Clinical Pediatrics
2015, Vol. 54(3) 264–272
© The Author(s) 2014
Reprints and permissions:
sagepub.com/journalsPermissions.nav
DOI: 10.1177/0009922814557785
cpj.sagepub.com

# Evaluation of Hypoallergenicity of a New, Amino Acid–Based Formula

**Anna Nowak-Węgrzyn, MD[1], Laura A. Czerkies, MS, RD[2], Barbara Collins, BSc, PhD[3], and José M. Saavedra, MD[2,4]**

**Abstract**

The American Academy of Pediatrics (AAP) defined a formula as hypoallergenic if it ensures with 95% confidence that 90% of infants with confirmed cow's milk allergy (CMA) will not react with defined symptoms under double-blind, placebo-controlled conditions. This study's objective was to determine whether a new amino acid–based formula (AAF) meets the AAP hypoallergenicity criteria. *Methods*. Children with CMA were randomized to double-blind placebo-controlled food challenges (DBPCFC) with a new AAF and a commercial AAF in crossover fashion followed by an at-home open challenge with the new AAF. Allergic reactions were assessed using a scoring system. *Results*. Thirty-three subjects completed DBPCFCs with both formulas without acute allergic reactions. The lower bound 95% confidence interval for hypoallergenicity was 91.3%. No unusual stool patterns, allergic symptoms, or signs of intolerance were reported during the open challenge. **Conclusion**. The new AAF meets AAP hypoallergenicity criteria and can be recommended for the management of CMA.

**Keywords**

hypoallergenic, infant formula, cow's milk allergy

## Introduction

Food allergy is an increasing problem in the United States and other Western countries.[1] The overall economic cost of childhood food allergy in the United States has been estimated at approximately $24.8 billion annually ($4184 per year per child).[2] It has been estimated that in the United States, food allergy prevalence is 8%, with 30% of those being multiple food allergies.[3] Cow's milk allergy (CMA) is one of the major food allergies that can present during infancy and childhood.[4,5] CMA affects 2% to 3% of infants and children in Western industrialized countries.[6-8] The allergic reactions that can occur following intact cow's milk ingestion vary from cutaneous symptoms to vomiting, wheezing, and severe anaphylaxis.[1,5]

While breast milk is the ideal source of nutrition, infants with CMA who consume infant formula as sole source of nutrition or as a supplement to breast milk, may require infant formulas whose protein base does not include intact cow's milk protein. A recent physician survey showed that soy-based formulas are frequently suggested by physicians to be used for their patients with CMA.[9] However, because of high frequency of infant sensitivity to both cow's milk and soy proteins, soy-based formulas are not recommended for use by the American Academy of Pediatrics (AAP) in infants with CMA.[10] In addition to infants, older children with CMA also commonly use hypoallergenic formulations that have been designed to provide nutrients in a cow's milk–protein-free diet that is safe for them.

Currently, extensively hydrolyzed formulas are an option for infants and children with CMA because although they are cow's milk–based, the proteins have been extensively broken down to substantially reduce their allergenicity. Some commercially available extensively hydrolyzed formulations in the United States have been deemed "hypoallergenic." The AAP[11] defines a formula as "hypoallergenic" on the basis of therapeutic use, not in preventive potential benefit. By AAP's criteria, a formula can be considered "hypoallergenic" only after being tested in subjects with hypersensitivity to cow's milk or cow's milk–based formula via properly

[1]Icahn School of Medicine at Mount Sinai, New York, NY, USA
[2]Nestlé Nutrition, Florham Park, NJ, USA
[3]Clinipace Worldwide, Morrisville, NC, USA
[4]Johns Hopkins University, Baltimore, MD

**Corresponding Author:**
José M. Saavedra, MD, Nestlé Nutrition, Avenue Nestlé 55, CH-1800, Vevey, Switzerland.
Email: jose.saavedra@nestle.com

Downloaded from cpj.sagepub.com at Infotrieve on March 3, 2015

conducted elimination-challenge tests.[11] These tests should ensure with 95% confidence that 90% of subjects with CMA will not react to formula under a double-blind placebo-controlled food challenge (DBPCFC).[12] After a successful DBPCFC, testing should include an open challenge using an objective scoring system to document allergic symptoms during a period of 7 days.[12]

Infants and children with the most severe CMA may still react to the extensively hydrolyzed formulas.[13,14] Such children require the use of amino acid–based formulas, whose protein source is synthetic amino acids, and therefore completely devoid of cow's milk protein.

A new amino acid–based nutritionally complete formula designed for the management of severe CMA and intolerance has been developed. This amino acid–based formula contains medium-chain triglycerides and structured lipids. The medium chain triglycerides are rapidly metabolized to provide a more readily available energy source, especially during times of gastrointestinal compromise. The structured lipids provide palmitic acid in the *sn*-2 position of the glycerol backbone, in keeping with its position in human breast milk.[15] The carbohydrate source is a blend of maltodextrin and potato starch to yield energy in easy-to-digest forms that does not contribute significantly to formula osmolality. Furthermore, the protein content is higher than it is in standard infant formulas in order to meet the nutritional needs of infants with compromised gastrointestinal function who may be experiencing malabsorption and severe diarrhea.

The present study was designed to evaluate whether or not a new amino acid–based formula provokes allergic reactions in infants and children between 2 months and 12 years of age with documented CMA who are otherwise healthy. The primary outcome was the occurrence of allergic reactions to a DBPCFC with the test product. Standardized approaches to CMA diagnosis and assessment of DBPCFCs were created and used for this study as a single set of diagnostic criteria for CMA are not widely accepted; nor are there standardized tools and criteria for DBPCFC currently used in clinical practice and studies.

## Materials and Methods

Infants and children of any ethnicity between 2 months and 12 years of age with documented CMA who were otherwise healthy were recruited from 6 sites around the United States from August to September 2012. For the purposes of this study, based on clinical practice at Mt Sinai in New York, CMA had to be documented within 6 months prior to enrollment by (*a*) reported convincing allergic symptoms following an exposure to milk or a milk-containing food product and detectable serum milk–specific IgE (>0.7 kIU/L) or positive skin prick test (wheal greater than or equal to 5 mm); (*b*) physician-supervised oral food challenge that elicited immediate allergic symptoms; (*c*) serum milk IgE ≥15 kIU/L or ≥5 kIU/L, if younger than 1 year; or (*d*) skin prick tests characterized by a wheal ≥10 mm.[16-18] These criteria are accepted as diagnostic of CMA without the requirement for performing a confirmatory oral food challenge. All subjects were observing strict milk elimination diet before and during the study. Potential subjects were excluded from study inclusion if they were consuming mother's milk at the time of study enrollment; had any chronic medical diseases, chromosomal or major congenital anomalies, or major gastrointestinal disease/abnormalities (other the CMPA); had immunodeficiency; antihistamine [excluding eye drops] use in 7 days prior to a food challenge or oral steroid use within 14 days prior to enrollment; unstable asthma; severe uncontrolled eczema; or severe anaphylactic reaction [requiring ≥2 doses of epinephrine] to milk or breast milk within the past 2 years; and/or current participation in another clinical study.

Subjects were asked to complete DBPCFCs of both a test (Nestle Health Science) and a control (Neocate, Nutricia) amino acid–based formula in a randomized, blinded, crossover fashion.[19] The macronutrient and micronutrient profiles of both formulas were similar (Table 1). Subjects were asked to eat nothing for 1 hour with allowance for light meals 2 hours prior to each session of the DBPCFC. For subjects ≤1 year of age, the initial dose was a lip smear with the assigned formula, followed by 5, 10, 20, 30, 30, 35, and 50 mL at 10- to 15-minute intervals. For subjects >1 year of age, the initial dose was a lip smear with the assigned formula, followed by 5, 10, 25, 45, 45, 45, and 65 mL at 10- to 15-minute intervals. The total volume ingested was 180 mL for subjects ≤1 year and 240 mL for subjects >1 year. A minimum postobservation period of 1 hour was required. Each site followed its own clinical and safety monitoring standards of procedure throughout the DBPCFCs. All sites documented any allergic signs or symptoms (skin, gastrointestinal, respiratory, cardiovascular) attributable to test or control formulas on a standardized DBPCFC data collection form (Figure 1) and adhered to a common definition of the criteria for the pass/fail for each symptom during the DBPCFC (Figure 2), as per recommendations of the Adverse Reactions to Food Committee of the American Academy of Allergy, Asthma, & Immunology.[19] The 2 DBPCFCs occurred 2 to 7 days apart. Subjects were instructed to follow a cow's milk–free diet for the entire study period and provided with emergency treatment plan and prescriptions for emergency medications, as appropriate.

Downloaded from cpj.sagepub.com at Infotrieve on March 3, 2015

**Table 1.** Nutrient Comparison of Test and Control Formulas.

| Per 100 kcal | Control Formula | Test Formula |
|---|---|---|
| Protein source | Amino acids | Amino acids |
| Protein (g/100 kcal, % kcal) | 3.1, 12.4% | 2.8, 11.2% |
| Fat source | Refined vegetable oil (medium chain triglycerides (palm kernel and/or coconut oil), high oleic sunflower, soy), ARA, DHA | Medium chain triglycerides, soybean oil, high oleic sunflower oil, esterified palm oil, ARA, DHA |
| Fat (g/100 kcal, % kcal) | 4.5, 41% | 4.9, 45% |
| MCT, % of fat as | 33% | 43% |
| Carbohydrate source | Corn syrup solids | corn syrup solids, potato starch |
| Carbohydrate (g/100 kcal, % kcal) | 11.7, 47% | 11.2, 44% |

Abbreviations: ARA, arachidonic acid; DHA, docosahexaenoic acid; MCT, medium-chain triglycerides.

If a subject passed (had no allergic reaction) both DBPCFCs, the subject was given the test formula and instructed to drink a minimum of 8 ounces daily for a period of 1 week (7-9 days). Subjects were provided with an emergency plan for any adverse reactions during the open challenge. During the open challenge, daily formula intake was recorded as well as daily stool frequency, color, consistency, or odor; frequency of flatulence; frequency of spit-up and/or vomiting; any symptoms of potential allergic etiology; and any adverse or serious adverse events.

The primary objective of this clinical trial was to demonstrate that the test formula ensures with 95% confidence that 90% of infants or children with confirmed CMA will not react under a double-blind, active (commercially available hypoallergenic formula) controlled study. Specifically, if the lower bound of the 1-sided exact 95% confidence interval for the proportion of infants or children without allergic reactions at the end of the study was at least 90%, hypoallergenicity of the test formula would be demonstrated. Based on an exact method for obtaining confidence intervals for a binomial proportion, for 45 infants with no allergic reactions, a sample size of 46 infants would support a 95% one-sided confidence interval for the nonallergic reaction rate to be at least 90%. The maximum adjusted sample size assuming a linear spending function with one interim analysis at information fraction $t = 0.6957$ is 51. The Lan and DeMets[20] method, assuming a linear spending function, $\alpha_2^*(t) = \alpha t$, where $\alpha = 0.05$ and $t$ is the information fraction, was used to adjust the $\alpha$ levels to account for the possible interim analysis. An interim analysis was allowed for, to be performed if and after 32 subjects successfully completed both test periods, that is, at information fraction $t = 0.6957$ (32/46). Using the linear $\alpha$ spending approach, $\alpha_2^*(0.6957) = 0.05 * 0.6957 = 0.034783$ would be spent at this interim analysis with a corresponding boundary of 1.81473. If there were no allergic reactions observed, the adjusted 1-sided exact lower bound confidence interval would be at least 90.04%, which would meet the standard specified by the AAP.[11] A subject was included in the per protocol population as long as both DBPCFCs for that subject were completed. The determination of AAP criteria was based on the per protocol population.

The study protocol was approved by Copernicus Institutional Review Board and the Mount Sinai School of Medicine Institutional Review Board. The study was registered through clinicaltrials.gov (#NCT01569776). Informed consent was obtained from the parents of the participating children, and child's assent was documented as appropriate. Good clinical practice was followed by all sites throughout the study.

## Results

Thirty-seven subjects were enrolled and randomized to formula sequence (Figure 3). Four subjects discontinued their first DBPCFC early, with 2 subjects stopping the DBPCFC of test formula and 2 subjects stopping the DBPCFC of control formula before completion due to the dislike of the formula taste. Subjects consumed 1 to 85 mL before withdrawing from the DBPCFC. No signs of a positive allergic reaction were noted in any of these subjects.

The majority of subjects were between the ages of 1 and 5 years, with the overall average of 4.6 years (Table 2). One subject was 13 years old, but deemed to still be appropriate for the study and the exception for his participation has been granted by the site isntitutional review board and the study sponsor. There were similar numbers of male and female subjects, and subjects were predominantly Caucasian. All subjects had documented CMA within the 6 months prior to enrollment: 16 subjects had reported convincing allergic symptoms following an exposure to milk or a milk-

Downloaded from cpj.sagepub.com at Infotrieve on March 3, 2015

**SUBJECT ID:** **DATE:** **PERSON ADMINISTERING CHALLENGE:**
**SESSION:** CHALLENGE 1 ☐ CHALLENGE 2 ☐ **FORMULA:** CODE: **WEIGHT:** . kg

*Doses should be given at 10-15 minute intervals after each ingestion. Scoring for any reaction to product reflected by skin, upper respiratory, lower respiratory (LR), gastrointestinal (GI), and/or cardiovascular (CV) signs or symptoms should be documented here. For IA, please indicate the % area of skin exhibiting erythematous rash. For all other sections, please provide a score 0 (absent), 1 (mild), 2 (moderate), 3 (severe) as defined by the "Symptom Scoring Key." Refer to "Guidelines for Clinical Determination of Pass/Fail Status for Food Challenge" as needed. A minimum of 1 hour post-observation time is required.*

| Dose Schedule | Time | Amt Ingested | SKIN | | | | UPPER RESPIRATORY | | | | LR | GI | | CV | COMMENTS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | IA (%) | IB | IC | ID | IIA | IIB | IIC | IID | IIIA | IVA | IVB | V | |
| Baseline | | | | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | |
| Lip smear | | | | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | |
| 5 ml | | | | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | |
| 10 ml | | | | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | |
| 25 ml | | | | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | |
| 45 ml | | | | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | |
| 45 ml | | | | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | |
| 45 ml | | | | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | |
| 65 ml | | | | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | |
| END OF POST-OB | | | | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | 0 ☐ 2 ☐ 1 ☐ 3 ☐ | |
| TOTAL INGESTED (ml) | | | | | | | | | | | | | | | |

**DID SUBJECT COMPLETE DBPCFC?** YES NO ☐ ☐

**DID SUBJECT PASS DBPCFC?** YES NO NA

_____

**Investigator Signature**

**Figure 1.** (A) Data collection form (for subjects >1 year of age) used to objectively assess each double-blind placebo-controlled food challenge (DBPCFC). (B) Symptom score key.

Downloaded from cpj.sagepub.com at Infotrieve on March 3, 2015

**I. Skin**
A. Erythematous Rash - % of area involved
B. Pruritus
0=Absent
1=Mild, occasional scratching
2=Moderate – scratching continuously for >2 minutes at a time
3=Severe – hard continuous scratching – excoriation
C. Urticaria/Angioedema
0=Absent
1=Mild - <3 hives
2=Moderate - <10 hives but >3
3=Severe – generalized involvement
D. Rash
0=Absent
1=Mild – few areas of faint erythema
2=Moderate – areas of erythema, macular and raised rash
3=Severe – generalized marked erythema (>50%), extensive raised lesion (>25%), vesiculation and/or piloerection

**II. Upper Respiratory**
A. Sneezing/Itching
0=Absent
1=Mild – rare bursts
2=Moderate – bursts <10, intermittent rubbing of nose, and/or eyes
3=Severe – continuous rubbing of nose and/or eyes, periocular swelling and/or long bursts of sneezing
B. Nasal Congestion
0=Absent
1=Mild – some hindrance to breathing
2=Moderate – nostrils feel blocked/breathes through mouth most of the time
3=Severe – nostrils occluded
C. Rhinorrhea
0=Absent
1=Mild – occasional sniffing
2=Moderate – frequent sniffing, requires tissues
3=Severe – nose runs freely despite sniffing and tissues
D. Laryngeal
0=Absent
1=Mild – throat clearing, occasional cough
2=Moderate – hoarseness, frequent dry cough
3=Severe – inspiratory stridor

**III. Lower Respiratory**
A. Wheezing
0=Absent
1=Mild – expiratory wheezing to auscultation
2=Moderate – dyspnea, inspiratory and expiratory wheezing
3=Severe – dyspnea, use of accessory muscles, audible wheezing

**IV. Gastrointestinal**
A. Subjective Complaints
0=Absent
1= Mild – complaints of nausea or abdominal pain, no change in activity, itchy mouth
2=Moderate – frequent c/o nausea or pain, decreased activity
3= Severe – patient in bed; crying or notably distressed
B. Objective Complaints
0=Absent
1=Mild – 1 episode of emesis or diarrhea
2=Moderate – 2-3 episodes of emesis or diarrhea or 1 of each
3=Severe - >3 episodes of emesis or diarrhea or 2 of each

**V. Cardiovascular**
0=Normal heart rate, or BP for age/baseline
1=Mild – color change, subjective response (weak, dizzy), mental status change, sustained tachycardia
2=Moderate – drop in blood pressure and/or >20% drop from baseline

Downloaded from cpj.sagepub.com at Infotrieve on March 3, 2015

| | Symptoms | 0 None | 1 Mild | 2 Moderate | 3 Severe |
|---|---|---|---|---|---|
| SKIN | IA Skin rash Erythematous Rash - % of area involved | | | | |
| | IB Pruritus | Absent | Occasional scratching | Scratching continuously for >2 minutes at a time | Hard continuous scratching – excoriation |
| | IC Urticaria | Absent | <3 hives | 3-10 hives | Generalized involvement |
| | ID Rash | Absent | Few areas of faint erythema | Areas of erythema, macular and raised rash | Marked erythema (>50%), extensive raised lesion (>25%) vesiculation and/or piloerection |
| UPPER RESPIRATORY | II A Sneezing | Absent | Rare bursts | Bursts <10, intermittent rubbing of nose, and/or eyes | Continuous rubbing of nose and/or eyes, periocular swelling and/or long bursts of sneezing |
| | IIB Nasal Congestion | Absent | Some hindrance to breathing | Nostrils feel blocked/breathes through mouth most of the time | Nostrils occluded |
| | IIC Rhinorrhea | Absent | Occasional sniffing | Frequent sniffing, requires tissues | Nose runs freely despite sniffing and tissues |
| | IID Laryngeal | Absent | Throat clearing, occasional cough | Hoarseness, frequent dry cough | Inspiratory stridor |
| L R | III Wheezing | Absent | Expiratory wheezing to auscultation | Dyspnea, inspiratory and expiratory wheezing | Dyspnea, use of accessory muscles, audible wheezing |
| GI | IVA Subjective | Absent | Complaints of nausea or abdominal pain, no change in activity, itchy mouth | Frequent c/o nausea or pain, decreased activity | Subject in bed; crying or notably distressed |
| | IVB Objective | Absent | 1 episode of diarrhea (ensure no diarrhea prior to challenge) or emesis | 2-3 episodes of emesis or diarrhea or 1 of each | >3 episodes of emesis or diarrhea or 2 of each |
| CV | V Cardiovascular | Normal heart rate, or BP for age/baseline | Color change, subjective response (weak, dizzy), mental status change, sustained tachycardia | Drop in blood pressure and/or >20% drop from baseline | Cardiovascular collapse, signs of impaired circulation (unconscious) |

**PASS/FAIL STATUS:**

| | |
|---|---|
| GREEN | Continue; Pass |
| YELLOW | Pass/Fail status based on clinical judgment. If symptoms occur mid-challenge, consider longer observation before next dose, and/or repeat the dose rather than increasing the dose. If no progression of symptoms, could continue with further dosing. If no additional symptoms develop this could be considered a passed challenge. |
| RED | STOP; failed challenge if:<br>1. Mild lower respiratory, laryngeal, cardiovascular or objective GI symptoms<br>2. Any moderate or severe symptoms<br>3. Two or more mild symptoms from different organ systems<br>4. Any challenge that resulted in treatment of allergic symptoms |

**Figure 2.** Guidelines for clinical determination of pass/fail for food challenge.

Downloaded from cpj.sagepub.com at Infotrieve on March 3, 2015

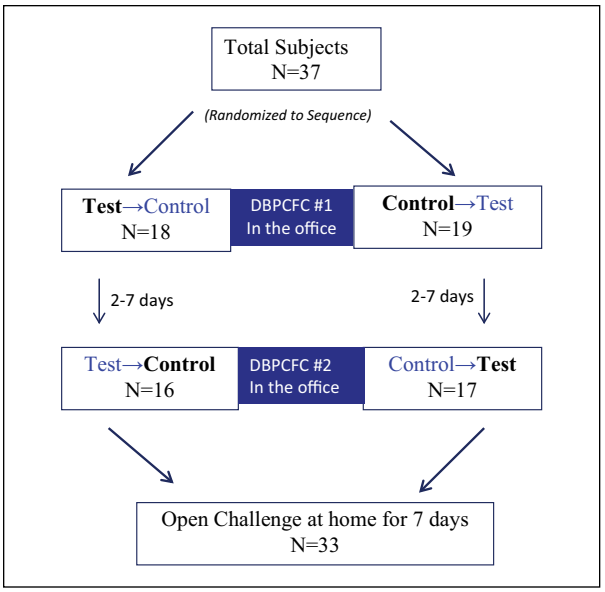



**Figure 3.** Study schematic. Eligible subjects were randomized to sequence of formulas to be used in double-blind placebo-controlled food challenge (DBPCFC). After successful completion of both DBPCFCs, subjects were asked to participate in a 1-week open challenge period with the test formula.

**Table 2.** Summary of Subject Demographic and Baseline Characteristics (Intention-to-Treat Population; N = 37).

| | |
|---|---|
| Gestational age at birth (weeks); min-max, mean (SD) | 33.0-41.0, 39.1 (1.646) |
| Current age (years); min-max, mean (SD) | 0.0-13.0, 4.6 (3.686) |
| Current age range (years); n (%) | |
| <1 | 5 (13.5) |
| 1-5 | 20 (54.1) |
| 6-12 | 11 (29.7) |
| >12 | 1 (2.7) |
| Gender; n (%) | |
| Male | 18 (48.6) |
| Female | 19 (51.4) |
| Race/ethnicity; n (%) | |
| Asian | 2 (5.4) |
| Black | 4 (10.8) |
| Caucasian | 21 (56.8) |
| Hispanic | 8 (21.6) |
| Other | 2 (5.4) |
| History of anaphylactic reaction to milk; n (%) | 9 (24.3) |
| Milk wheal size (mm); min-max, mean (SD), n | 5-32, 11.9 (6.9), 27 subjects |
| Milk IgE level (kIU/L); min-max, mean (SD), n | 3.87-100.0, 34.9 (29.2), 11 subjects |

containing food product and detectable serum milk–specific IgE (>0.7 kIU/L) and/ or positive skin prick test (wheal ≥5 mm); 1 subject had a physician-supervised oral food challenge that elicited immediate allergic symptoms; 6 subjects had serum milk IgE ≥15 kIU/L or ≥5 kIU/L if younger than 1 year; and 14 subjects had skin prick tests mean wheal ≥10 mm (Table 2). Twelve of the 37 subjects reported having multiple food allergies, including peanuts, nuts, eggs, soy, corn, wheat, shellfish, rye, apple, beef, oat, pea, lamb, and banana.

Out of 33 DBPCFCs completed with both the test and control formulas, no subjects experienced an allergic reaction during DBPCFCs of either product. As planned, an interim analysis occurred at this point in the study. The interim analysis revealed the lower bound of the 95% confidence interval was 91.3%, above the 90% requirement set forth by the AAP.[11]

Of the 33 subjects participating in the open challenge, 30 consumed at least 8 oz on at least 5 days during the open challenge. No unusual stool patterns or characteristics, incidence of flatulence or spit-up were reported. Four subjects reported vomiting on at least 1 occasion during the open challenge; these were attributed to palatability. Three of the subjects reporting vomiting were <1 year old, and the fourth subject was 3 years old who reported 1 episode of vomiting on the fifth day of the open food challenge. During the open challenge, 1 subject reported erythema around the mouth, 1 subject reported itchy skin on the back for 5 days, and another subject reported a mild stomachache on 4 days of the open challenge. All of these reported events were self-resolving, and none of these subjects ceased consumption of the test formula throughout the open challenge. One subject reported an upper respiratory infection not related to study formula that resolved after 6 days. There were no serious adverse events reported in this study.

## Discussion

This new formula with a different carbohydrate and lipid profile from existing formulas performed well in the study. The AAP hypoallergenicity criteria[11] were met for the test formula. No allergic reactions were noted to either the test formula or the control formula. This study documents the hypoallergenicity of another amino acid–based formula option for infants/children with CMA.

Of the 33 subjects participating in the open challenge of test formula, 30 were able to consume a minimum of 8 oz test formula daily for at least 5 days. While 4 subjects did report episodes of vomiting, itchy back, erythema, or stomachache, all these events were self-resolving. None of the events were considered to be

Downloaded from cpj.sagepub.com at Infotrieve on March 3, 2015

allergic reactions by the respective site investigators, and none of these subjects stopped consuming the study formula. As these events occurred during the open challenge while subjects were at home in an uncontrolled environment, and not relying exclusively on the formula for nutrition, other factors could have been related to any of these events.

This study involved multiple clinical practice study sites from across the United States. The study employed strict inclusion criteria that ensured enrolment of children with test results that exceeded the diagnostic decision points of above 95% likelihood of clinical IgE-mediated milk allergy. In children meeting these inclusion criteria, oral food challenges are considered superfluous. The DBPCFC is the gold standard for diagnosis of the food allergy. The study utilized the standardized DBPCFC protocol, including an instrument for recording the conduct of the DBPCFC, symptoms, and treatments to assure that each subject's challenge was conducted, monitored, and interpreted in the uniform manner. Tools like those used in this study (Figures 1 and 2) could be used in future multicenter studies where allergic reactions may be an outcome.

The current management of food allergy is limited to strict dietary avoidance, nutritional counseling, and emergency treatment of adverse reactions.[1] Allergen-specific (oral, sublingual, and epicutaneous immunotherapy) and non–allergen-specific (probiotics, toll-like receptor agonists) therapies to food allergy treatment are currently under investigation.[21] Until these therapeutic approaches are proven as effective as dietary avoidance and become more readily available, hypoallergenic nutrition formulas can help in providing adequate nutrition support to children with CMA.

## Conclusion

In conclusion, the primary objective of this study was met; the test formula meets the AAP criteria for hypoallergenicity of a formula. The new test formula offers another option of a free amino acid formula available to children with severe milk allergy who may require the use of such a formula. The CMA criteria and tools used for standardization of assessing DBPCFCs in this clinical study may be useful in future studies and clinical applications in allergic infants and children.

### Acknowledgments

The authors would like to acknowledge Clinipace Worldwide (Morrissville, NC) for their contract research organization management; Dr David Fuentes of TTS Research (Boerne, TX) and staff; Dr Majed Koleilat of Deaconess Clinic (Evansville, IN) and staff; Dr Paul Qaqundah of Pediatric Care Medical Group, Inc (Huntington Beach, CA) and staff; Dr Michael Welch of Allergy & Asthma Medical Group and Research Center, A P.C. (San Diego, CA) and staff.

### Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Laura A. Czerkies and José M. Saavedra are employees of Nestlé Nutrition.

### Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: All phases of this study were supported by Nestle Health Science.

### References

1. Burks AE, Jones SM, Boyce JA, et al. NIAID-sponsored 2010 guidelines for managing food allergy: applications in the pediatric population. *Pediatrics*. 2011;128:955-965.
2. Gupta R, Holdford D, Bilayer L, Dyer A, Holl JL, Meltzer D. The economic impact of childhood food allergy in the United States. *JAMA Pediatr*. 2013;167:1026-1031.
3. Gupta RD, Springston EE, Warrier MR, et al. The prevalence, severity and distribution of childhood food allergy in the United States. *Pediatrics*. 2011;128:e9-e17.
4. Høst A. Cow's milk protein allergy and intolerance in infancy. Some clinical, epidemiological and immunological aspects. *Pediatr Allergy Immunol*. 1994;5(5 suppl):1-36.
5. Sampson HA. Food allergy. Part 1: immunopathogenesis and clinical disorders. *J Allergy Clin Immunol*. 1999;103:717-728.
6. Bock SA. Prospective appraisal of complaints of adverse reactions to foods in children during the first 3 years of life. *Pediatrics*. 1987;79:683-688.
7. Host A, Halken S. A prospective study of cow milk allergy in Danish infants during the first 3 years of life. Clinical course in relation to clinical and immunological type of hypersensitivity reaction. *Allergy*. 1990;45:587-596.
8. Schrander JJ, Van Den Bogart JP, Forget PP, Schrander-Stumpel CT, Kuijten RH, Kester AD. Cow's milk protein intolerance in infants under 1 year of age: a prospective epidemiological study. *Eur J Pediatr*. 1993;152:640-644.
9. Saavedra JM, Boguniewicz M, Chamlin S, et al. Patterns of clinical management of atopic dermatitis in infants and toddlers: a survey of three physician specialties in the United States. *J Pediatr*. 2013;163:1747-1753.
10. Bhatia J, Greer F, American Academy of Pediatrics Committee on Nutrition. Use of soy protein-based formulas in infant feeding. *Pediatrics*. 2008;121:1062-1068.
11. American Academy of Pediatrics, Committee on Nutrition. Hypoallergenic infant formulas. *Pediatrics*. 2000;106 (2 pt 1):346-349.
12. Kleinman RE, Bahna S, Powell GF, Sampson HA. Use of infant formulas in infants with cow milk allergy: a

Downloaded from cpj.sagepub.com at Infotrieve on March 3, 2015

review and recommendations. *Pediatr Allergy Immunol*. 1991;2:146-155.
13. Saylor JD, Bahna SL. Anaphylaxis to casein hydrolysate formula. *J Pediatr*. 1991;118:71-74.
14. Hill DJ, Cameron DJ, Francis DE, Gonzalez-Andaya AM, Hosking CS. Challenge confirmation of late-onset reactions to extensively hydrolyzed formulas in infants with multiple food protein intolerance. *J Allergy Clin Immunol*. 1995;96:386-394.
15. Cruz-Hernandez C, Thakkar SK, Moulin J, et al. Benefits of structured and free monoacylglycerols to deliver eicosapentaenoic (EPA) in a model of lipid malabsorption. *Nutrients*. 2012;4:1781-1793.
16. García-Ara C, Boyano-Martínez T, Díaz-Pena JM, Martín-Muñoz F, Reche-Frutos M, Martín-Esteban M. Specific IgE levels in the diagnosis of immediate hypersensitivity to cow's milk protein in the infant. *J Allergy Clin Immunol*. 2001;107:185-190.
17. Sampson HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. *J Allergy Clin Immunol*. 2001;107:891-896.
18. Sporik R, Hill DJ, Hosking CS. Specificity of allergen skin testing in predicting positive open food challenges to milk, egg and peanut in children. *Clin Exp Allergy*. 2000;30:1540-1546.
19. Nowak-Węgrzyn A, Assa'ad AH, Bahna SL, Bock SA, Sicherer SH, Teuber SS; Adverse Reactions to Food Committee of American Academy of Allergy, Asthma & Immunology. Work Group report: oral food challenge testing. *J Allergy Clin Immunol*. 2009;123(6 suppl): S365-S383.
20. Lan KKG, DeMets DL. Discrete sequential boundaries for clinical trials. *Biometrika*. 1983;70:659-663.
21. Nowak-Węgrzyn A, Muraro A. Food allergy therapy: is a cure within reach? *Pediatr Clin North Am*. 2011;58: 511-530.

Downloaded from cpj.sagepub.com at Infotrieve on March 3, 2015