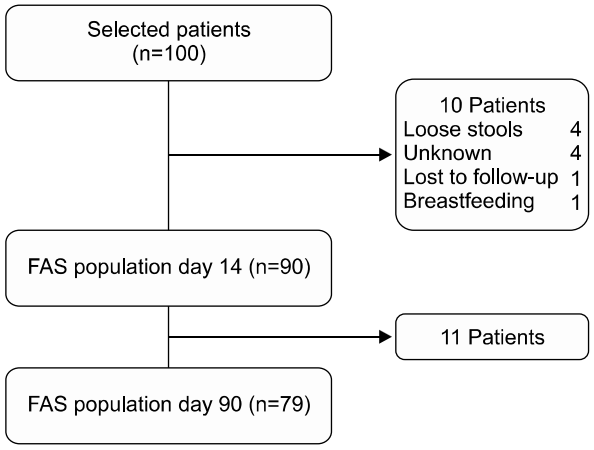

Fig. 1. Flow diagram.

Table 2. Evolution of the Daily Mean Number of Regurgitations and Vandenplas Score from Inclusion to 14 Days

Daily number of regurgitations	Inclusion	7.3+3.4
	Day 3	1.2+1.5
	Day 14	1.1+1.3
	p (day 3-baseline)	<0.001*
	p (day 14-baseline)	<0.001*
Vandenplas score	Inclusion	1.8+0.9
	Day 14	0.2+0.6
	p (day 14-baseline)	<0.001*

Values are presented as mean±standard deviation.
*By Wilcoxon test.

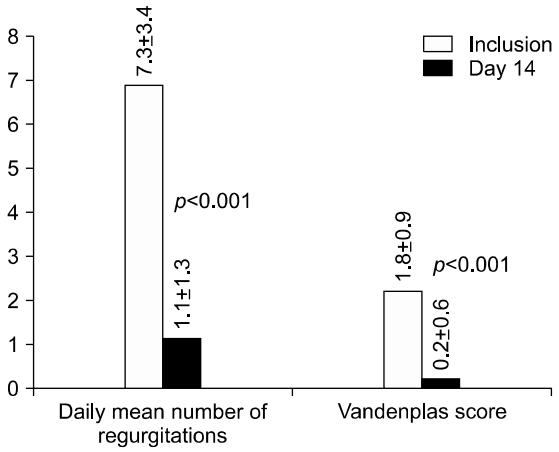

Fig. 2. Evolution of the daily mean number of regurgitations and Vandenplas score from inclusion to 14 days.

Table 3. Detail of the Stool Consistency at Inclusion and after 14 Days

	Inclusion	Day 14
Hard (Bristol scale 1, 2)	14.4%	3.3%
Formed (Bristol scale 3, 4)	36.7%	51.1%
Soft (Bristol scale 5, 6)	42.2%	36.7%
Loose (Bristol scale 7)	6.7%	8.9%

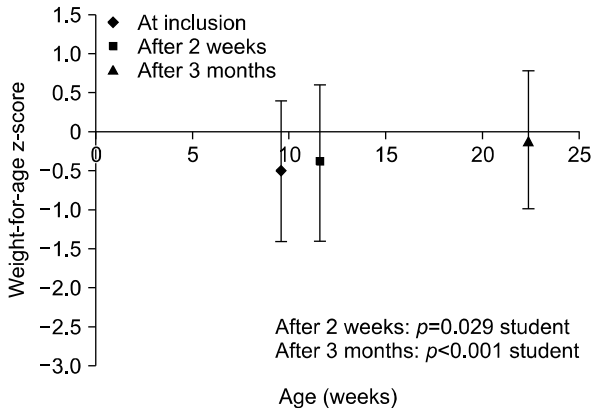

Fig. 3. Evolution of the weight for age z-score.

stool consistency with 78.9% of infants having soft/mushy or formed stools at inclusion and 87.8% after 14 days (Table 3). The percentage of formed stools increased after 14 days from 36.7% to 51.1% (p=0.053).

Growth was within normal range, with an increase of the weight for age z-score

(-0.5 ± 1.0 at inclusion and -0.1 ± 0.9 after 3 months, $p<0.001$), length for age z-score

(-0.5 ± 1.2 at inclusion and -0.0 ± 1.2 after 3 months, $p<0.001$) and BMI for age z-score

(-0.3 ± 1.0 at inclusion, and -0.2 ± 1.1 after 3 months, not significant) (Fig. 3).

After 14 days, 66 parents answered the question regarding general satisfaction and 61 (92.4%) were in overall satisfied or very satisfied. Seventy-one parents answered the question about efficacy on regurgitation, and 67 (94.3%) said to be satisfied or very satisfied. Regarding the digestive comfort and general well-being of their child, as of day 3, respectively 76.1% (54/72) and 80.3% (57/71) of the parents reported to be satisfied or very satisfied. After 2 weeks, 87.8% (79/90) and 94.4% (85/90) of the paediatricians reported to be satisfied or very satisfied regarding respectively the digestive comfort and general well-being.

DISCUSSION

Thickening of infant formula is considered as an option to reduce regurgitation [1]. However, the efficacy and risk for adverse effects of all thickeners is not equal. For example, according to the meta-analysis by Horvath et al. [12], only corn starch thickened formulas have an impact on reflux index.

This pilot study has shown that the AR formula with the new thickening agent is effective in the treatment of regurgitation. As the study was not controlled and regurgitations do naturally decrease over time, the primary endpoint was evaluated only two weeks after inclusion. Actually, parents reported a significant decrease of the number of episodes of regurgitation as of day three, what minimalizes the effect of natural evolution. Moreover, the decrease in regurgitation was similar in infants already under AR formula but still regurgitating more than five times a day at inclusion. A major weakness of this open observational study is the lack of a control group. However, the short observation period of only two weeks and the fact that the reduction in regurgitation was similar in the group that had been treated before with an AR formula without success and new untreated infants makes it unlikely that the reduction in episodes of regurgitation is due to the natural evolution. A placebo effect of a formula on infant regurgitation seems also unlikely. Another shortcoming is that no information has been acquired on irritability or time needed for feeding. As the main objective of this study was to evaluate the impact on regurgitation and on stools, for which the record by the parents was very important, it was decided to keep the parents' diary as simple as possible to ensure a better quality of the data recorded. Therefore parents were not required to report precise information such as the duration of each feeding nor on the daily crying time. Irritability was evaluated through two questions about digestive comfort and global well-being.

This formula is thickened with a complex of fibers containing pectins. Pectins are safe and naturally present in fruits such as apple, frequently eaten by young infants. According to European Union regulation, pectins can be used in baby foods, cereals or food for special medical purposes for newborns.

Moreover, special formulae containing pectins have been used in recent clinical trials on thickened formulae specially designed for the treatment of cow's milk allergy; they all show an adapted growth of the infants fed such formula for a duration up to 6 months [13,14].

Depending on the nature of the thickening agent, the impact on the stool frequency and consistency can differ. For example with a formula thickened with locust bean, Iacono et al. [9] reported diarrhea to occur in 16.7% (14/84) of infants. Although the new formula is thickened with fibres and even though four children have left the study before day 14 due to loose stools, this formula seems to have a regulatory effect on stool composition. Indeed, 87.8% of the infants had soft or formed stools after 14 days, and 97.5% after three months.

It is important to assure optimal nutritional intake, especially in regurgitating infants. This new formula allowed a satisfactory growth over the three months of observation. This study shows that a formula containing a new thickening agent gives promising results not only in term of regurgitation but also without causing diarrhea or constipation.

ACKNOWLEDGEMENTS

Christophe Dupont received fees from Nestlé, United Pharmaceuticals, Danone, Sodilac; Yvan Vandenplas is consultant for Aspen, Ausnutria, Biocodex, Nestle Nutrition Institute and United Pharmaceuticals.

United Pharmaceuticals provided the tested formula and financial support.

REFERENCES

1. Vandenplas Y, Rudolph CD, Di Lorenzo C, Hassall E, Liptak G, Mazur L, et al. Pediatric gastroesophageal reflux clinical practice guidelines: joint recommendations of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN). J Pediatr Gastroenterol Nutr 2009;49:498-547.
2. Martin AJ, Pratt N, Kennedy JD, Ryan P, Ruffin RE, Miles H, et al. Natural history and familial relationships of infant spilling to 9 years of age. Pediatrics 2002;109:1061-7.
3. Nelson SP, Chen EH, Syniar GM, Christoffel KK. Prevalence of symptoms of gastroesophageal reflux during infancy. A pediatric practice-based survey. Pediatric Practice Research Group. Arch Pediatr Adolesc Med 1997;151:569-72.
4. Vandenplas Y, Abkari A, Bellaiche M, Benninga M, Chouraqui JP, Çokura F, et al. Prevalence and health outcomes of functional gastrointestinal symptoms in infants from birth to 12 months of age. J Pediatr Gastroenterol Nutr 2015;61:531-7.
5. Vandenplas Y, Lifshitz JZ, Orenstein S, Lifschitz CH, Shepherd RW, Casaubón PR, et al. Nutritional management of regurgitation in infants. J Am Coll Nutr 1998;17:308-16.
6. Vandenplas Y, Gutierrez-Castrellon P, Velasco-Benitez C, Palacios J, Jaen D, Ribeiro H, et al. Practical algorithms for managing common gastrointestinal symptoms in infants. Nutrition 2013;29:184-94.
7. Wenzl TG, Schneider S, Scheele F, Silny J, Heimann G, Skopnik H. Effects of thickened feeding on gastroesophageal reflux in infants: a placebo-controlled crossover study using intraluminal impedance. Pediatrics 2003;111:e355-9.
8. Vandenplas Y, Hachimi-Idrissi S, Casteels A, Mahler T, Loeb H. A clinical trial with an "anti-regurgitation" formula. Eur J Pediatr 1994;153:419-23.
9. Iacono G, Vetrano S, Cataldo F, Ziino O, Russo A, Lorello D, et al. Clinical trial with thickened feeding for treatment of regurgitation in infants. Dig Liver Dis 2002;34:532-3.
10. Lewis SJ, Heaton KW. Stool form scale as a useful guide to intestinal transit time. Scand J Gastroenterol 1997;32:920-4.
11. World Health Organization.WHO Multicentre Growth Reference Study Group: WHO Child Growth Standards: length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: methods and development. Geneva: World Health Organization; 2006. Available from: http://www.who.int/childgrowth/standards/en/.
12. Horvath A, Dziechciarz P, Szajewska H. The effect of thickened-feed interventions on gastroesophageal reflux in infants: systematic review and meta-analysis of randomized, controlled trials. Pediatrics 2008;122:e1268-77.
13. Vandenplas Y, De Greef E, Hauser B; Paradice Study Group. Safety and tolerance of a new extensively hydro-

lyzed rice protein-based formula in the management of infants with cow's milk protein allergy. Eur J Pediatr 2014;173:1209-16.
14. Dupont C, Kalach N, Soulaines P, Bradatan E, Lachaux A, Payot F, 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;61: 456-63.

Safety of a New Amino Acid Formula in Infants Allergic to Cow's Milk and Intolerant to Hydrolysates

*Christophe Dupont, †Nicolas Kalach, *Pascale Soulaines, ‡Elena Bradatan, §Alain Lachaux, §François Payot, ||Frédéric De Blay, ¶Lydie Guénard-Bilbault, #Riad Hatahet, **Sandra Mulier, ††Nathalie Kapel, ††Anne-Judith Waligora-Dupriet, and ††Marie-José Butel

See ''Primum Non Nocere'' by Agostoni on page 381.

ABSTRACT

Objectives: Amino acid–based formulas (AAFs) are recommended for children with cow's-milk allergy (CMA) failing to respond to extensively hydrolysed formulas (eHFs). We evaluated the effects of a new thickened AAF (TAAF, Novalac), containing a pectin-based thickener, and a reference AAF (RAAF, Neocate) on allergy symptoms and safety, through blood biochemistry analysis and growth.

Methods: Infants (ages < 18 months) with CMA symptoms failing to respond to eHFs were randomised in a double-blind manner to receive TAAF or RAAF for 3 months. All of the infants were then fed TAAF for 3 additional months. Paediatric visits occurred at 1, 3, and 6 months. Blood samples were collected at inclusion and 3 months.

Results: Results at 1 month were previously described. The 75 infants with proven CMA and eHF intolerance tolerated their allocated formula. At 3 months, the dominant allergic symptom had disappeared in 76.2% of the infants with TAAF and in 51.5% of the infants with RAAF ($P = 0.026$). The Scoring Atopic Dermatitis Index significantly improved more with TAAF than with RAAF (-27.3 ± 2.3 vs -20.8 ± 2.2, $P = 0.048$). Of the infants, 92.9% had normal stools (soft or formed consistency) with TAAF vs 75.8% with RAAF ($P = 0.051$). More infants in TAAF group had better quality of nighttime sleep ($P = 0.036$) and low frequency of irritability signs ($P < 0.001$). With both formulas, all of the biochemical parameters were within normal ranges. There were no differences between the 2 groups in any of the anthropometric z scores.

Conclusions: The new TAAF was tolerated by all of the infants with CMA and intolerance to eHFs. Anthropometric and clinical data showed that both formulas were safe.

Key Words: amino acid formula, growth, infants with cow's-milk allergy and intolerance to extensively hydrolysed formulas, safety, Scoring Atopic Dermatitis Index

(*JPGN* 2015;61: 456–463)

What Is Known

- Guidelines recommend amino acid–based formulas for children with cow's-milk allergy symptoms persisting on extensively hydrolysed formulas.
- No randomised controlled study was ever conducted in this specific population.
- Data on the impact of amino acid–based formulas on daily family life are scarce.

What Is New

- Tolerance and safety of the thickened amino acid–based formula were shown in this specific population.
- Thickened amino acid–based formula significantly reduced more Scoring Atopic Dermatitis Index and the number of infants with skin dryness than the reference amino acid–based formula.
- Thickened amino acid–based formula provided additional comfort through improvement of stool consistency, less irritability signs, and better nighttime sleep quality in more infants.

Cow's-milk allergy (CMA) manifests by clinical symptoms related to the abnormal immune response of the host after ingestion of milk proteins and affects 2% to 7% of children (1).

Received December 16, 2014; accepted March 24, 2015.

From the *Pediatric Gastroenterology, Hepatology and Nutrition Department, Necker Children's Hospital, Paris, France, the †Saint Antoine Clinics of Pediatrics, Saint Vincent de Paul Hospital, Groupement des Hôpitaux de l'Institut Catholique, Lille, France, the ‡Department of Pediatrics, Regional Hospital, Namur, Belgium, the §Gastroenterology, Hepatology and Nutrition Unit, University and Pediatric Hospital of Lyon, Lyon, France, the ||Pulmonology and Allergology Department, Regional University Hospital, Strasbourg, France, ¶private allergy medical practice, Illkirch-Graffenstaden, #private allergy medical practice, Forbach, France, the **Allergology Department, Queen Fabiola Children's University Hospital, Brussels, Belgium, and the ††Intestinal Ecosystem, Probiotics, Antibiotics (EA4065), Sorbonne Paris Cité, DHU Risks in Pregnancy, Paris Descartes University, Paris, France.

Address correspondence and reprint requests to Christophe Dupont, Pediatric Gastroenterology, Hepatology, and Nutrition Department, Necker Children's Hospital, 149, Rue de Sèvres, 75015 Paris, France (e-mail: christophe.dupont@nck.aphp.fr).

Supplemental digital content is available for this article. Direct URL citations appear in the printed text, and links to the digital files are provided in the HTML text of this article on the journal's Web site (*www.jpgn.org*).

The authors (or their institutions) received honoraria from United Pharmaceuticals, Paris, France, for their work in this study. This study was funded by United Pharmaceuticals, Paris, France. C.D. reports personal fees and nonfinancial support from Danone and personal fees from Nestlé, Sodilac, and Wyeth Nutrition; F.P. reports nonfinancial support from Nutricia and Mead Johnson Nutrition; A.L. reports personal fees from Mead Johnson Nutrition.

www.clinicaltrials.gov registration number: NCT01940068.

DOI: 10.1097/MPG.0000000000000803

Guidelines for the dietary management of infants with diagnosed CMA recommend the substitution with extensively hydrolysed casein or whey protein formulas for cow's milk (2–4). Some studies have highlighted the allergenicity of hydrolysates in highly allergic children, caused by residual immunologically active protein (5–7). For these patients, dietary treatment with an amino acid–based formula (AAF) is required (2–4). Several studies assessed the impact of AAFs on the growth of healthy infants (8,9), but few open noncontrolled studies reported growth data in infants with the dual condition of CMA and intolerance to extensively hydrolysed formulas (eHFs), that is, allergy symptoms persisting with eHFs (6,10). Regarding hypoallergenicity and clinical efficacy, published results only refer to retrospective observational studies including a limited number of patients (6,7,10,11). Moreover, controlled trials showing efficacy, tolerance, and safety of AAFs were carried out either in children with proven CMA but no intolerance to eHFs (8,9,12–14) or in children only part of whom also had documented intolerance to eHFs (11).

Results at 1 month of a double-blind, placebo-controlled, randomised study comparing a thickened AAF (TAAF) to a reference AAF (RAAF) on hypoallergenicity/tolerance, efficacy, and safety in 75 infants with CMA failing to respond to eHFs were previously published (15). Here, the results obtained in the same population at 3 and 6 months are reported.

In addition, because of the possible relation between allergic diseases and gut microbiota (16) and the presence of fibre in the TAAF that may affect colonic microbiota composition (17), the main changes in faecal microbiota after 3 months of AAF feeding were investigated. Noninvasive faecal markers have a reliable place in gastroenterology by evaluating the intestinal inflammatory responses. Hence, faecal eosinophil-derived neurotoxin (EDN) as intestinal marker of eosinophilic infiltration was also assessed (18).

METHODS

Study Design

The methodology of the study was detailed in 2014 (15). Briefly, infants (ages < 18 months) with allergic symptoms persistent under eHF feeding with 1 or several commercial eHFs available in France or Belgium for ≥2 weeks were selected. CMA diagnosis had to be proven by double-blind placebo-controlled food challenge (DBPCFC), positive skin prick test (SPT) (wheal diameter >6 mm), specific immunoglobulin E ([s]IgE) to milk >5 kU/L, or a combination of both positive cutaneous tests and (s)IgE (19,20). Infants were randomised to the TAAF group (Novalac; United Pharmaceuticals, Paris, France) or to the RAAF group (Neocate; Nutricia, Erlangen, Germany). The TAAF had a similar nitrogen content (1.9 g/100 mL) and differed mostly by the presence of a patented thickening mixture including fibres (0.5 g/100 mL), mainly composed of pectin, which thickens at gastric pH. Infants were fed study formulas in a double-blind manner for 3 months. Then the TAAF was used during 3 supplementary months for both groups to collect anthropometric data. Following analysis of the primary outcome, that is, tolerance/hypoallergenicity of the TAAF at 1 month (15), paediatric visits were programmed 3 and 6 months after dietary treatment initiation.

The objectives of the present analysis were the evaluation at 3 and 6 months of the tolerance/hypoallergenicity of the TAAF and the evolution of symptoms characteristic of CMA. Other secondary outcomes included general symptoms associated with CMA and having an impact on daily family life, the safety through growth (evaluated in accordance with World Health Organization [WHO] growth curves) and biological (including plasma amino acids) parameters, as well as intestinal microbiota and faecal EDN.

At inclusion, the dominant allergic symptom was identified for each infant. Anthropometric measurements and all of the symptoms of CMA, including skin, respiratory, and gastrointestinal tract manifestations were recorded by paediatricians at inclusion and each follow-up visit. Severity of eczema, stool consistency, and regurgitations were assessed by Scoring Atopic Dermatitis (SCORAD) Index (21), Bekkali scale (22) and Vandenplas score (23), respectively. General symptoms associated with CMA were also registered (15): sleep quality, daily crying and sleeping time, irritability signs, and crying frequency. Adverse events were recorded. Parents' satisfaction and infants' acceptability of the product (ie, parents' perception of the appreciation of the formula taste by their infant) as well as presence of gas and intestinal bloating were assessed through parents' diaries.

Blood samples were obtained by venipuncture from a subset of infants at inclusion and at 3 months; usual laboratory parameters were analysed at entry and at 3 months, and amino acid plasma concentrations were determined at 3 months.

Faecal Analyses

For each child, a sample of faeces was collected from the diapers within 3 hours after defecation and then stored at −80°C until assayed. Frozen stool samples were thawed at room temperature immediately before analysis. Faecal microbiota was assessed using quantitative real-time polymerase chain reaction (qPCR) as described in 2006 (24). Extraction of total DNA from faecal content was performed using guanidium isothiocyanate and the mechanical bead-beating method. TaqMan (Applied Biosystems, Saint-Aubin, France) qPCR was used to quantify total bacteria populations and the dominant (>1% of faecal bacteria population) bacterial groups, and genera: *Clostridium* cluster IV (*C leptum* group), *Bacteroides/Prevotella* group, and *Bifidobacterium*. qPCR using SYBR-Green (Applied Biosystems) was performed to quantify *Lactobacillus/Leuconostoc/Pediococcus* group, *Clostridium* cluster XIVa (*C coccoides* group), *Clostridium* cluster XI, *Clostridium* cluster I/II, *Staphylococcus*, *Enterococcus*, and *Escherichia coli*. Primers and probes are available upon request. Standard curves were obtained from serial dilutions of a known concentration of plasmid DNA containing a *16S rRNA* gene insert from each species or group. The coefficients of correlation between $\log_{10}$ CFU and *rRNA* gene copy numbers for each species and group were obtained from ribosomal RNA operon copy number database (25), enabling calculation of the number of colony-forming unit per gram of faeces. The detection limits depended on the bacterial groups and ranged between 10^4 and 10^6 CFU/g.

The concentration of faecal EDN was assayed in duplicate using a ''sandwich''-type enzyme ELISA method which uses a polyclonal antibody system (Immundiagnostik, Bensheim, Germany) according to the manufacturer's instructions. The quantitation limit for EDN was 120 ng/g of stools.

Statistical Analysis

The number of subjects to be included was calculated based on the requirement that a hypoallergenic formula must be tolerated by ≥90% of infants with an overt CMA (95% confidence interval [CI]). The number of subjects needed was 29 per group (8). Considering the study design, allowing the CMA confirmation within 2 months after inclusion, 15% of dropouts and inappropriate selections were anticipated, which led to include 35 infants per group. The tolerance/hypoallergenicity was assessed in infants with confirmed CMA and intolerance to eHFs, defined as the tolerance/hypoallergenicity population. The other secondary endpoints were analysed on the full analysis set (FAS) population, defined as infants

from tolerance/hypoallergenicity population with evaluation of the dominant allergic symptom at 1 month. Safety was assessed on the intention-to-treat (ITT) population, defined as infants enrolled who took study formula.

For quantitative parameters, change from baseline was compared between groups by analysis of covariance (ANCOVA) (or nonparametric ANCOVA in case of nonnormality, assessed by Shapiro-Wilk test) using the baseline value as a covariate. Intragroup changes were analysed using the Student *t* test or Wilcoxon test (nonnormal data). For qualitative parameters, change from baseline within treatment groups was analysed by symmetry test, or by McNemar test for binary variables. The difference between groups for the qualitative parameters' change was analysed using the χ^2, the Fisher, or the Cochran-Mantel-Haenszel (CMH) test. Statistical analyses were conducted using SAS version 9.2. Body mass index (BMI) was calculated for each infant. *z* scores of weight-for-age, length-for-age, weight-for-length, BMI-for-age, and head circumference–for-age were computed based on WHO growth data (26). For microbiota, when species or targeted taxonomic groups were not detected, the arbitrary value of 1.5 $\log_{10}$ colony-forming unit per gram of faecal content was used. Significance was set at $P < 0.05$.

Ethics

The study design was approved by an institutional review board for each country: Ile-de-France III, Paris, France and QFCUH ethics committee, Brussels, Belgium. This study was conducted in accordance with the ethical standards laid down by the Declaration of Helsinki. All of the parents of participating infants provided written informed consent.

RESULTS

Study Population

The characteristics of the study population at entry and at 1 month were described in 2014 (15). Briefly, 86 patients with suspected CMA (ITT population) were included and 75 were diagnosed as allergic to cow's milk and intolerant to eHFs (tolerance/hypoallergenicity population), 42 in the TAAF group, and 33 in the RAAF group (mean age 6.2 ± 4.3 months, 44% boys). They were all assessed by paediatricians at 1 (FAS population) and at 3 months of entering the study. A total of 8 infants dropped out of the study after 3 months because of parents' refusal to continue: 3 in the TAAF group and 5 in the RAAF group, leading respectively to 39 and 28 infants in the TAAF and RAAF groups assessed by paediatricians at 6 months. In addition to CMA, food allergies (egg and/or wheat) were diagnosed in 5 patients based on (s)IgE (27,28) and sensitisation was diagnosed in 30 patients based on either positive (s)IgE or cutaneous tests (soy, egg, wheat, and/or peanut).

Tolerance/Hypoallergenicity

No infant from the tolerance/hypoallergenicity population dropped out for intolerance during the 6-month study, including those who switched from RAAF to TAAF at 3 months.

Efficacy

At 3 months, complete resolution of the dominant allergic symptom was seen in a significantly higher number of subjects in the TAAF group (76.2%) than in the RAAF group (51.5%, $P = 0.026$, χ^2 test). Noticeably, in the 5 infants in the RAAF group for whom the dominant allergic symptom was persistent at 3 months, a change was seen at 6 months under TAAF, with resolution in 4 and improvement in 1. At 3 months, SCORAD Index, eczema, and skin dryness significantly improved in both the groups (Table 1, Fig. 1, Table S1 [*http://links.lww.com/MPG/A458*]). The SCORAD index decreased significantly more in the TAAF group than in the reference group, and skin dryness was resolved in significantly more patients ($P = 0.019$, χ^2 test). Rhinitis and wheezing significantly improved in the TAAF group (Fig. 1, Table S1 [*http://links.lww.com/MPG/A458*]). Regurgitation scores significantly decreased in both the groups (Table 1), and regurgitations disappeared completely in 66.7% of infants with the TAAF and 44.0% with the RAAF. Percentage of infants with soft/formed stools significantly increased in both the groups, from 47.6% to 92.9% and from 51.5% to 75.8% in the TAAF and RAAF groups, respectively ($P < 0.001$ and $P = 0.032$, McNemar test); more infants had normal or improved stools with TAAF than with RAAF (Table 1). Moreover, all of the 7 infants from the RAAF group with hard or liquid stools at 3 months exhibited soft or formed stools with the TAAF at 6 months.

At 3 months, general symptoms associated with CMA showed significant improvement with the TAAF such as frequency of crying, irritability, and sleeping time and quality, and, with the RAAF only quality of daytime sleep (Table 1, Fig. 2, Table S2 [*http://links.lww.com/MPG/A459*]). In both the groups, daily crying time significantly decreased (Table 1). Significant differences between the 2 groups were observed for frequency of irritability and quality of nighttime sleep, with the TAAF being more effective (respectively $P < 0.001$ and $P = 0.036$, χ^2 test and Fisher test).

Growth Data

At 3 months, infants' growth was similar between the 2 groups with no significant differences for weight, length, weight-for-length, BMI, and head circumference *z* scores. Compared with baseline, growth during 6 months showed significant improvement of weight-for-age *z* score in the group initially fed the TAAF (mean ± SD 0.3 ± 0.6, Fig. 3). In the same group, length, weight-for-length, BMI, and head circumference *z* scores increased by 0.1 (±0.8), 0.1 (±0.8), 0.4 (±0.9), and 0.3 (±0.8), respectively, during the 6-month study. As infants of the RAAF group switched to TAAF during the last 3 months, mean changes of anthropometric data between 3 and 6 months were adjusted with baseline values as covariate. Based on these analyses, at 6 months, no significant differences were noted between groups. Between 3 and 6 months, the mean weight-for-age *z* score significantly increased by 0.1 ± 0.3 in the RAAF group ($P = 0.028$, Wilcoxon test).

Safety

The most common adverse events were gastrointestinal tract affections and infections and were not related to the study product. Incidence of adverse events was not different between groups. A total of 4 serious adverse effects were recorded between 1 and 3 months: 3 in the TAAF group (gastroenteritis, pneumonia, and gastrooesophageal reflux) and 1 in the RAAF group (gastroenteritis); 3 were recorded between 3 and 6 months (malaise, gastroenteritis, and pneumonia). None were related to the study formula and none led to study drop out. In 2014, Dupont et al (15) reported 2 nonserious adverse events that led to study termination within the first month in the RAAF group. Parents' satisfaction with the allocated formula was high in both the groups (90.9% vs 79.0% at 1 month and 90.0% vs 91.7% at 3 months for TAAF and RAAF groups, respectively). Infants' acceptability of the allocated formula was judged as very good or good by more parents in the TAAF group than in the RAAF group at 1 and 3 months (76.5% vs 58.6% at 1 month and 91.4% vs 61.9% at 3 months, not significant CMH

TABLE 1. Change from baseline in SCORAD index scores, regurgitations scores, stool consistency, daily crying, and sleeping time at 3 months

	TAAF, N = 42	RAAF, N = 33	Total, N = 75
SCORAD Index			
N	25	27	52
Mean ± SD	−27.3 ± 2.3*	−20.8 ± 2.2*	−23.9 ± 20.9
Median (minimum, maximum)	−26.5 (−59.0, 5.9)	−25.4 (−72.5, 20.7)	−25.9 (−72.5, 20.7)
P value vs baseline	<0.001†	<0.001†	<0.001†
P value between groups			0.048§
Regurgitation score			
N	27	25	52
Mean ± SD	−1.9 ± 1.7	−1.7 ± 1.9	−1.8 ± 1.7
Median (minimum, maximum)	−1 (−6, 0)	−1 (−6, 2)	−1 (−6, 2)
P value vs baseline	<0.001‡	<0.001†	<0.001‡
P value between groups			0.159
Stool consistency, N (%)			
N	42	33	75
Aggravated** or not formed	3 (7.1)	8 (24.2)	11 (14.7)
Improved or formed	39 (92.9)	25 (75.8)	64 (85.3)
P value between groups			0.051‖
Daily crying time, min			
N	36	28	64
Mean (SD)	−126.4 ± 195.2	−45.7 ± 102.2	−91.1 ± 165.2
Median (minimum, maximum)	−45.0 (−780.0, 75.0)	−17.5 (−300.0, 140.0)	−42.5 (−780.0, 140.0)
P value vs baseline	<0.001‡	0.025†	<0.001‡
P value between groups			0.827¶
Daily sleeping time, min			
N	39	30	69
Mean ± SD	67.2 ± 157.0	35.0 ± 143.8	53.2 ± 151.2
Median (minimum, maximum)	60.0 (−240.0, 420.0)	0.0 (−360.0, 420.0)	30.0 (−360.0, 420.0)
P value vs baseline	0.011†	0.193†	0.005†
P value between groups			0.623§

ANCOVA = analysis of covariance; N = number of subjects; RAAF = reference amino acid–based formula; SCORAD = Scoring Atopic Dermatitis Index; SD = standard deviation; TAAF = thickened amino acid–based formula.

* Adjusted means.

** Stool consistency change from soft/formed to liquid/hard.

† Student *t* test.

‡ Wilcoxon test.

§ ANCOVA.

‖ Fisher test.

¶ ANCOVA based on ranks.

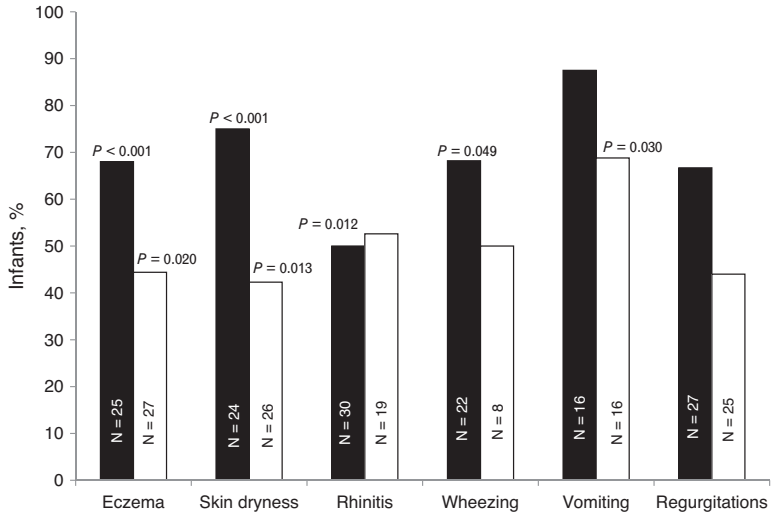

FIGURE 1. Proportion of infants with resolution of allergic symptoms at 3 months. All of the *P* values are versus baseline. Black column: TAAF; white column: RAAF. N = number of subjects; RAAF = reference amino acid–based formula; TAAF = thickened amino acid–based formula.

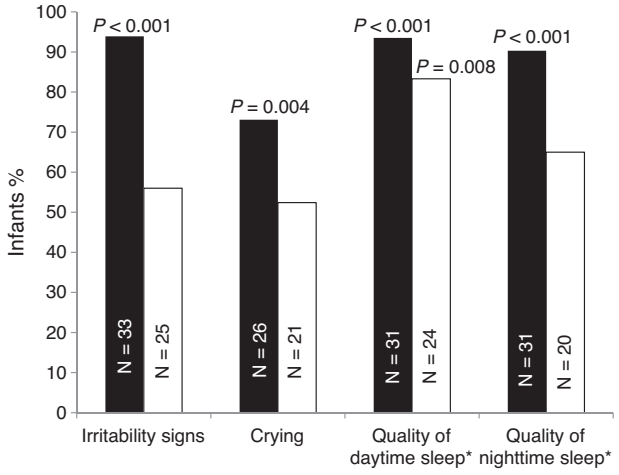

FIGURE 2. Proportion of infants with resolution (∗improvement) of general symptoms at 3 months. All of the *P* values are versus baseline. Black column: TAAF; white column: RAAF. N = number of subjects; RAAF = reference amino acid–based formula; TAAF = thickened amino acid–based formula.

test). No differences between groups were noted concerning the presence of gas and intestinal bloating at 1 and 3 months.

From inclusion to 3 months, there was a significant fall in mean plasma eosinophils concentrations in both the groups: from 0.49 (±0.55) to 0.28 (±0.22) 10^9/L in the TAAF group (n = 32, $P = 0.017$) and from 0.53 (±0.47) to 0.22 (±0.19) 10^9/L in the RAAF group (n = 29, $P < 0.001$, Wilcoxon test), with no significant difference between the groups. All of the other mean biochemical parameters, IgG, IgA, IgM, serum ferritin, and complete blood count were within normal range values at 3 months. In the subset of infants (25 in the TAAF group and 22 in the RAAF group) with plasma amino acid evaluation at 3 months, there were no differences between both the groups in plasma essential amino acid concentrations except for valine, higher in the RAAF group ($P = 0.049$, Wilcoxon test) (Fig. S1, *http://links.lww.com/MPG/A460*).

Faecal Analysis Data

At 3 months, infants of both the groups were colonized at high levels by bacterial groups usually encountered in the dominant microbiota of infants, that is, *Bacteroides/Prevotella*, *C coccoides* group, and *Bifidobacterium* (median $>10^9$ CFU/g of faeces, Fig. S2 [*http://links.lww.com/MPG/A461*]). A total of 2 infants, however, in the RAAF group were not colonized by bifidobacteria. Concerning the subdominant microbiota (median levels comprised between $10^{5.5}$ and 10^9 CFU/g of faeces), >90% of the infants were colonized by *E coli*, *Enterococcus*, and *Clostridium* cluster I/II and cluster XI. By contrast, colonization occurred less frequently with the *C leptum* group, *Lactobacillus/Leuconostoc* group, and *Staphylococcus* (53% to 83% of the infants depending on the bacterial groups and the formula group). After 3 months of AAF feeding, the evolution in the total bacteria counts was significantly different between TAAF and RAAF groups ($P = 0.021$), with a stable bacterial count in the TAAF group and a slight increase in the RAAF group. Despite no significant differences between groups for any genera, some different trends in the evolution were observed. Bifidobacteria decreased in both the groups, but the decrease was moderate in the TAAF group, -0.21 $\log_{10}$ CFU/g (±0.45), and higher in the RAAF group, -1.15 $\log_{10}$ CFU/g (±0.52) (adjusted means). When expressed as percentage of total bacteria, this trend was more marked, with bifidobacteria remaining stable in the TAAF group, 1.7% (±8.7), and decreasing in the RAAF group, −20.3% (±10.1). Similar trend was observed for the *Lactobacillus/Leuconostoc* group: -0.43 $\log_{10}$ CFU/g (±0.51) in the TAAF group versus -1.01 $\log_{10}$ CFU/g (±0.59) in the RAAF group. Likewise, there were increases in percentages of *Bacteroides/Prevotella* and *C coccoides* groups in the TAAF group (Fig. S3, *http://links.lww.com/MPG/A462*). These modifications tended to modify the balance of the microbiota, with a trend to a higher abundance of the bifidobacteria, *Bacteroides/Prevotella*, and *C coccoides* groups in the TAAF group compared with the RAAF group at 3 months.

EDN values ranged from <120 to 3475 ng/g at inclusion and from <120 to 3324 ng/g at 3 months, showing a high interindividual variability (n = 38). The trend (median, range) was similar with a decrease for both the groups: −196 ng/g (−1954 to 2026) in the TAAF group and −166 ng/g (−2469 to 2832) in the RAAF group, with no significant difference between the groups.

DISCUSSION

This study demonstrates the efficacy and safety in the long term of both AAFs in infants with proven CMA and intolerance to eHFs. All of the infants tolerated their allocated AAF for 3 months, and the TAAF was also tolerated by all of the infants who completed the 6-month study, including the infants who switched from RAAF to TAAF at 3 months. As reported in 2014, at 1 month, a complete resolution of the major CMA symptom occurred in 61.9% and 51.5% of infants in TAAF and RAAF groups, respectively (15). Results presented here confirm that at 3 months, both AAF formulas improved the major CMA symptom, the percentage of resolution being significantly higher with the TAAF.

One could argue that a DBPCFC, considered as the criterion standard (3) for CMA diagnosis, was not performed in all of the patients. Only a minority of subjects (26.7%) had no challenge and diagnosis based on (s)IgE assay and SPT values above validated cutoff levels for active CMA (19). This disposition in the protocol was chosen to favour the enrolment process of families dealing with an already complicated medical history (previous failure with 1 or more eHFs). In fact, the primary endpoint of this study, the tolerance/hypoallergenicity of the TAAF at 1 month in >90% (with 95% CI) of infants with both CMA and intolerance to eHFs, requires a sample size of ≥29 subjects with no reaction (2). This minimum number of subjects was reached in the TAAF group, even in the subgroup of subjects with CMA proven by a DBPCFC (15), and 100% of them tolerated the TAAF for ≥3 months. In addition, the percentage of the major CMA symptom resolution did not statistically differ between the infants with CMA proven by (s)IgE/SPT and those with CMA proven by a DBPCFC at 1 and 3 months; in the latter, this percentage remained significantly higher in the TAAF group compared with the RAAF group at 3 months.

In case of CMA, recommendations are first dietary treatment with eHFs to eliminate cow's-milk protein in the diet (1,2,4). eHFs have been successfully used to treat most of the infants with cow's-milk allergy. Some infants, however, are sensitive to these formulas, so their CMA symptoms persist with eHFs. AAFs are the recommended choice for these infants. Several studies reported hypoallergenicity and tolerance of AAF in infants with proven CMA (8,14,29) or in infants intolerant to eHFs (6,10), but no randomised controlled trial had ever been carried out in infants with CMA and allergy symptoms persisting with eHFs (3).

Beyond testing the tolerance of the AAFs, the aim was to quantify their efficacy in a large cohort of infants with both CMA and intolerance to eHFs. As CMA is characterized by a multitude of symptoms, including fussiness, irritability, emesis, poor feeding, and diarrhoea at presentation (1,4,30), paediatricians had to

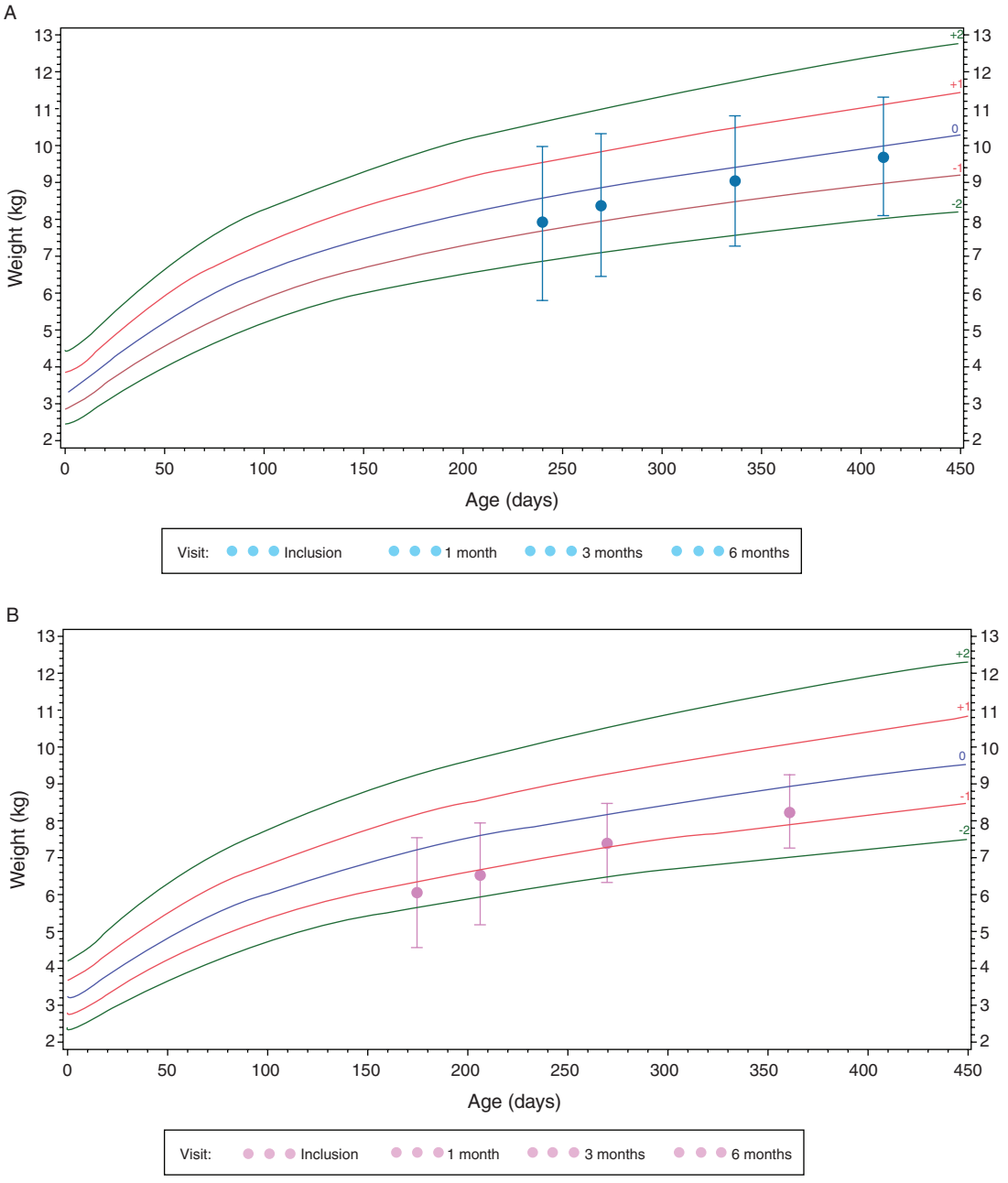

FIGURE 3. Mean weight (±SD) at each visit shown in comparison with the WHO growth standards for boys (A) and girls (B) initially fed TAAF. TAAF = thickened amino acid–based formula; WHO = World Health Organization.

determine in this study the dominant allergic symptom for each subject at inclusion and to assess its evolution at each follow-up visit. This dominant allergic symptom, however, did not reflect the complete clinical situation of each patient: for the subjects in which this symptom was not improved after 1 month, other allergic symptoms were resolved or improved. In addition, in 1995, Hill et al (31) evaluated children with multiple food intolerance, including CMA, and requiring an AAF feeding. Their allergic symptoms were scored during an eHFs challenge and compared with an AAF, as placebo; the score was most of the time lower with the AAF than with the eHF but never equal to zero. Recently, another tool, the Cow's Milk-related Symptom Score (CoMiSS), was proposed to allow the assessment and quantification of the evolution of CMA symptoms during a therapeutic intervention (32). Several trials, in which children with CMA on an eviction diet were followed, reported the evolution of CoMiSS values; after 1 month of dietary treatment, these values significantly decreased but were not equal to zero (33–35). Finally, the percentages of resolution of the dominant allergic symptom did not differ at 1 month between the TAAF and the RAAF, the latter having been considered for decades as the reference for severe CMA treatment (6,7).

The wide array of symptoms studied allowed a large documentation of symptom evolution. Regarding cutaneous symptoms, mean SCORAD index values observed in infants with CMA at

baseline ranged from 19.4 ± 16.1 (29) to 21 (95% CI 16–26) (5). Niggemann et al (13) reported a median SCORAD index of 18.5. All of these trials showed decreases of SCORAD index with AAFs. The present results confirm the efficacy of AAFs to reduce eczema severity in infants with CMA and intolerance to eHFs, the TAAF reducing significantly more the SCORAD index than the RAAF. The major difference between both AAFs and that may explain the observed difference is the presence of a pectin-based thickener in the TAAF. To the best of our knowledge, no clinical study has reported a significant effect of a pectin-based complex on skin health improvement in allergic infants. An earlier study found that a prebiotic mix, containing in particular acidic pectin oligosaccharides, had an effect on eczema prevention in infants with no atopy risk but no impact on eczema severity (36). In addition, results on the effect of prebiotic and probiotic blends on SCORAD evolution in infants and young children with atopic dermatitis are inconsistent (37,38). Given these contradictory results, the possible role of pectin on skin symptoms is unclear, requiring further investigation.

Previous studies suggest that pectin could play an integral role in improving stool consistency (34,35), and this was shown by a significant decrease in the number of liquid and hard stools in the TAAF group. Pectin is a dietary fibre known for having an impact on faecal weight (39). Other mechanisms could be considered such as enhanced colonic fluid absorption through the production of short-chain fatty acids (40) by colon microbiota (41), which may explain the positive effects of a pectin-based diet on diarrhoea resolution (42).

Very little data are available on the effect of AAFs on general symptoms related to CMA and affecting daily family life. Vanderhoof et al (7) reported the evolution of crying time and duration of sleep patterns in such patients. Fifteen days after AAF initiation, crying time was significantly reduced, but no change in the sleep duration was observed. The present results confirm the impact of AAF on reduced crying time but also show a significant increase in daily sleeping time. In addition, the TAAF showed a better improvement of irritability signs and quality of night sleep, compared with the RAAF.

Compared with healthy infants, allergic infants may have impaired growth, which is partially linked to improper food substitutions following allergen elimination (43). Moreover, CMA may also increase energy requirements because of inflammation (ie, skin or gastrointestinal) and disrupted sleep, and reduce the absorption of major nutrients (ie, CMA-induced enteropathy) (44). Mean weight and length z scores of the infants included in this study were < -0.5 at inclusion, showing poor weight and length gains in these infants, as already evidenced by other clinical studies (5,10,34,35,45,46). Previous clinical studies assessed the impact of AAF on the growth of healthy infants (8,9), or infants with CMA (5,13,29), but only 2 open noncontrolled studies reported growth data in infants with CMA and intolerance to eHFs (6,10). They all showed improvement of anthropometric data with AAF. In the present study, growth of infants fed the TAAF was similar to that of infants fed the RAAF, confirming the nutritional safety of this formula.

Essential amino acid plasma concentrations did not differ between the groups, except for valine, which was closer to breast-fed concentrations in the TAAF group, and slightly higher in the RAAF group compared with the TAAF group, however not clinically significant. They were all in the range of the concentrations measured in 6-month healthy infants (47) and similar to those in breast-fed infants (48).

Three months after AAF initiation, plasma eosinophils significantly decreased in both the groups. Same results were reported with AAF feeding but in older children (11,49). EDN is a faecal marker of intestinal immune stimulation related to allergic inflammatory responses, especially eosinophilic infiltration (18). Kalach et al (18) showed that infants with intestinal symptoms during a cow's milk challenge had higher faecal EDN levels than those with other symptoms or those tolerating cow's milk, suggesting an eosinophilic degranulation in the intestine. Our study confirms the high level of faecal EDN in this population. Three months after AAF initiation, children from both the groups showed a decrease of their EDN values, which may reflect a decrease of their intestinal inflammation.

Microbiota composition is in both groups in accordance with previous studies performed in infants at a similar age (50). The intervariability observed between infants is likely because of the diversity in age at sampling and in the perinatal determinants of the studied infants which are known to affect the bacterial colonization (50). No significant differences were observed between both the formulas throughout the study. Although the number of samples may not have been sufficient to detect a statistical difference, in the TAAF group, however, bifidobacteria percentages remained stable throughout the study whereas a slight decrease was observed in the RAAF group. Such observation was reported by Thompson-Chagoyan et al (51) who showed in CMA infants followed for 6 months on an elimination diet a significant increase in percentages in lactobacilli and a significant decrease in bifidobacteria. In TAAF, pectin, which is known to increase bifidobacteria counts (41), may have counterbalanced the effect of an elimination diet on decreasing bifidobacteria percentages.

This study presents several limitations. First, the number of faecal samples collected was low compared with the number of subjects included and may explain the absence of statistical differences observed between the groups. Secondly, our population may not have been homogenous enough regarding the CMA symptomatology to detect a possible impact of the dietary treatment on EDN values. In fact, this marker being associated with eosinophils intestinal infiltration, it may be more relevant in patients with gastrointestinal symptoms (18).

In conclusion, we have shown that the TAAF is hypoallergenic, efficient to alleviate symptoms, nutritionally adequate, and able to support growth during long-term feeding in these infants.

REFERENCES

1. Vandenplas Y, Koletzko S, Isolauri E, et al. Guidelines for the diagnosis and management of cow's milk protein allergy in infants. *Arch Dis Child* 2007;92:902–8.
2. American Academy of Pediatrics. Committee on Nutrition. Hypoallergenic infant formulas. *Pediatrics* 2000;106 (2 pt 1):346–9.
3. Dupont C, Chouraqui JP, de Boissieu D, et al. Dietary treatment of cows' milk protein allergy in childhood: a commentary by the Committee on Nutrition of the French Society of Paediatrics. *Br J Nutr* 2012;107:325–38.
4. 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.
5. Isolauri E, Sütas Y, Mäkinen-Kiljunen S, et al. Efficacy and safety of hydrolysed cow milk and amino acid–derived formulas in infants with cow milk allergy. *J Pediatr* 1995;127:550–7.
6. de Boissieu D, Matarazzo P, Dupont C. Allergy to extensively hydrolyzed cow milk proteins in infants: identification and treatment with an amino acid–based formula. *J Pediatr* 1997;131:744–7.
7. Vanderhoof JA, Murray ND, Kaufman SS, et al. Intolerance to protein hydrolysate infant formulas: an underrecognized cause of gastrointestinal symptoms in infants. *J Pediatr* 1997;131:741–4.
8. Burks W, Jones SM, Berseth CL, et al. Hypoallergenicity and effects on growth and tolerance of a new amino acid–based formula with docosahexaenoic acid and arachidonic acid. *J Pediatr* 2008;153:266–71.
9. Harvey BM, Langford JE, Harthoorn LF, et al. Effects on growth and tolerance and hypoallergenicity of an amino acid–based formula with synbiotics. *Pediatr Res* 2014;75:343–51.

10. 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.
11. Sicherer SH, Noone SA, Koerner CB, et al. 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.
12. Sampson HA, James JM, Bernhisel-Broadbent J. Safety of an amino acid–derived infant formula in children allergic to cow milk. *Pediatrics* 1992;90:463–5.
13. Niggemann B, Binder C, Dupont C, et al. Prospective, controlled, multicenter 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.
14. Berni Canani R, Nocerino R, Leone L, et al. Tolerance to a new free amino acid–based formula in children with IgE or non-IgE-mediated cow's milk allergy: a randomized controlled clinical trial. *BMC Pediatr* 2013;13:24.
15. Dupont C, Kalach N, Soulaines P, et al. A thickened amino-acid formula in infants with cow's milk allergy failing to respond to protein hydrolysate formulas: a randomized double-blind trial. *Paediatr Drugs* 2014;16:513–22.
16. Penders J, Stobberingh EE, van den Brandt PA, et al. The role of the intestinal microbiota in the development of atopic disorders. *Allergy* 2007;62:1223–36.
17. Scott KP, Duncan SH, Flint HJ. Dietary fibre and the gut microbiota. *Nutr Bull* 2008;33:201–11.
18. Kalach N, Kapel N, Waligora-Dupriet AJ, et al. Intestinal permeability and fecal eosinophil–derived neurotoxin are the best diagnosis tools for digestive non-IgE-mediated cow's milk allergy in toddlers. *Clin Chem Lab Med* 2013;51:351–61.
19. du Toit G, Meyer R, Shah N, et al. Identifying and managing cow's milk protein allergy. *Arch Dis Child Educ Pract Ed* 2010;95:134–44.
20. Dupont C. Diagnosis of cow's milk allergy in children: determining the gold standard? *Expert Rev Clin Immunol* 2014;10:257–67.
21. Consensus Report of the European Task Force on Atopic Dermatitis. Severity scoring of atopic dermatitis: the SCORAD index. *Dermatology* 1993;186:23–31.
22. Bekkali N, Hamers SL, Reitsma JB, et al. Infant stool form scale: development and results. *J Pediatr* 2009;154:521-6.e1.
23. Vandenplas Y, Hachimi-Idrissi S, Casteels A, et al. A clinical trial with an ''anti-regurgitation'' formula. *Eur J Pediatr* 1994;153:419–23.
24. Mangin I, Suau A, Magne F, et al. Characterization of human intestinal bifidobacteria using competitive PCR and PCR-TTGE. *FEMS Microbiol Ecol* 2006;55:28–37.
25. Klappenbach JA, Saxman PR, Cole JR, et al. rrnDB: the Ribosomal RNA operon copy number database. *Nucleic Acids Res* 2001;29:181–4.
26. WHO Multicentre Growth Reference Study Group. *WHO Child Growth Standards: Length/Height-for-Age, Weight-for-Age, Weight-for-Length, Weight-for-Height and Body Mass Index-for-Age: Methods and Development.* Geneva: World Health Organization; 2006.
27. Sicherer SH, Sampson HA. 9. Food allergy. *J Allergy Clin Immunol* 2006;117 (2 suppl mini-primer):S470–5.
28. Sampson HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. *J Allergy Clin Immunol* 2001;107:891–6.
29. Niggemann B, von Berg A, Bollrath C, et al. Safety and efficacy of a new extensively hydrolyzed formula for infants with cow's milk protein allergy. *Pediatr Allergy Immunol* 2008;19:348–54.
30. Allen KJ, Davidson GP, Day AS, et al. Management of cow's milk protein allergy in infants and young children: an expert panel perspective. *J Paediatr Child Health* 2009;45:481–6.
31. Hill DJ, Cameron DJ, Francis DE, et al. Challenge confirmation of late-onset reactions to extensively hydrolyzed formulas in infants with multiple food protein intolerance. *J Allergy Clin Immunol* 1995; 96:386–94.
32. Vandenplas Y, Dupont C, Eigenmann P, et al. A workshop report on the development of the Cow's Milk-related Symptom Score awareness tool for young children. *Acta Paediatr* 2015;104:334–9.
33. Vandenplas Y, Althera Study Group, Steenhout P, et al. A pilot study on the application of a symptom-based score for the diagnosis of cow's milk protein allergy. *SAGE Open Med* 2014;2:2050312114523423.
34. Vandenplas Y, De Greef E, Hauser B, et al. Safety and tolerance of a new extensively hydrolyzed rice protein–based formula in the management of infants with cow's milk protein allergy. *Eur J Pediatr* 2014;173:1209–16.
35. Vandenplas Y, De Greef E. ALLAR study group. Extensive protein hydrolysate formula effectively reduces regurgitation in infants with positive and negative challenge tests for cow's milk allergy. *Acta Paediatr* 2014;103:e243–50.
36. Grüber C, van Stuijvenberg M, Mosca F, et al. Reduced occurrence of early atopic dermatitis because of immunoactive prebiotics among low-atopy-risk infants. *J Allergy Clin Immunol* 2010;126:791–7.
37. van der Aa LB, Heymans HS, van Aalderen WM, et al. Effect of a new synbiotic mixture on atopic dermatitis in infants: a randomized-controlled trial. *Clin Exp Allergy* 2010;40:795–804.
38. Gerasimov SV, Vasjuta VV, Myhovych OO, et al. Probiotic supplement reduces atopic dermatitis in preschool children: a randomized, double-blind, placebo-controlled, clinical trial. *Am J Clin Dermatol* 2010;11: 351–61.
39. Cummings C. The effect of dietary fiber on fecal weight and composition. In: Spiller GA, ed. *CRC Handbook of Dietary Fiber in Human Nutrition.* 3rd ed. Boca Raton, FL: CRC Press; 2001.
40. Binder HJ. Role of colonic short-chain fatty acid transport in diarrhea. *Annu Rev Physiol* 2010;72:297–313.
41. Yang J, Martínez I, Walter J, et al. In vitro characterization of the impact of selected dietary fibers on fecal microbiota composition and short chain fatty acid production. *Anaerobe* 2013;23:74–81.
42. Rabbani GH, Teka T, Zaman B, et al. Clinical studies in persistent diarrhea: dietary management with green banana or pectin in Bangladeshi children. *Gastroenterology* 2001;121:554–60.
43. Isolauri E, Sutas Y, Salo MK, et al. Elimination diet in cow's milk allergy: risk for impaired growth in young children. *J Pediatr* 1998;132:1004–9.
44. Meyer R, Venter C, Fox AT, et al. Practical dietary management of protein energy malnutrition in young children with cow's milk protein allergy. *Pediatr Allergy Immunol* 2012;23:307–14.
45. Savino F, Castagno E, Monti G, et al. *Z*-score of weight for age of infants with atopic dermatitis and cow's milk allergy fed with a rice-hydrolysate formula during the first two years of life. *Acta Paediatr Suppl* 2005;94:115–9.
46. Agostoni C, Fiocchi A, Riva E, et al. Growth of infants with IgE-mediated cow's milk allergy fed different formulas in the complementary feeding period. *Pediatr Allergy Immunol* 2007;18:599–606.
47. Lepage N, McDonald N, Dallaire L, et al. Age-specific distribution of plasma amino acid concentrations in a healthy pediatric population. *Clin Chem* 1997;43:2397–402.
48. Picone TA, Benson JD, Moro G, et al. Growth, serum biochemistries, and amino acids of term infants fed formulas with amino acid and protein concentrations similar to human milk. *J Pediatr Gastroenterol Nutr* 1989;9:351–60.
49. Colson D, Kalach N, Soulaines P, et al. The impact of dietary therapy on clinical and biologic parameters of pediatric patients with eosinophilic esophagitis. *J Allergy Clin Immunol Pract* 2014;2:587–93.
50. Bergström A, Skov TH, Bahl MI, et al. Establishment of intestinal microbiota during early life: a longitudinal, explorative study of a large cohort of Danish infants. *Appl Environ Microbiol* 2014;80:2889–900.
51. Thompson-Chagoyan OC, Vieites JM, Maldonado J, et al. Changes in faecal microbiota of infants with cow's milk protein allergy—a Spanish prospective case-control 6-month follow-up study. *Pediatr Allergy Immunol* 2010;21 (2 Pt 2):e394–400.