# Extensively hydrolyzed casein formula supplemented with *Lactobacillus rhamnosus* GG maintains hypoallergenic status: Randomized double-blind, placebo-controlled crossover trial

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# Extensively hydrolyzed casein formula supplemented with *Lactobacillus rhamnosus* GG maintains hypoallergenic status: Randomized double-blind, placebo-controlled crossover trial

Antonella Muraro,[1] Maarten O Hoekstra,[2] Yolanda Meijer,[3] Carlos H Lifschitz,[4] Jennifer L Wampler,[5] Cheryl L Harris,[5] and Deolinda MF Scalabrin[5]

[1]Department of Pediatrics, Università degli Studi di Padova and Regione Veneto, Food Allergy Centre, Padova, Italy

[2]Department of Paediatrics, University Medical Centre St Radboud, Nijmegen, The Netherlands

[3]Wilhelmina Childrens' Hospital, University Medical Centre, Utrecht, The Netherlands

[4]Departamento de Pediatria, Hospital Italiano de Buenos Aires, Buenos Aires, Argentina

[5]Clinical Research, Department of Medical Affairs, Mead Johnson Nutrition, Evansville, IN

**Correspondence to**

Dr Jennifer L Wampler, Department of Medical Affairs, Mead Johnson Nutrition, 2400 West Lloyd Expressway, Evansville, IN, 47714. E-mail: jennifer.wampler@mjn.com.

**Keywords**

cow's milk protein, cow's milk allergy, extensively hydrolyzed formula, double-blind placebo-controlled food challenge, hypoallergenic formula, infant, *Lactobacillus* GG

## ABSTRACT

**Objective:** To evaluate the hypoallergenicity of an extensively hydrolyzed (EH) casein formula supplemented with *Lactobacillus rhamnosus* GG (LGG).

**Design:** A prospective, randomized, double-blind, placebo-controlled crossover trial.

**Setting:** Two study sites in Italy and The Netherlands.

**Study participants:** Children with documented cow's milk allergy (CMA) were eligible for inclusion in this trial.

**Interventions:** After a 7-day period of strict avoidance of cow's milk protein (CMP) and other suspected food allergens, participants were tested with an EH casein formula with demonstrated hypoallergenicity (control, EHF) and a formula of the same composition with LGG added at $10^8$ colony-forming units/g powder (EHF-LGG) in randomized order in a double-blind, placebo-controlled, food challenge (DBPCFC). After absence of adverse reactions in the DBPCFC, an open challenge was performed with EHF-LGG, followed by a 7-day home feeding period with the same formula.

**Main outcome measure:** Clinical assessment of any adverse reactions to ingestion of study formulas during the DBPCFC.

**Results:** For all participants with confirmed CMA (n = 31), the DBPCFC and open challenge were classified as negative.

**Conclusion:** The EH casein formula supplemented with LGG is hypoallergenic and can be recommended for infants and children allergic to cow's milk who require an alternative to formulas containing intact CMP.

**Trial registration:** ClinicalTrials.gov Identifier: NCT01181297

**Article focus**

- Hypoallergenic, extensively hydrolyzed (EH) cow's milk-based or amino acid-based formulas are recommended for management of cow's milk allergy (CMA) in formula-fed infants.
- Although *Lactobacillus rhamnosus* GG (LGG) has over 25 years of safe use as a dietary probiotic, the safety and hypoallergenic status of EH casein formula supplemented with LGG has not yet been demonstrated.

**Key messages**

- Supplementing the EH casein formula with LGG to provide additional benefits does not change its hypoallergenic status.
- The LGG-supplemented EH formula can be safely used for management of CMA in infants and children.

**Strengths and limitations of this study**

- Testing the LGG-supplemented EH formula in a properly designed double-blind, placebo-controlled, food challenge (DBPCFC) in accordance with accepted European Society of Pediatric Gastroenterology and Nutrition (ESPGHAN) and American Academy of Pediatrics (AAP) standards to establish hypoallergenicity is a major strength of this study.
- One limitation is the potentially low novelty of our finding. Because LGG is the most used dietary probiotic, accumulated safety data for LGG as a stand-alone dietary supplement in infants and adults is available.

## INTRODUCTION

Breast milk is the gold standard for infant nutrition and is recommended for most infants.[1 2] A cow's milk-based infant formula is most commonly used if a breast milk substitute is needed during the first year of life.[1] However, allergy to cow's milk protein (CMP) affects 2.2 to 2.8% of all infants.[3 4] Diagnostic confirmation of cow's milk allergy (CMA) is based on clinical history, physical exam, and controlled elimination of CMP followed by challenge procedures, including double-blind placebo-controlled food challenge (DBPCFC).[5] Quantification of specific IgE to cow's milk (CM) is used to diagnose IgE-mediated CMA and may eliminate the need to perform a DBPCFC for confirmation.[5 6] A child may be considered allergic to CM with no need for DBPCFC confirmation if the specific IgE concentration by CAP RAST is ≥ the 95% positive predictive value as established in earlier studies (5 $kU_A$/L and 15 $kU_A$/L, for children ≤1 year of age and >1 year of age, respectively).[6 7] Management of CMA is based on complete avoidance of intact CMP. One alternative, soy-based formula, is generally not recommended, particularly for infants younger than 6 months of age with non-IgE mediated manifestations of CMA, who are more likely to develop concomitant soy allergy.[8 9] Thus, formulas with reduced allergenicity, such as those with extensively hydrolyzed (EH) protein, are recommended for formula-fed infants with CMA.[2 8 10] EH casein formula has a long history of demonstrated efficacy and safety to manage infants and children with CMA.[11-13]

Determination of β-lactoglobulin (βLG) level, a major CM allergen, is a first assessment of the suitability of a substitute infant formula for infants and children with CMA.[14] The minute amount of βLG detected in EH casein formula[14] is in the lower range of the amounts detected in breast milk (0.9 to 150 μg/L).[15] According to the European Society of Pediatric Gastroenterology and Nutrition (ESPGHAN) and the American Academy of Pediatrics (AAP), a

formula must be tested in a properly designed DBPCFC and can be considered hypoallergenic when demonstrated with 95% confidence that at least 90% of infants and children with confirmed CMA would have no reaction to the formula under double-blind, placebo-controlled conditions.[10 14 16] To control for possible false-negatives, a negative DBPCFC should be followed by an open challenge (OC) with the tested formula.[5] After negative challenges, further assessment of tolerance to the tested formula during a 7-day feeding period to detect potential late-onset reactions is also recommended.[10 14]

Probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit to the host.[17] *Lactobacillus rhamnosus* GG (LGG) is the most studied probiotic, with demonstrated benefits when added to an EH formula, including decreased severity of atopic dermatitis (AD),[18 19] reduced intestinal inflammation [18 20] and faster induction of tolerance[21] in infants with CMA, and improved recovery from allergic colitis.[20] We previously demonstrated LGG was well tolerated, promoted normal growth, and transiently colonized the intestine when added to an EH casein formula fed to healthy term infants.[22 23] An EH formula with the same casein hydrolysate and many years of clinical experience of safety use in children with CMA was demonstrated to be hypoallergenic in those children in a DBPCFC trial.[13] However, the hypoallergenic status of the EH casein formula with added LGG has not yet been demonstrated. In the current study, we evaluated if LGG addition to this EH casein formula affected its hypoallergenic status for use in management of confirmed CMA in infants and children.

## METHODS

### Study design and participants

A randomized, controlled, prospective trial was conducted at two study sites to assess the hypoallergenicity of an EH casein formula with the same formulation of a previously existing formula (Nutramigen®, Mead Johnson & Company, Evansville, IN; control, EHF) that differed only in supplementation with LGG at $10^8$ colony-forming units/g of powder (EHF-LGG). Each powdered formula provided 2.8 g protein/100 kcal. The LGG raw material used in the formula demonstrated absence of βLG, as determined by an ELISA test with a detection limit of 0.1 μg/g (data on file).

Infants and children ≤14 years of age with confirmed CMA were eligible for this study if their allergic manifestations were under sufficient control so that a positive response to a food challenge would be recognizable. In addition, participants should have successfully consumed the control formula within 1 week of study enrollment. Exclusion criteria were presence of systemic disease or illness that could compromise participation in the study, use of beta-blockers within 12 hours of DBPCFC, use of short-acting, medium-acting, or long-acting antihistamines more than once within 3, 7, or 21 days of DBPCFC, respectively, or oral steroids within 21 days of DBPCFC. Adverse events were recorded throughout the study.

### Confirmation of CMA

Confirmation of CMA required one of the following criteria: 1) a positive DBPCFC to CM or CM-based formula within 6 months of study enrollment; 2) a positive confirmatory value of CAP RAST (Pharmacia, Uppsala, Sweden) to CM within 6 months of study enrollment (≥5 $kU_A/L$ in participants ≤1 year of age or ≥15 $kU_A/L$ in participants >1 year of age); 3) a documented significant adverse reaction to inadvertent ingestion of CM or CM-based formula

within 6 months of enrollment plus a positive DBPCFC or a confirmatory CAP RAST value to CM within 12 months of enrollment; or 4) a physician-documented anaphylaxis to CM or CM-based formula within 6 months of study enrollment plus a confirmatory CAP RAST value to CM within 12 months of enrollment.

**DBPCFC and Open Challenge (OC)**

The hypoallergenicity of the EHF-LGG formula was evaluated in a DBPCFC and OC, as described previously.[24] A 7-day period of strict elimination of CMP and other suspected food allergens preceded the DBPCFC (Figure 1). On Study Day 1 prior to the beginning of the DBPCFC and OC, participants underwent a physical examination and medical history and status of allergic diseases was recorded. Participants were either asymptomatic or allergic manifestations had been stabilized for a minimum of 7 days prior to the DBPCFC. The study sponsor had issued a list of 6-digit participant numbers to each study site and the study coordinator sequentially assigned a participant number to each participant. The sponsor also created a separate computer-generated randomization list of participant numbers that indicated the order in which each study formula should be offered in the DBPCFC challenge. At both study sites, the participant number was provided to a third-party pharmacist who referenced the number against the randomization list in order to prepare the EHF and EHF-LGG formulas in the assigned randomized order for each participant.

In the DBPCFC, the EHF and EHF-LGG formulas fed in randomized order were administered in an initial 5-10 mL aliquot followed by gradually increasing volumes over a maximum period of 120 minutes to provide a cumulative volume of 150 mL. A minimum interval of approximately 120 minutes between the end of the challenge with the first formula separated the beginning of the challenge with the second formula. Times of consumption and

amounts of study formula consumed during each challenge were recorded. Any signs or symptoms present before (baseline), during, or after the DBPCFC and OC were recorded using a scoring system to rate severity. The skin was observed for rash, urticaria/angioedema, or pruritus, with the percentage of body area affected recorded. The upper respiratory system was assessed for sneezing/itching, nasal congestion, rhinorrhea, or laryngeal symptoms, and the lower respiratory system was assessed for wheezing. The gastrointestinal system was evaluated for subjective symptoms such as nausea and abdominal pain and objective symptoms such as vomiting and diarrhea. Any changes in signs or symptoms from baseline would have resulted in classifying the challenge as positive and discontinuing the participant from the study. If the DBPCFC was negative, an OC with 150 to 250 mL of the EHF-LGG followed.

**Home feeding period**

To assess long-term tolerance and reveal any false-negative results to the challenges, all participants with negative responses to both the DBPCFC and OC consumed a minimum of 240 mL of EHF-LGG formula/day during a 7-day home feeding period. Participants' parents recorded in a daily diary volume of formula consumed; presence and severity of vomiting, diarrhea, rash, runny nose, wheezing, or any other symptoms (rated as mild, moderate, or excessive); number of bowel movements; and overall formula acceptance and tolerance (rated as satisfactory or unsatisfactory). The investigator completed a final evaluation at the end of the 7-day home feeding period.

**~~Statistical analysis~~Sample size determination**

In a study with a binomial outcome (reaction versus no reaction), the sample size can be determined by calculating a binomial confidence interval (CI) for p, the probability of having a reaction, as demonstrated previously.[13] In the case of 0 observed reactions, the upper 95% CI for

p is less than 0.10 when the sample size is 29 participants. Thus studying at least 29 participants and having none classified as positive in the DBPBFC allows the conclusion that the study provided 95% confidence that at least 90% of children with confirmed CMA who ingest the tested formula would have no reaction.[10 16] Data was prepared using SAS® version 8 (Cary, NC).

**Ethics approval**

The research protocol and informed consent form observing the Declaration of Helsinki (including October 1996 amendment) were approved by the Medical Ethics Committees of the Department of Pediatrics, Università degli Studi di Padova and Regione Veneto, Food Allergy Centre, Padova, Italy, and the Wilhelmina Children's Hospital, University Medical Centre, Utrecht, The Netherlands. The study complied with good clinical practices.

## RESULTS

Of the 34 children enrolled in the study between April 2003 and February 2004, a total of 33 (males, 19; females, 14) completed the DBPCFC, OC, and 7-day home feeding period (one participant who was enrolled but did not meet inclusion criteria was discontinued from the study) (Figure 1). Two participants were excluded from further analyses because CAP RAST to CM was lower than the confirmatory value for CMA. Neither participant experienced allergic reactions to the study formulas. Of the remaining 31 participants, 13 were <1 year, 17 were 1 to 3 years, and 1 was 11 years of age. The primary criterion used to confirm ongoing CMA, values for CAP RAST to CM, and symptoms evoked after the most recent inadvertent CMP intake or DBPCFC are summarized in table 1 for these participants.

Ongoing allergic diseases including AD, asthma, and/or allergic rhinitis were noted in 29 participants at study entry. Two participants reported a history of AD but no active allergic manifestation at study entry. Participants' status of allergic manifestations and presence of food allergies other than CMA at enrollment are shown in figures 2a and 2b, respectively. Ongoing allergy to multiple foods was reported for 29 participants, with 18 participants having 2 or more reported food allergies in addition to CMA.

After the pre-challenge 7-day period of CMP elimination, 29 of 31 participants had no allergic symptoms and remained asymptomatic throughout the DBPCFC and OC. Of the two remaining participants, one had no change in the mild rhinorrhea reported at baseline, and one had an improvement in the pruritus and rash reported at baseline. The DBPCFC and OC were thus classified as negative for all participants. Parent-recorded diaries during the home feeding period were returned for 30 participants and indicated that overall acceptance and tolerance of the EHF-LGG formula was generally good. Mean daily intake (mL/day±SD) reported was

546±251 and 522±132 for participants <1 year and 1 to 3 years of age, respectively, and 561 for the 11-year-old participant. Mean daily stool frequencies (± SD) were 1.9±0.5 and 1.7±0.9 for participants <1 year and 1 to 3 years of age, respectively, and 1.3 for the 11-year-old. No serious adverse events were reported during the DBPCFC, OC, or home feeding period.

## DISCUSSION

These findings demonstrate that a hypoallergenic EH casein hydrolysate formula remains hypoallergenic following the addition of LGG, satisfying both ESPHGAN and AAP guidelines. In this study all 31 study participants with confirmed CMA had a previous history of experiencing one or more types of allergic symptoms in the skin, respiratory and/or gastrointestinal systems, or had systemic anaphylaxis after ingestion of CMP, meeting recognized criterion to confirm CMA using a combination of convincing symptoms upon exposure to CMP and a strongly positive confirmatory value of specific IgE to CM by RAST.[6 7 24] In accordance with reports of sensitization to other food allergens commonly observed in children with CMA,[13 24] allergy to one or more foods in addition to CM was reported in 94% of participants in this study. After 7 days of strict CMP elimination from the diet, 29 of 31 participants had no allergic symptoms and remained asymptomatic throughout the DBPCFC and OC, whereas the other two had mild symptoms that either did not change or improved during the challenges. No serious adverse events were reported during the DBPCFC, OC, or the 7-day home feeding period.

The addition of probiotics in formula used for management of CMA requires that they be proven safe and are well tolerated. LGG has over 25 years of safe use[25] including administration to preterm infants[26] or to infants perinatally who were at high risk of allergy, in whom normal growth was demonstrated up to 2 to 4 years of age.[27 28] To justify use, addition of a probiotic must also be shown to be of benefit. Early gut microbial colonization is associated with modulation of inflammation and expression of allergy.[18 20 29 30] LGG administration to atopic pregnant women followed by postnatal administration to their infants was associated with lower incidence of AD at 2, 4, and 7 years of age compared to placebo.[30] Additionally, anti-

inflammatory effects of LGG accompanied by amelioration of symptoms were observed in infants experiencing AD as a manifestation of CMA.[19 31] In a study using fecal calprotectin as a marker of intestinal inflammation, infants with presumptive allergic colitis were randomized to receive an EH formula with or without LGG and the same casein hydrolysate as the formulas in the current study.[20] After a 4-week feeding period, blood in stools characteristic of allergic colitis disappeared in all infants in the LGG-supplemented group versus only 63% in the non-supplemented group. The LGG-supplemented group also experienced a larger decrease in fecal calprotectin level. In a recent study, EH casein formula with LGG was demonstrated to accelerate the time of acquisition of tolerance to CMP in infants with CMA after 6 and 12 months of feeding.[21]

We previously demonstrated that LGG added to an EH casein formula was well tolerated and transiently colonized the intestinal tract of healthy, term infants.[22] Growth and other nutrition parameters, including circulating fatty acid levels, were demonstrated to be normal in healthy term infants who received this formula up to 4 months of age.[23] Available data suggests that the LGG-supplemented EH casein formula assessed in the current study provides additional benefits of better management of allergic colitis, as well as faster tolerance acquisition, in infants with CMA that are not observed with the non-supplemented formula. We tested the EH casein formula supplemented with LGG according to established criteria and demonstrated its hypoallergenic status is maintained. Therefore, this formula can be recommended for infants and children with CMA who require an alternative to formulas containing intact CMP.

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**Competing interests**

AM, MOH, and YM have received research support from Mead Johnson Nutrition. CHL is a former employee of Mead Johnson Nutrition. JLW, CLH, and DMFS work in the Department of Medical Affairs at Mead Johnson Nutrition.

**Data sharing statement**

No additional data available.

**Contributorship**

AM, YM, and MOH helped design the study, assessed study participants and collected study data, interpreted data, and reviewed and revised the manuscript. CHL interpreted data and reviewed and revised the manuscript. JLW and DMFS interpreted data and drafted the manuscript. CLH prepared and interpreted data, and reviewed the manuscript. All authors contributed to the intellectual content and approved the final version.

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**Table 1** Participants with confirmed CMA: primary criterion used to confirm CMA, age at CAP RAST to CM and CAP RAST values, and symptoms evoked by the participants' most recent exposure to CMP

| Primary criterion to confirm CMA | Age (years) | CAP RAST to CM ($kU_A/L$) | Symptoms evoked after most recent inadvertent CMP intake or DBPCFC to CMP |
|---|---|---|---|
| Positive DBPCFC to CMP within 6 mo of study enrollment | 0.7 | 70 | pruritus, rash, urticaria/angioedema, rhinorrhea |
| | 0.9 | 12.2 | pruritus, rash, urticaria/angioedema |
| | 1.1 | <0.35 | pruritus, rash |
| | 1.3 | >100 | pruritus, rash, urticaria/angioedema |
| | 1.4 | 9.9 | pruritus, rash, urticaria/angioedema |
| | 1.6 | 15.7 | urticaria/angioedema, sneezing/itching, laryngeal edema |
| | 11.6 | 12.3 | laryngeal edema |
| Confirmatory CAP RAST to CM within 6 mo of study enrollment | 0.6 | 7.16 | pruritus, rash, urticaria/angioedema, wheezing |
| | 0.8 | 17.1 | pruritus, rash, urticaria/angioedema |
| | 1.5 | 22.4 | pruritus, rash, urticaria/angioedema, sneezing/itching |
| | 1.6 | 34.5 | pruritus, rash, vomiting |
| | 2.3 | >100 | * |
| | 2.4 | 61.8 | pruritus, rash, urticaria/angioedema, vomiting |
| Adverse reaction to inadvertent CMP intake within 6 mo and positive CAP RAST to CM within 12 mo of study enrollment | 0.3 | 68.3 | pruritis, rash, urticaria/angioedema, nasal congestion, sneezing/itching |
| | 0.4 | 6.09 | pruritus, rash, urticaria/angioedema, vomiting |
| | 0.5 | 4.59† | pruritus, rash, urticaria/angioedema, nasal congestion, sneezing/itching |
| | 0.6 | 10.5 | pruritus, rash, urticaria/angioedema, vomiting |
| | 0.6 | 9.01 | pruritus, urticaria/angioedema, nasal congestion, sneezing/itching |
| | 0.6 | 57.3 | pruritus, rash, urticaria/angioedema, nasal congestion, sneezing/itching, laryngeal edema |
| | 0.7 | 7.46 | pruritus, rash, urticaria/angioedema, nasal congestion, rhinorrhea, sneezing/itching |
| | 0.7 | 9.05 | pruritus, rash, urticaria/angioedema, rhinorrhea, sneezing/itching |
| | 0.8 | 6.84 | pruritus, rash, wheezing, vomiting |
| | 1.0 | 29.1 | pruritus, rash, urticaria/angioedema |
| | 1.0 | 29.5 | pruritus, rash, urticaria/angioedema |
| | 1.1 | 60.8 | pruritus, rash, urticaria/angioedema, vomiting |
| | 1.3 | >100 | urticaria/angioedema, rhinorrhea, wheezing, diarrhea, vomiting |
| | 1.4 | 23.9 | pruritus, rash, urticaria/angioedema, vomiting |
| | 1.5 | 25.0 | pruritus, rash, urticaria/angioedema, vomiting |
| | 1.6 | 30.5 | pruritus, rash, urticaria/angioedema, rhinorrhea, sneezing/itching |
| Anaphylaxis to CMP within 6 mo and positive CAP RAST to CM within 12 mo of study enrollment | 0.3 | 8.01 | pruritus, rash, urticaria/angioedema, systemic anaphylaxis |
| | 0.4 | 5 | pruritus, rash, urticaria/angioedema, nasal congestion, rhinorrhea, sneezing/itching, systemic anaphylaxis |

*Participant had a history suggestive of CMA beginning at 6 mo of age and ongoing symptoms of AD at enrollment

†Participant had sufficient evidence of CMA (exhibited multiple symptoms upon inadvertent CM intake within 3 mo of enrollment) although CAP RAST to CM was slightly < 5 $kU_A/L$

## FIGURES

**Figure 1** Flow of participants through the double-blind placebo-controlled food challenge (DBPCFC), open challenge (OC), and home feeding period (EHF: control formula; EHF-LGG: tested formula; CMP: cow's milk protein; CMA: cow's milk allergy)

**Figure 2** Medical history of participants with confirmed CMA (n=31): 2a) Ongoing and resolved clinical allergic manifestations at enrollment; 2b) Number of participants who reported allergy to foods other than cow's milk at enrollment.

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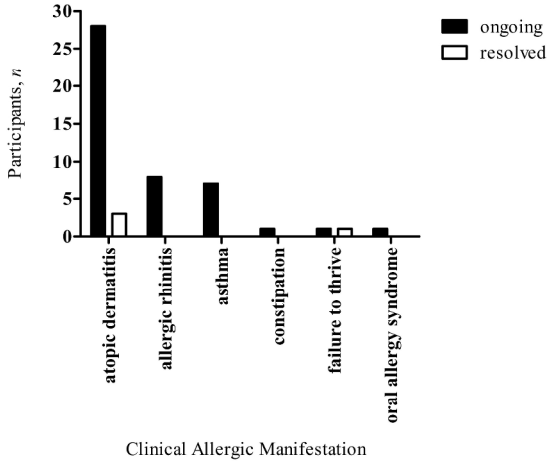



**Figure 2a**

Medical history of participants with confirmed CMA (n=31): 2a) Ongoing and resolved clinical allergic manifestations at enrollment.
270x352mm (300 x 300 DPI)

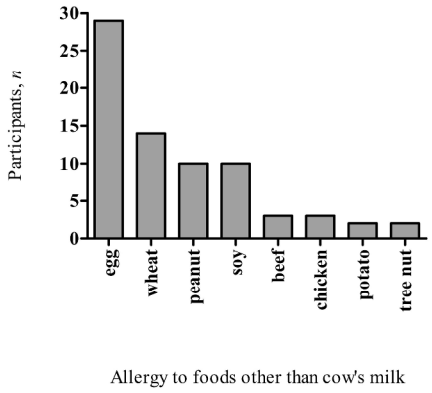
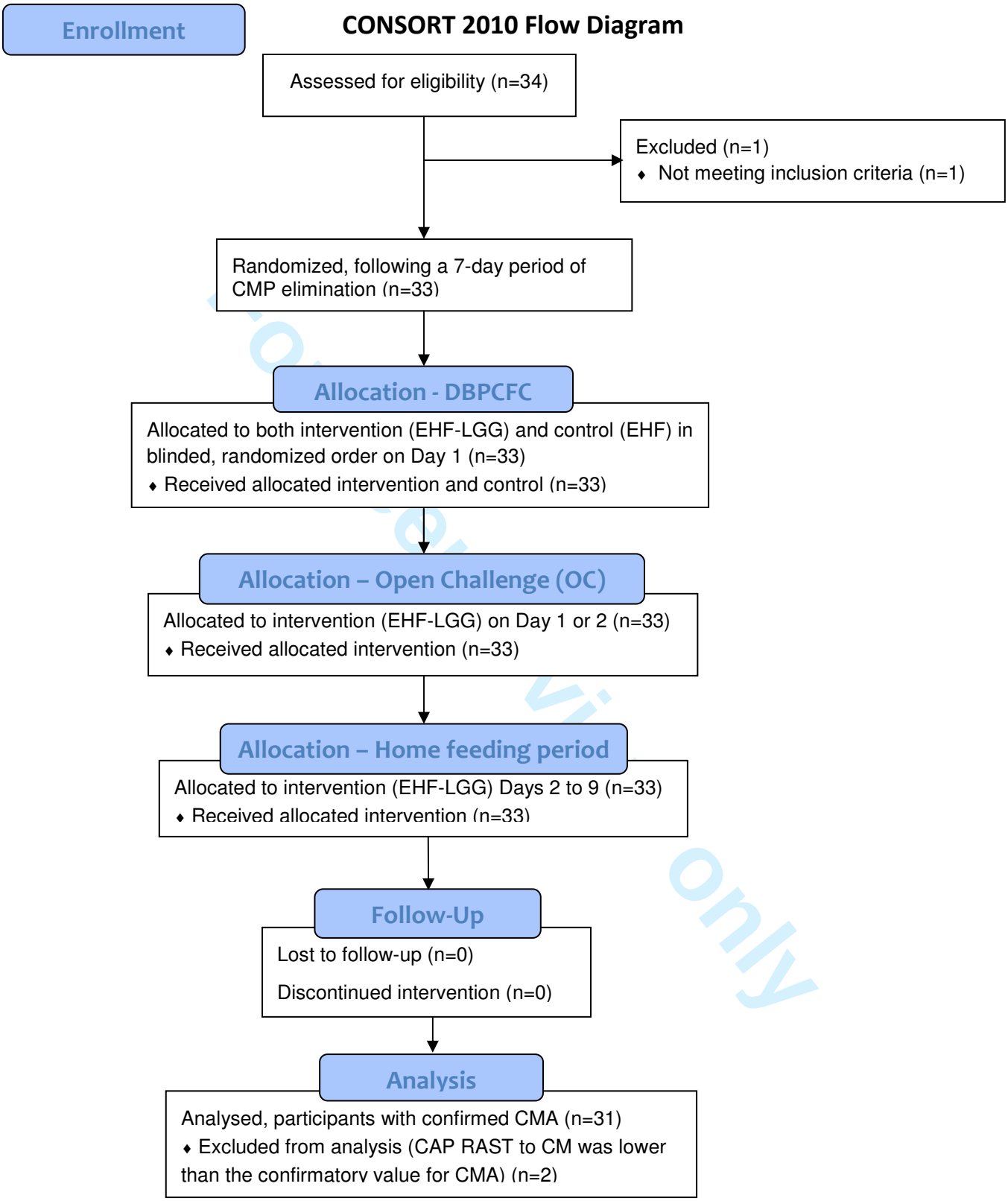



**Figure 2b**

2b) Number of participants who reported allergy to foods other than cow's milk at enrollment.
270x352mm (300 x 300 DPI)



# CONSORT 2010 checklist of information to include when reporting a randomised trial*

| Section/Topic | Item No | Checklist item | Reported on page No |
|---|---|---|---|
| **Title and abstract** | | | |
| | 1a | Identification as a randomised trial in the title | 1 |
| | 1b | Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts) | 2 |
| **Introduction** | | | |
| Background and objectives | 2a | Scientific background and explanation of rationale | 4-5 |
| | 2b | Specific objectives or hypotheses | 4 |
| **Methods** | | | |
| Trial design | 3a | Description of trial design (such as parallel, factorial) including allocation ratio | 6-7 |
| | 3b | Important changes to methods after trial commencement (such as eligibility criteria), with reasons | n/a |
| Participants | 4a | Eligibility criteria for participants | 6 |
| | 4b | Settings and locations where the data were collected | 10 |
| Interventions | 5 | The interventions for each group with sufficient details to allow replication, including how and when they were actually administered | 6-8 |
| Outcomes | 6a | Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed | 8 |
| | 6b | Any changes to trial outcomes after the trial commenced, with reasons | n/a |
| Sample size | 7a | How sample size was determined | 8-9 |
| | 7b | When applicable, explanation of any interim analyses and stopping guidelines | n/a |
| Randomisation: | | | |
| Sequence generation | 8a | Method used to generate the random allocation sequence | 7 |
| | 8b | Type of randomisation; details of any restriction (such as blocking and block size) | 7 |
| Allocation concealment mechanism | 9 | Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned | 7 |
| Implementation | 10 | Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions | 7 |
| Blinding | 11a | If done, who was blinded after assignment to interventions (for example, participants, care providers, those | 7 |

| | | | |
|---|---|---|---|
| | | assessing outcomes) and how | |
| | 11b | If relevant, description of the similarity of interventions | 6 |
| Statistical methods | 12a | Statistical methods used to compare groups for primary and secondary outcomes | 8-9 |
| | 12b | Methods for additional analyses, such as subgroup analyses and adjusted analyses | n/a |
| **Results** | | | |
| Participant flow (a diagram is strongly recommended) | 13a | For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome | 11 |
| | 13b | For each group, losses and exclusions after randomisation, together with reasons | 11 |
| Recruitment | 14a | Dates defining the periods of recruitment and follow-up | 11 |
| | 14b | Why the trial ended or was stopped | n/a |
| Baseline data | 15 | A table showing baseline demographic and clinical characteristics for each group | 16 |
| Numbers analysed | 16 | For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups | n/a |
| Outcomes and estimation | 17a | For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval) | 11 |
| | 17b | For binary outcomes, presentation of both absolute and relative effect sizes is recommended | n/a |
| Ancillary analyses | 18 | Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory | n/a |
| Harms | 19 | All important harms or unintended effects in each group (for specific guidance see CONSORT for harms) | 12 |
| **Discussion** | | | |
| Limitations | 20 | Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses | 13 |
| Generalisability | 21 | Generalisability (external validity, applicability) of the trial findings | 13-14 |
| Interpretation | 22 | Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence | 13-14 |
| **Other information** | | | |
| Registration | 23 | Registration number and name of trial registry | 15 |
| Protocol | 24 | Where the full trial protocol can be accessed, if available | n/a |
| Funding | 25 | Sources of funding and other support (such as supply of drugs), role of funders | 15 |

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

# Extensively hydrolyzed casein formula supplemented with *Lactobacillus rhamnosus* GG maintains hypoallergenic status: Randomized double-blind, placebo-controlled crossover trial

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|---|---|
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| Primary Subject Heading: | Paediatrics |
| Secondary Subject Heading: | Nutrition & metabolism, Immunology (including allergy) |
| Keywords: | Allergy < THORACIC MEDICINE, PAEDIATRICS, NUTRITION & DIETETICS |
| | |

# Extensively hydrolyzed casein formula supplemented with *Lactobacillus rhamnosus* GG maintains hypoallergenic status: Randomized double-blind, placebo-controlled crossover trial

Antonella Muraro,[1] Maarten O Hoekstra,[2] Yolanda Meijer,[3] Carlos H Lifschitz,[4] Jennifer L Wampler,[5] Cheryl L Harris,[5] and Deolinda MF Scalabrin[5]

[1]Department of Pediatrics, Università degli Studi di Padova and Regione Veneto, Food Allergy Centre, Padova, Italy

[2]Department of Paediatrics, University Medical Centre St Radboud, Nijmegen, The Netherlands

[3]Wilhelmina Childrens' Hospital, University Medical Centre, Utrecht, The Netherlands

[4]Departamento de Pediatria, Hospital Italiano de Buenos Aires, Buenos Aires, Argentina

[5]Clinical Research, Department of Medical Affairs, Mead Johnson Nutrition, Evansville, IN

**Correspondence to**

Dr Jennifer L Wampler, Department of Medical Affairs, Mead Johnson Nutrition, 2400 West Lloyd Expressway, Evansville, IN, 47714. E-mail: jennifer.wampler@mjn.com.

**Keywords**

cow's milk protein, cow's milk allergy, extensively hydrolyzed formula, double-blind placebo-controlled food challenge, hypoallergenic formula, infant, *Lactobacillus* GG

## ABSTRACT

**Objective:** To evaluate the hypoallergenicity of an extensively hydrolyzed (EH) casein formula supplemented with *Lactobacillus rhamnosus* GG (LGG).

**Design:** A prospective, randomized, double-blind, placebo-controlled crossover trial.

**Setting:** Two study sites in Italy and The Netherlands.

**Study participants:** Children with documented cow's milk allergy (CMA) were eligible for inclusion in this trial.

**Interventions:** After a 7-day period of strict avoidance of cow's milk protein and other suspected food allergens, participants were tested with an EH casein formula with demonstrated hypoallergenicity (control, EHF) and a formula of the same composition with LGG added at $10^8$ colony-forming units/g powder (EHF-LGG) in randomized order in a double-blind, placebo-controlled, food challenge (DBPCFC). After absence of adverse reactions in the DBPCFC, an open challenge was performed with EHF-LGG, followed by a 7-day home feeding period with the same formula.

**Main outcome measure:** Clinical assessment of any adverse reactions to ingestion of study formulas during the DBPCFC.

**Results:** For all participants with confirmed CMA (n = 31), the DBPCFC and open challenge were classified as negative.

**Conclusion:** The EH casein formula supplemented with LGG is hypoallergenic and can be recommended for infants and children allergic to cow's milk who require an alternative to formulas containing intact cow's milk protein.

**Trial registration:** ClinicalTrials.gov Identifier: NCT01181297

**Article focus**

- Hypoallergenic, extensively hydrolyzed (EH) cow's milk-based or amino acid-based formulas are recommended for management of cow's milk allergy (CMA) in formula-fed infants.
- Although *Lactobacillus rhamnosus* GG (LGG) has over 25 years of safe use as a dietary probiotic, the safety and hypoallergenic status of EH casein formula supplemented with LGG has not yet been demonstrated.

**Key messages**

- Supplementing the EH casein formula with LGG to provide additional benefits does not change its hypoallergenic status.
- The LGG-supplemented EH formula can be safely used for management of CMA in infants and children.

**Strengths and limitations of this study**

- Testing the LGG-supplemented EH formula in a properly designed double-blind, placebo-controlled, food challenge (DBPCFC) in accordance with accepted European Society of Pediatric Gastroenterology and Nutrition (ESPGHAN) and American Academy of Pediatrics (AAP) standards to establish hypoallergenicity is a major strength of this study.
- One limitation is the potentially low novelty of our finding. Because LGG is the most used dietary probiotic, accumulated safety data for LGG as a stand-alone dietary supplement in infants and adults is available.

## INTRODUCTION

Breast milk is the gold standard for infant nutrition and is recommended for most infants.[1 2] A cow's milk-based infant formula is most commonly used if a breast milk substitute is needed during the first year of life.[1] However, allergy to cow's milk protein affects 2.2 to 2.8% of all infants.[3 4] Diagnostic confirmation of cow's milk allergy (CMA) is based on clinical history, physical exam, and controlled elimination of cow's milk protein followed by challenge procedures, including double-blind placebo-controlled food challenge (DBPCFC).[5] Quantification of specific IgE to cow's milk is used to diagnose IgE-mediated CMA and may eliminate the need to perform a DBPCFC for confirmation.[5 6] A child may be considered allergic to cow's milk with no need for DBPCFC confirmation if the specific IgE concentration by CAP RAST is ≥ the 95% positive predictive value as established in earlier studies (5 $kU_A$/L and 15 $kU_A$/L, for children ≤1 year of age and >1 year of age, respectively).[6 7] Management of CMA is based on complete avoidance of intact cow's milk protein. One alternative, soy-based formula, is generally not recommended, particularly for infants younger than 6 months of age with non-IgE mediated manifestations of CMA, who are more likely to develop concomitant soy allergy.[8 9] Thus, formulas with reduced allergenicity, such as those with extensively hydrolyzed (EH) protein, are recommended for formula-fed infants with CMA.[2 8 10] EH casein formula has a long history of demonstrated efficacy and safety to manage infants and children with CMA.[11-13]

Determination of β-lactoglobulin (βLG) level, a major cow's milk allergen, is a first assessment of the suitability of a substitute infant formula for infants and children with CMA.[14] The minute amount of βLG detected in EH casein formula[14] is in the lower range of the amounts detected in breast milk (0.9 to 150 μg/L).[15] According to the European Society of Pediatric Gastroenterology and Nutrition (ESPGHAN) and the American Academy of Pediatrics (AAP), a

formula must be tested in a properly designed DBPCFC and can be considered hypoallergenic when demonstrated with 95% confidence that at least 90% of infants and children with confirmed CMA would have no reaction to the formula under double-blind, placebo-controlled conditions.[10 14 16] To control for possible false-negatives, a negative DBPCFC should be followed by an open challenge (OC) with the tested formula.[5] After negative challenges, further assessment of tolerance to the tested formula during a 7-day feeding period to detect potential late-onset reactions is also recommended.[10 14]

Probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit to the host.[17] *Lactobacillus rhamnosus* GG (LGG) is the most studied probiotic, with demonstrated benefits when added to an EH formula, including decreased severity of atopic dermatitis (AD),[18 19] reduced intestinal inflammation [18 20] and faster induction of tolerance[21] in infants with CMA, and improved recovery from allergic colitis.[20] We previously demonstrated LGG was well tolerated, promoted normal growth, and transiently colonized the intestine when added to an EH casein formula fed to healthy term infants.[22 23] An EH formula with the same casein hydrolysate and many years of clinical experience of safety use in children with CMA was demonstrated to be hypoallergenic in those children in a DBPCFC trial.[13] However, the hypoallergenic status of the EH casein formula with added LGG has not yet been demonstrated. In the current study, we evaluated if LGG addition to this EH casein formula affected its hypoallergenic status for use in management of confirmed CMA in infants and children.

## METHODS

### Study design and participants

A randomized, controlled, prospective trial was conducted at two study sites to assess the hypoallergenicity of an EH casein formula with the same formulation of a previously existing formula (Nutramigen®, Mead Johnson & Company, Evansville, IN; control, EHF) that differed only in supplementation with LGG at $10^8$ colony-forming units/g of powder (EHF-LGG). Each powdered formula provided 2.8 g protein/100 kcal. The LGG raw material used in the formula demonstrated absence of βLG, as determined by an ELISA test with a detection limit of 0.1 μg/g (data on file).

Infants and children ≤14 years of age with confirmed CMA were eligible for this study if their allergic manifestations were under sufficient control so that a positive response to a food challenge would be recognizable. In addition, participants should have successfully consumed the control formula within 1 week of study enrollment. Exclusion criteria were presence of systemic disease or illness that could compromise participation in the study, use of beta-blockers within 12 hours of DBPCFC, use of short-acting, medium-acting, or long-acting antihistamines more than once within 3, 7, or 21 days of DBPCFC, respectively, or oral steroids within 21 days of DBPCFC. Adverse events were recorded throughout the study.

### Confirmation of CMA

Confirmation of CMA required one of the following criteria: 1) a positive DBPCFC to cow's milk or cow's milk-based formula within 6 months of study enrollment; 2) a positive confirmatory value of CAP RAST (Pharmacia, Uppsala, Sweden) to cow's milk within 6 months of study enrollment (≥5 $kU_A$/L in participants ≤1 year of age or ≥15 $kU_A$/L in participants >1 year of age); 3) a documented significant adverse reaction to inadvertent ingestion of cow's milk

or cow's milk-based formula within 6 months of enrollment plus a positive DBPCFC or a confirmatory CAP RAST value to cow's milk within 12 months of enrollment; or 4) a physician-documented anaphylaxis to cow's milk or cow's milk-based formula within 6 months of study enrollment plus a confirmatory CAP RAST value to cow's milk within 12 months of enrollment.

**DBPCFC and Open Challenge (OC)**

The hypoallergenicity of the EHF-LGG formula was evaluated in a DBPCFC and OC, as described previously.[24] A 7-day period of strict elimination of cow's milk protein and other suspected food allergens preceded the DBPCFC (Figure 1). On Study Day 1 prior to the beginning of the DBPCFC and OC, participants underwent a physical examination and medical history and status of allergic diseases was recorded. Participants were either asymptomatic or allergic manifestations had been stabilized for a minimum of 7 days prior to the DBPCFC. The study sponsor had issued a list of 6-digit participant numbers to each study site and the study coordinator sequentially assigned a participant number to each participant. The sponsor also created a separate computer-generated randomization list of participant numbers that indicated the order in which each study formula should be offered in the DBPCFC challenge. At both study sites, the participant number was provided to a third-party pharmacist who referenced the number against the randomization list in order to prepare the EHF and EHF-LGG formulas in the assigned randomized order for each participant.

In the DBPCFC, the EHF and EHF-LGG formulas fed in randomized order were administered in an initial 5-10 mL aliquot followed by gradually increasing volumes over a maximum period of 120 minutes to provide a cumulative volume of 150 mL. A minimum interval of approximately 120 minutes between the end of the challenge with the first formula separated the beginning of the challenge with the second formula. Times of consumption and

amounts of study formula consumed during each challenge were recorded. Any signs or symptoms present before (baseline), during, or after the DBPCFC and OC were recorded using a scoring system to rate severity. The skin was observed for rash, urticaria/angioedema, or pruritus, with the percentage of body area affected recorded. The upper respiratory system was assessed for sneezing/itching, nasal congestion, rhinorrhea, or laryngeal symptoms, and the lower respiratory system was assessed for wheezing. The gastrointestinal system was evaluated for subjective symptoms such as nausea and abdominal pain and objective symptoms such as vomiting and diarrhea. Any changes in signs or symptoms from baseline would have resulted in classifying the challenge as positive and discontinuing the participant from the study. If the DBPCFC was negative, an OC with 150 to 250 mL of the EHF-LGG followed.

**Home feeding period**

To assess long-term tolerance and reveal any false-negative results to the challenges, all participants with negative responses to both the DBPCFC and OC consumed a minimum of 240 mL of EHF-LGG formula/day during a 7-day home feeding period. Participants' parents recorded in a daily diary volume of formula consumed; presence and severity of vomiting, diarrhea, rash, runny nose, wheezing, or any other symptoms (rated as mild, moderate, or excessive); number of bowel movements; and overall formula acceptance and tolerance (rated as satisfactory or unsatisfactory). The investigator completed a final evaluation at the end of the 7-day home feeding period.

**Sample size determination**

In a study with a binomial outcome (reaction versus no reaction), the sample size can be determined by calculating a binomial confidence interval (CI) for p, the probability of having a reaction, as demonstrated previously.[13] In the case of 0 observed reactions, the upper 95% CI for

p is less than 0.10 when the sample size is 29 participants. Thus studying at least 29 participants and having none classified as positive in the DBPBFC allows the conclusion that the study provided 95% confidence that at least 90% of children with confirmed CMA who ingest the tested formula would have no reaction.[10 16] Data was prepared using SAS® version 8 (Cary, NC).

**Ethics approval**

The research protocol and informed consent were approved by the Medical Ethics Committees of the Department of Pediatrics, Università degli Studi di Padova and Regione Veneto, Food Allergy Centre, Padova, Italy, and the Wilhelmina Children's Hospital, University Medical Centre, Utrecht, The Netherlands. The study complied with good clinical practice guidelines and the 1996 version of the Declaration of Helsinki.

## RESULTS

Of the 34 children enrolled in the study between April 2003 and February 2004, a total of 33 (males, 19; females, 14) completed the DBPCFC, OC, and 7-day home feeding period (one participant who was enrolled but did not meet inclusion criteria was discontinued from the study) (Figure 1). Two participants were excluded from further analyses because CAP RAST to cow's milk was lower than the confirmatory value for CMA. Neither participant experienced allergic reactions to the study formulas. Of the remaining 31 participants, 13 were <1 year, 17 were 1 to 3 years, and 1 was 11 years of age. The primary criterion used to confirm ongoing CMA, values for CAP RAST to cow's milk, and symptoms evoked after the most recent inadvertent cow's milk protein intake or DBPCFC are summarized in table 1 for these participants.

Ongoing allergic diseases including AD, asthma, and/or allergic rhinitis were noted in 29 participants at study entry. Two participants reported a history of AD but no active allergic manifestation at study entry. Participants' status of allergic manifestations and presence of food allergies other than CMA at enrollment are shown in figures 2a and 2b, respectively. Ongoing allergy to multiple foods was reported for 29 participants, with 18 participants having 2 or more reported food allergies in addition to CMA.

After the pre-challenge 7-day period of cow's milk protein elimination, 29 of 31 participants had no allergic symptoms and remained asymptomatic throughout the DBPCFC and OC. Of the two remaining participants, one had no change in the mild rhinorrhea reported at baseline, and one had an improvement in the pruritus and rash reported at baseline. The DBPCFC and OC were thus classified as negative for all participants. Parent-recorded diaries during the home feeding period were returned for 30 participants and indicated that overall acceptance and tolerance of the EHF-LGG formula was generally good. Mean daily intake (mL/day±SD)

reported was 546±251 and 522±132 for participants <1 year and 1 to 3 years of age, respectively, and 561 for the 11-year-old participant. Mean daily stool frequencies (± SD) were 1.9±0.5 and 1.7±0.9 for participants <1 year and 1 to 3 years of age, respectively, and 1.3 for the 11-year-old. No serious adverse events were reported during the DBPCFC, OC, or home feeding period.

## DISCUSSION

These findings demonstrate that a hypoallergenic EH casein hydrolysate formula remains hypoallergenic following the addition of LGG, satisfying both ESPHGAN and AAP guidelines. In this study all 31 study participants with confirmed CMA had a previous history of experiencing one or more types of allergic symptoms in the skin, respiratory and/or gastrointestinal systems, or had systemic anaphylaxis after ingestion of cow's milk protein, meeting recognized criterion to confirm CMA using a combination of convincing symptoms upon exposure to cow's milk protein and a strongly positive confirmatory value of specific IgE to cow's milk by RAST.[6 7 24] In accordance with reports of sensitization to other food allergens commonly observed in children with CMA,[13 24] allergy to one or more foods in addition to cow's milk was reported in 94% of participants in this study. After 7 days of strict cow's milk protein elimination from the diet, 29 of 31 participants had no allergic symptoms and remained asymptomatic throughout the DBPCFC and OC, whereas the other two had mild symptoms that either did not change or improved during the challenges. No serious adverse events were reported during the DBPCFC, OC, or the 7-day home feeding period.

The addition of probiotics in formula used for management of CMA requires that they be proven safe and are well tolerated. LGG has over 25 years of safe use[25] including administration to preterm infants[26] or to infants perinatally who were at high risk of allergy, in whom normal growth was demonstrated up to 2 to 4 years of age.[27 28] To justify use, addition of a probiotic must also be shown to be of benefit. Early gut microbial colonization is associated with modulation of inflammation and expression of allergy.[18 20 29 30] LGG administration to atopic pregnant women followed by postnatal administration to their infants was associated with lower incidence of AD at 2, 4, and 7 years of age compared to placebo.[30] Additionally, anti-

inflammatory effects of LGG accompanied by amelioration of symptoms were observed in infants experiencing AD as a manifestation of CMA.[19 31] In a study using fecal calprotectin as a marker of intestinal inflammation, infants with presumptive allergic colitis were randomized to receive an EH formula with or without LGG and the same casein hydrolysate as the formulas in the current study.[20] After a 4-week feeding period, blood in stools characteristic of allergic colitis disappeared in all infants in the LGG-supplemented group versus only 63% in the non-supplemented group. The LGG-supplemented group also experienced a larger decrease in fecal calprotectin level. In a recent study, EH casein formula with LGG was demonstrated to accelerate the time of acquisition of tolerance to cow's milk protein in infants with CMA after 6 and 12 months of feeding.[21]

We previously demonstrated that LGG added to an EH casein formula was well tolerated and transiently colonized the intestinal tract of healthy, term infants.[22] Growth and other nutrition parameters, including circulating fatty acid levels, were demonstrated to be normal in healthy term infants who received this formula up to 4 months of age.[23] Available data suggests that the LGG-supplemented EH casein formula assessed in the current study provides additional benefits of better management of allergic colitis, as well as faster tolerance acquisition, in infants with CMA that are not observed with the non-supplemented formula. We tested the EH casein formula supplemented with LGG according to established criteria and demonstrated its hypoallergenic status is maintained. Therefore, this formula can be recommended for infants and children with CMA who require an alternative to formulas containing intact cow's milk protein.

**Acknowledgements**

The authors wish to thank study site staff for their cooperation. The participation of parents and infants in this study is greatly acknowledged.

**Funding**

The study was funded by the study sponsor, Mead Johnson Nutrition (study number 3369-2). Mead Johnson Nutrition provided logistical support during the trial. Employees of the sponsor worked with study investigators to prepare the protocol, summarize the collected data, and write the manuscript.

**Competing interests**

AM, MOH, and YM have received research support from Mead Johnson Nutrition. CHL is a former employee of Mead Johnson Nutrition. JLW, CLH, and DMFS work in the Department of Medical Affairs at Mead Johnson Nutrition.

**Data sharing statement**

No additional data available.

**Contributorship**

AM, YM, and MOH helped design the study, assessed study participants and collected study data, interpreted data, and reviewed and revised the manuscript. CHL interpreted data and reviewed and revised the manuscript. JLW and DMFS interpreted data and drafted the manuscript. CLH prepared and interpreted data, and reviewed the manuscript. All authors contributed to the intellectual content and approved the final version.

**Table 1** Participants with confirmed CMA: primary criterion used to confirm CMA, age at CAP RAST to cow's milk (CM) and CAP RAST values, and symptoms evoked by the participants' most recent exposure to cow's milk protein (CMP)

| Primary criterion to confirm CMA | Age (years) | CAP RAST to CM ($kU_A/L$) | Symptoms evoked after most recent inadvertent CMP intake or DBPCFC to CMP |
|---|---|---|---|
| Positive DBPCFC to CMP within 6 mo of study enrollment | 0.7 | 70 | pruritus, rash, urticaria/angioedema, rhinorrhea |
| | 0.9 | 12.2 | pruritus, rash, urticaria/angioedema |
| | 1.1 | <0.35 | pruritus, rash |
| | 1.3 | >100 | pruritus, rash, urticaria/angioedema |
| | 1.4 | 9.9 | pruritus, rash, urticaria/angioedema |
| | 1.6 | 15.7 | urticaria/angioedema, sneezing/itching, laryngeal edema |
| | 11.6 | 12.3 | laryngeal edema |
| Confirmatory CAP RAST to CM within 6 mo of study enrollment | 0.6 | 7.16 | pruritus, rash, urticaria/angioedema, wheezing |
| | 0.8 | 17.1 | pruritus, rash, urticaria/angioedema |
| | 1.5 | 22.4 | pruritus, rash, urticaria/angioedema, sneezing/itching |
| | 1.6 | 34.5 | pruritus, rash, vomiting |
| | 2.3 | >100 | * |
| | 2.4 | 61.8 | pruritus, rash, urticaria/angioedema, vomiting |
| Adverse reaction to inadvertent CMP intake within 6 mo and positive CAP RAST to CM within 12 mo of study enrollment | 0.3 | 68.3 | pruritis, rash, urticaria/angioedema, nasal congestion, sneezing/itching |
| | 0.4 | 6.09 | pruritus, rash, urticaria/angioedema, vomiting |
| | 0.5 | 4.59† | pruritus, rash, urticaria/angioedema, nasal congestion, sneezing/itching |
| | 0.6 | 10.5 | pruritus, rash, urticaria/angioedema, vomiting |
| | 0.6 | 9.01 | pruritus, urticaria/angioedema, nasal congestion, sneezing/itching |
| | 0.6 | 57.3 | pruritus, rash, urticaria/angioedema, nasal congestion, sneezing/itching, laryngeal edema |
| | 0.7 | 7.46 | pruritus, rash, urticaria/angioedema, nasal congestion, rhinorrhea, sneezing/itching |
| | 0.7 | 9.05 | pruritus, rash, urticaria/angioedema, rhinorrhea, sneezing/itching |
| | 0.8 | 6.84 | pruritus, rash, wheezing, vomiting |
| | 1.0 | 29.1 | pruritus, rash, urticaria/angioedema |
| | 1.0 | 29.5 | pruritus, rash, urticaria/angioedema |
| | 1.1 | 60.8 | pruritus, rash, urticaria/angioedema, vomiting |
| | 1.3 | >100 | urticaria/angioedema, rhinorrhea, wheezing, diarrhea, vomiting |
| | 1.4 | 23.9 | pruritus, rash, urticaria/angioedema, vomiting |
| | 1.5 | 25.0 | pruritus, rash, urticaria/angioedema, vomiting |
| | 1.6 | 30.5 | pruritus, rash, urticaria/angioedema, rhinorrhea, sneezing/itching |
| Anaphylaxis to CMP within 6 mo and positive CAP RAST to CM within 12 mo of study enrollment | 0.3 | 8.01 | pruritus, rash, urticaria/angioedema, systemic anaphylaxis |
| | 0.4 | 5 | pruritus, rash, urticaria/angioedema, nasal congestion, rhinorrhea, sneezing/itching, systemic anaphylaxis |

*Participant had a history suggestive of CMA beginning at 6 mo of age and ongoing symptoms of AD at enrollment

†Participant had sufficient evidence of CMA (exhibited multiple symptoms upon inadvertent CM intake within 3 mo of enrollment) although CAP RAST to CM was slightly < 5 $kU_A/L$

## FIGURES

**Figure 1** Flow of participants through the double-blind placebo-controlled food challenge (DBPCFC), open challenge (OC), and home feeding period (EHF: control formula; EHF-LGG: tested formula; CMP: cow's milk protein; CMA: cow's milk allergy)

**Figure 2** Medical history of participants with confirmed CMA (n=31): 2a) Ongoing and resolved clinical allergic manifestations at enrollment; 2b) Number of participants who reported allergy to foods other than cow's milk at enrollment.

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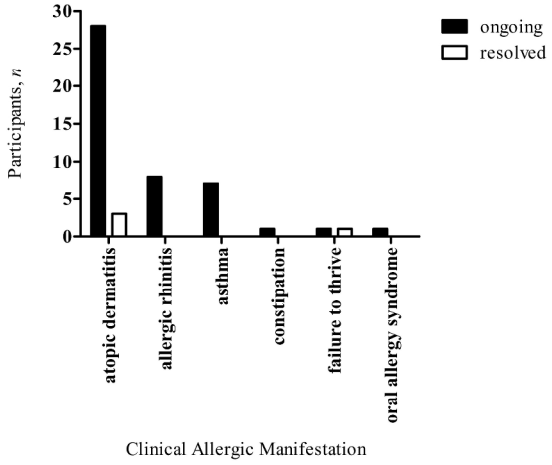



**Figure 2a**

Medical history of participants with confirmed CMA (n=31): 2a) Ongoing and resolved clinical allergic manifestations at enrollment.
270x352mm (300 x 300 DPI)

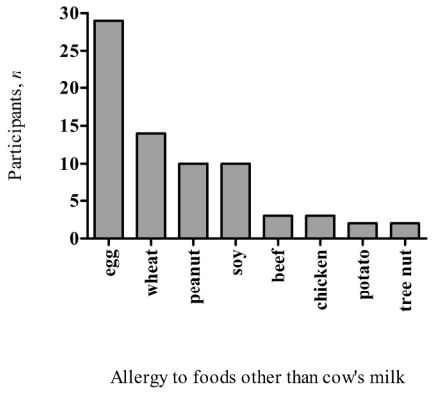
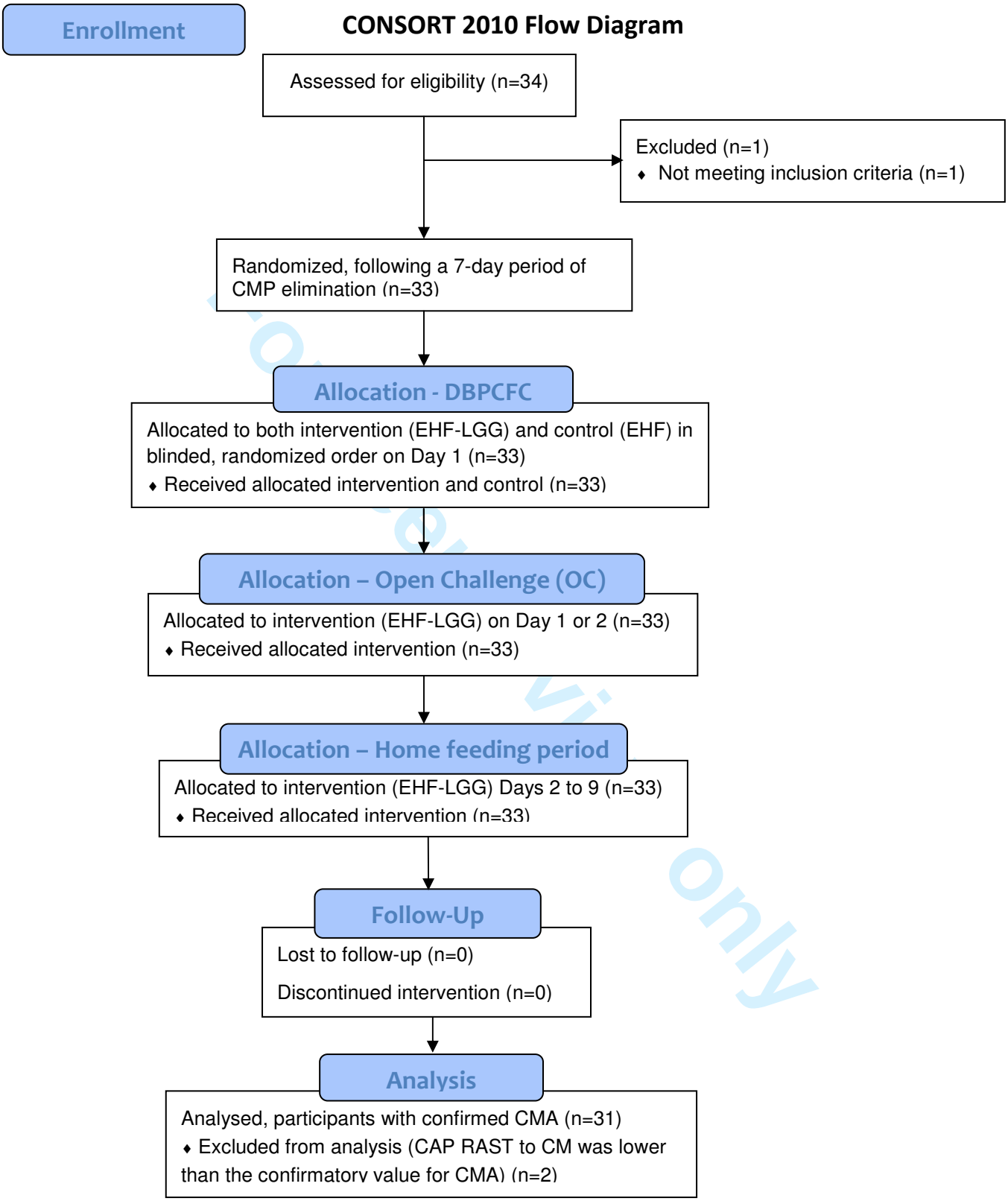



**Figure 2b**

2b) Number of participants who reported allergy to foods other than cow's milk at enrollment.
270x352mm (300 x 300 DPI)



# CONSORT 2010 checklist of information to include when reporting a randomised trial*

| Section/Topic | Item No | Checklist item | Reported on page No |
|---|---|---|---|
| **Title and abstract** | | | |
| | 1a | Identification as a randomised trial in the title | 1 |
| | 1b | Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts) | 2 |
| **Introduction** | | | |
| Background and objectives | 2a | Scientific background and explanation of rationale | 4-5 |
| | 2b | Specific objectives or hypotheses | 4 |
| **Methods** | | | |
| Trial design | 3a | Description of trial design (such as parallel, factorial) including allocation ratio | 6-7 |
| | 3b | Important changes to methods after trial commencement (such as eligibility criteria), with reasons | n/a |
| Participants | 4a | Eligibility criteria for participants | 6 |
| | 4b | Settings and locations where the data were collected | 10 |
| Interventions | 5 | The interventions for each group with sufficient details to allow replication, including how and when they were actually administered | 6-8 |
| Outcomes | 6a | Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed | 8 |
| | 6b | Any changes to trial outcomes after the trial commenced, with reasons | n/a |
| Sample size | 7a | How sample size was determined | 8-9 |
| | 7b | When applicable, explanation of any interim analyses and stopping guidelines | n/a |
| Randomisation: | | | |
| Sequence generation | 8a | Method used to generate the random allocation sequence | 7 |
| | 8b | Type of randomisation; details of any restriction (such as blocking and block size) | 7 |
| Allocation concealment mechanism | 9 | Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned | 7 |
| Implementation | 10 | Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions | 7 |
| Blinding | 11a | If done, who was blinded after assignment to interventions (for example, participants, care providers, those | 7 |

| | | | |
|---|---|---|---|
| | | assessing outcomes) and how | |
| | 11b | If relevant, description of the similarity of interventions | 6 |
| Statistical methods | 12a | Statistical methods used to compare groups for primary and secondary outcomes | 8-9 |
| | 12b | Methods for additional analyses, such as subgroup analyses and adjusted analyses | n/a |
| **Results** | | | |
| Participant flow (a diagram is strongly recommended) | 13a | For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome | 11 |
| | 13b | For each group, losses and exclusions after randomisation, together with reasons | 11 |
| Recruitment | 14a | Dates defining the periods of recruitment and follow-up | 11 |
| | 14b | Why the trial ended or was stopped | n/a |
| Baseline data | 15 | A table showing baseline demographic and clinical characteristics for each group | 16 |
| Numbers analysed | 16 | For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups | n/a |
| Outcomes and estimation | 17a | For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval) | 11 |
| | 17b | For binary outcomes, presentation of both absolute and relative effect sizes is recommended | n/a |
| Ancillary analyses | 18 | Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory | n/a |
| Harms | 19 | All important harms or unintended effects in each group (for specific guidance see CONSORT for harms) | 12 |
| **Discussion** | | | |
| Limitations | 20 | Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses | 13 |
| Generalisability | 21 | Generalisability (external validity, applicability) of the trial findings | 13-14 |
| Interpretation | 22 | Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence | 13-14 |
| **Other information** | | | |
| Registration | 23 | Registration number and name of trial registry | 15 |
| Protocol | 24 | Where the full trial protocol can be accessed, if available | n/a |
| Funding | 25 | Sources of funding and other support (such as supply of drugs), role of funders | 15 |

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.