Effects on growth and tolerance and hypoallergenicity of an amino acid–based formula with synbiotics

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BACKGROUND: To evaluate the effects of an amino acid–based formula (AAF) with synbiotics on growth and tolerance in healthy infants. The hypoallergenicity of this AAF with synbiotics was evaluated in subjects with cow's milk allergy (CMA).

METHODS: Study 1: 115 full-term, healthy infants randomly received an AAF with synbiotics or a commercially available AAF for 16 wk. Subjects' weight, length, and head circumference were primary outcome measures. Stool characteristics and gastrointestinal (GI) symptoms were secondary outcome measures. Clinical examinations, dietary intake, clinical laboratory results, and adverse events were recorded. Study 2: hypoallergenicity of the AAF with synbiotics was evaluated in 30 infants and children with immunoglobulin E (IgE)–mediated CMA using a double-blind, placebo-controlled food challenge, and a 7-d feeding period.

RESULTS: Study 1: comparable results in growth parameters and tolerance were observed for both groups. Minimal differences were observed in stool characteristics and GI symptoms throughout the study. Study 2: all 30 subjects with IgE-mediated CMA completed the study with no allergic reactions detected to challenges.

CONCLUSION: These studies demonstrate that an AAF with synbiotics is safe and well tolerated and promotes normal growth when fed to healthy full-term infants as the sole source of nutrition and is hypoallergenic in subjects with CMA.

Food allergies are a growing problem in developed countries, with rates highest in infancy and childhood (5–8%), though decreasing with age (1–2% in adults) (1) as tolerance gradually develops. Cow's milk allergy (CMA) is common with estimated prevalence during infancy, ranging from 2–5% (1). The long-term prognosis for CMA is good, with the majority of children outgrowing their allergies by age of 5 y (2); however, in recent years, it has been noted that some allergies persist into later life (3). CMA can be broadly split into IgE and non-IgE mediated and is associated with the development of other allergies (1), when poorly managed it can lead to a detrimental effect on growth (4). CMA generally affects more than one organ system, with the most common being gastrointestinal (GI), cutaneous, and respiratory systems.

CMA is managed by avoiding cow's milk, using alternative formulae based on soy or hydrolyzed cow's milk protein. However, these are not always completely effective, especially in the more severe CMA cases, as hydrolyzation does not completely eliminate the cow's milk antigens (5), while cosensitivity to soy has been observed in 50% of infants with non-IgE–mediated CMA (6); therefore, with severe CMA the use of an amino acid–based formula (AAF) is often preferred. The use of an AAF in severe CMA has been proven to provide effective, rapid symptom relief whilst providing good infant growth rates (7).

Neocate Infant DHA and ARA (also known as Neocate LCP) (Nutricia, SHS International, Liverpool, UK) is a commercially available, nutritionally complete hypoallergenic AAF with docosahexaenoic acid (DHA) and arachidonic acid (ARA) used for the dietary management of infants with severe CMA and multiple food allergies (7).

Previous studies suggest that the composition of the intestinal microbiota is important in the long-term development of allergies (8), which is consistent with the "hygiene hypothesis" (9). Several factors influence the composition of the microbiota in an infant's gut, including mode of delivery and type of nutrition (breast milk vs. infant formula). Differences in the gut microbiota of breast and formula fed babies have been found, with the former having more strict anaerobes such as *bifidobacteria* and *lactobacilli* and the latter more facultative anaerobes (10). *Bifidobacteria* and *lactobacilli* are thought to be beneficial, modulating the immune system, whereas *coliforms* and *clostridia* are thought of as detrimental (11). Positive clinical results have led to the development of food supplements and infant formula containing prebiotic and probiotic ingredients. A prebiotic is defined as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or more of a limited number of bacteria in the colon that can improve the host health" (12). Prebiotic oligosaccharides are naturally occurring in human breast milk. Probiotics are defined as living micro-organisms that survive passage through the GI tract and have beneficial

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effects on the host (13). Synbiotics refer to the addition of a combination of prebiotic and probiotic ingredients. Prebiotics and synbiotics have been demonstrated to play a role in both the prevention and treatment of allergies (14,15).

Being highly sensitive, patients with severe CMA who require an AAF have not had a synbiotic formula commercially available to them as many probiotics are cultured in the presence of milk protein which have been proven to elicit an allergic response in CMA infants (16). In two studies, we test a new version of Neocate Infant DHA and ARA containing an added synbiotic blend (Neo-Syn, Nutricia, SHS International, Liverpool, UK).

The prebiotic blend is a combination of neutral fructo-oligosaccharides and pectin-derived acidic oligosaccharides in a ratio of 85:15 to mimic the molecular weight profile of human milk oligosaccharides. The safety of prebiotic infant formula has been well documented, with a number of studies published demonstrating efficacy in infants (17,18). The probiotic included is *Bifidobacterium breve* M-16V strain originally isolated from a healthy infant. This strain has been identified as an effective probiotic strain when compared to other probiotic bacteria in terms of antiallergic activity demonstrating superior activity for lung hyper-responsiveness and skin reactivity in allergic mouse models (19). It has been tested in several clinical trials in neonates and low birth weight infants (15,20–22) where it showed a reduction in cutaneous and total allergic score in infants with atopic dermatitis (AD) (20).

The present studies were designed to evaluate Neo-Syn on overall growth and tolerance in healthy full-term infants. Furthermore, the hypoallergenicity of Neo-Syn was evaluated in infants and children with documented IgE-mediated CMA based on criteria developed by the American Academy of Pediatrics' (AAP) subcommittee on Nutrition and Allergic diseases (Clinical trial registration number NCT00664768).

RESULTS

Study 1

In total, 115 infants were enrolled in the study between 7 November 2008 and 21 March 2011, 70 (60.9%) infants completed the study, 32 (54.2%) in the test group (Neocate Infant DHA and ARA with synbiotics (Neo-Syn)) and 38 (67.9%) in the control group (commercially available Neocate Infant DHA and ARA (Neo)). The dropout rate was relatively high, with a total of 45 subjects (39.1%) withdrew from the study early due to the occurrence of an adverse event ($n = 22$), withdrawal of informed consent ($n = 20$) and lost to follow-up ($n = 3$) (see **Figure 1**). Reasons for early withdrawal were not different between the study groups. All 115 infants were included in the main Intention to Treat (ITT) analysis; however, 32 subjects were excluded for the per protocol (PP) analysis because they did not solely consume the formula they were randomized to during the study.

The demographic data and subject characteristics at baseline for all ITT subjects are summarized in **Table 1**. Subjects were well balanced over the study groups with respect to baseline characteristics; similar mean measures were observed for the PP population. **Figure 2** plots the ratios of the weight, length, and head circumference growth parameters over time for the two groups. There was no statistically significant difference in weight gain, achieved length, or achieved head circumference between the two formula groups for the ITT and PP analyses. At the end of the study (week 16) the ratio (90% CI) in weight gain (Neo-Syn/Neo) was 0.98 (0.95, 1.01) (ITT) and 1.00 (0.97, 1.04) (PP). For length increase these ratios (90% CI) were 0.99 (0.98, 1.00) (ITT) and 0.99 (0.98, 1.01) (PP). For head circumference, these ratios (90% CI) were 1.01 (1.00, 1.02) both for ITT and PP. Z-scores adjusted for age to weight ratio were similarly different from the average mean for the Neo-Syn and Neo groups at each time point and for each growth parameter.

Parents and guardians reported a similar mean (±SD) number of formula exposure days (75.5 ± 51.5 for Neo-Syn and 79.1 ± 45.4 for Neo groups) and a similar amount of mean (±SD) formula consumed (11.8 ± 4.3 ounces per day for Neo-Syn and 11.2 ± 4.2 ounces per day for Neo).

There were significant differences in stool characteristics, but minimal differences in GI symptoms (**Table 2**) between the formula groups. Overall significant associations were found between study product and frequent categories recorded for stool consistency and stool color with a movement towards more watery/soft pudding–like and more yellow/brown stools in the Neo-Syn group (**Table 2**). There were no associations between study product and the most frequent category recorded for spitting up, vomiting, and colic. No associations were found between study product and the most frequent category recorded for flatulence at all time points except at week 2 ($P < 0.001$) (**Table 2**).

More infants had at least one reported adverse event in the Neo (49 (88%)) compared to the Neo-Syn (37 (63%)) group. **Table 3** summarizes all adverse events by system order class and related adverse events by preferred term. The majority of adverse events were categorized as mild and unrelated to the formulations. Six serious adverse events were reported to be unrelated to the formula intake (four unrelated with Neo, two unrelated with Neo-Syn), whereas one (dehydration) was considered possibly related to Neo by the investigator (**Table 3**). This serious adverse event occurred with gastroenteritis and is thus related to the concurrent adverse event. Two serious adverse events resulted in early discontinuation of the study. The most frequent type of adverse events considered formula related were classified as GI disorders. These were highest in the Neo (34 (61%)) vs. the Neo-Syn (24 (41%)) group. The number of infants with reported constipation was higher for the Neo (15 (27%)) compared to the Neo-Syn group (3 (5%)). Reported flatulence was similar between the Neo-Syn and Neo groups (13 (22%)) and 11 (20%), respectively), was classed as mild-moderate and generally decreased over the first weeks of the study. Laboratory parameters were within the specified normal ranges and were not statistically different between the study groups.

Study 2

All 30 infants and children (age range, birth to 3 y) with IgE-mediated CMA included in the study (ITT) completed the double-blind placebo-controlled formula challenge (DBPCFC). Of

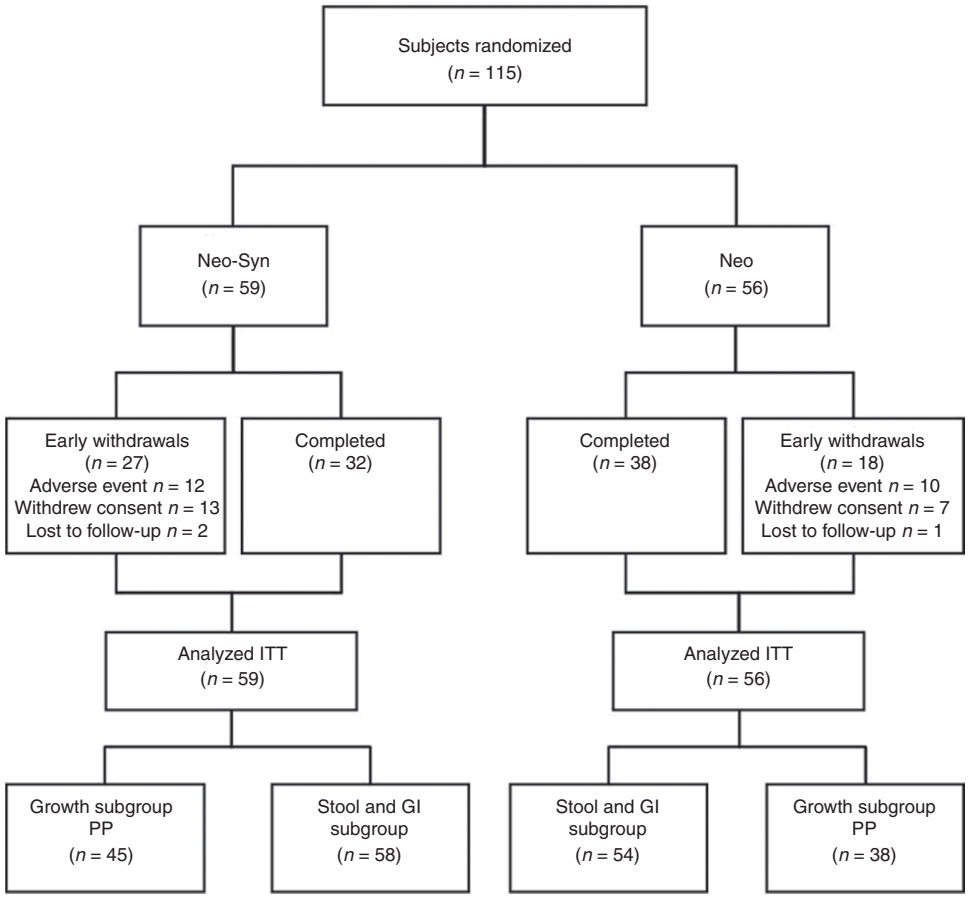

Figure 1. Subjects flowchart of study 1. Intention to treat (ITT), per protocol (PP). GI, gastrointestinal.

Table 1. Study 1: demographic data and subject characteristics at baseline in the study groups; intention to treat (ITT) population

		Neo-Syn (*n* = 59)	Neo (*n* = 56)
Age (months)	Mean (SD)	10.6 (4.1)	10.5 (4.3)
	Min–max	4–16	3–16
Gender			
Male	*n* (%)	35 (59%)	35 (63%)
Female	*n* (%)	24 (41%)	21 (38%)
Length (cm)	Mean (SD)	51.3 (2.3)	51.6 (2.5)
Weight (kg)	Mean (SD)	3.45 (0.45)	3.54 (0.46)
Head circumference (cm)	Mean (SD)	35.1 (1.6)	35.4 (1.3)

Denominator for % is number of subjects in treatment group with nonmissing data.

these, 24 subjects completed the full study PP including the following 7-d feeding period. Four completed 6 d, 1 completed 5 d, and 1 completed 4 d of the 7-d feeding period due to difficulty to protocol adherence.

A clinical history of CMA was confirmed in all 30 subjects who completed the DBPCFC and open challenge; in addition, 25 subjects were diagnosed with a positive reaction to cow's milk protein skin prick test (SPT), and 5 subjects were diagnosed with positive cow milk-specific IgE levels. Diagnosis, formula at study entry, sex, and allergy history are summarized in **Table 4**. Of the 30 subjects who completed the DBPCFC and 7-d feeding period, 23 subjects had other food allergies as reported by their parent or guardian.

All of the 30 subjects in the DBPCFC and open challenge had negative responses to both tests. There were no serious adverse events during the DBPCFC or extended 7-d feeding period reported for infants receiving Neo-Syn. The study provided 95% confidence that at least 90% of infants and children with CMA would have no reaction to Neo-Syn, thus demonstrating the hypoallergenicity of Neo-Syn in infants and children with documented CMA.

DISCUSSION

Global guidelines advise that infants who develop food allergies can benefit from the use of an extensively hydrolyzed formula (eHF) or an AAF (23,24). Clinical data are beginning to suggest not only that the addition of synbiotics to food promotes a healthy gut microbiota but that these microbiota might influence the severity and occurrence of some food allergies by modulating the immune system (8,25,26). The two studies reported herein were designed to evaluate the safety, tolerability, and nutritional impact of a new formulation of the clinically proven Neocate AAF, to which carefully selected synbiotics have been added (Neo-Syn), and to demonstrate the continued

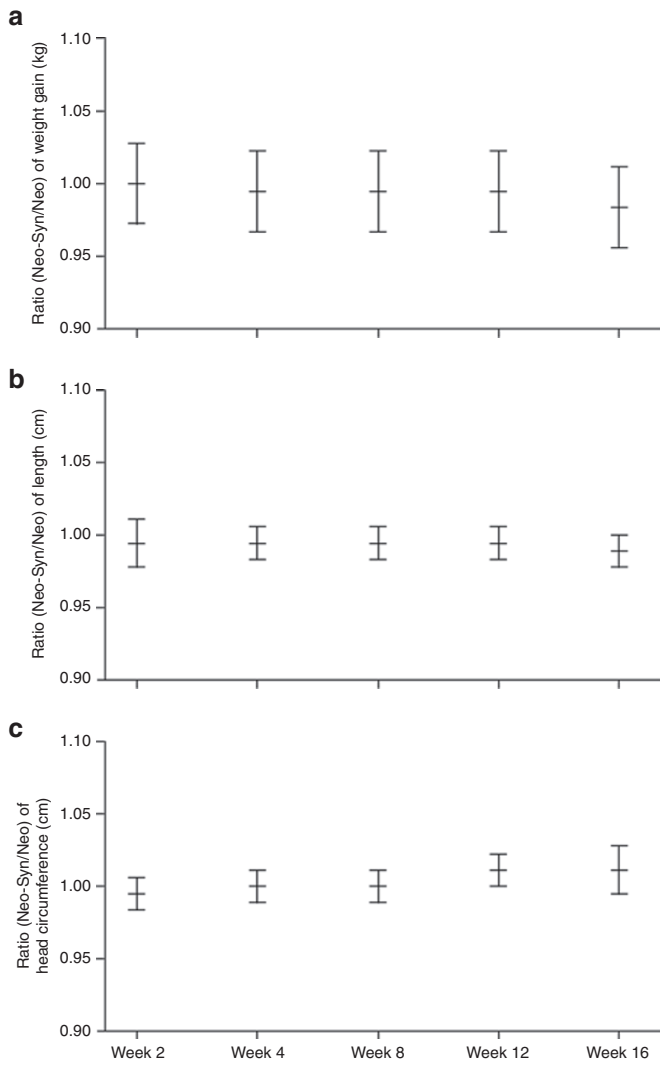

Figure 2. Growth parameter treatment ratios over time of the intention to treat (ITT) population of study 1 for (**a**) weight gain (kg), (**b**) length (cm), and (**c**) head circumference (cm).

hypoallergenicity of Neo-Syn. The results of these studies demonstrate that the Neo-Syn is hypoallergenic in CMA infants and children, safe, well tolerated, and promotes normal growth when fed to healthy full-term infants from birth to 16 wk.

In Study 1, the ability of Neo-Syn to promote adequate growth was comparable to Neo from birth to 16 wk, with no significant differences noted between achieved weight, length and head circumference. Furthermore, similar results were observed when infants who had consumed food other than their allocated formula were excluded.

The dropout rate in Study 1 was high, with the majority of these occurring within the first 2–4 wk of the study and due to early occurrence of a mild adverse event or withdrawal of informed consent; however, it was comparable to other AAF trials in healthy infants with dropout rates of 37% (data not shown) and 33% (27).

Most of the adverse events were unrelated to the formulations. Furthermore, it appears that trials of this nature are

Table 2. Study 1: stool characteristics and gastrointestinal symptoms

		Neo-Syn ($N = 59$)[a]	Neo ($N = 56$)[b]	
		Median (range)	Median (range)	*P* value
Stool characteristics				
Consistency[c]	Week 2	1.88 (1.00, 3.71)	2.70 (1.31, 4.50)	<0.001
	Week 4	1.93 (1.00, 3.00)	2.57 (1.21, 5.00)	<0.001
	Week 8	2.00 (1.00, 3.00)	2.29 (1.71, 3.20)	0.017
	Week 12	2.00 (1.00, 3.00)	2.64 (1.00, 4.00)	0.017
	Week 16	2.00 (1.00, 3.00)	2.57 (2.00, 4.00)	0.047
Frequency[d]	Week 2	2.50 (0.42, 6.14)	2.50 (0.89, 6.50)	0.350
	Week 4	2.38 (0.71, 5.05)	2.50 (0.75, 4.50)	1.000
	Week 8	2.50 (0.71, 5.36)	2.50 (1.43, 2.50)	0.236
	Week 12	2.50 (0.71, 3.07)	2.50 (0.36, 7.07)	0.613
	Week 16	2.50 (0.71, 6.50)	2.50 (1.43, 2.50)	0.048
Color[e]	Week 2	2.29 (1.29, 3.50)	2.00 (1.00, 4.60)	0.115
	Week 4	2.50 (1.00, 3.00)	1.67 (1.00, 3.64)	0.001
	Week 8	2.71 (1.00, 3.00)	1.71 (1.00, 5.00)	0.004
	Week 12	2.40 (1.00, 3.00)	1.64 (1.00, 4.00)	0.033
	Week 16	2.43 (1.00, 3.29)	1.57 (1.00, 4.00)	0.001
Gastrointestinal symptoms				
Spitting up[f]	Week 2	1.86 (1.00, 4.00)	2.00 (1.00, 4.00)	0.787
	Week 4	1.71 (1.00, 2.46)	1.92 (1.00, 4.00)	0.635
	Week 8	1.71 (1.00, 2.50)	2.00 (1.00, 3.00)	0.171
	Week 12	1.43 (1.00, 3.00)	2.00 (1.00, 3.86)	0.313
	Week 16	1.71 (1.00, 3.00)	1.71 (1.00, 3.57)	0.791
Vomiting[f]	Week 2	1.00 (1.00, 4.00)	1.07 (1.00, 4.00)	0.072
	Week 4	1.00 (1.00, 2.07)	1.00 (1.00, 2.50)	0.721
	Week 8	1.00 (1.00, 2.43)	1.00 (1.00, 2.43)	1.000
	Week 12	1.00 (1.00, 1.43)	1.00 (1.00, 4.00)	1.000
	Week 16	1.00 (1.00, 1.86)	1.00 (1.00, 3.00)	1.000
Flatulence[g,h]	Week 2	2.64 (1.00, 4.14)	2.07 (1.00, 3.67)	<0.001
	Week 4	2.46 (1.00, 4.00)	2.21 (1.00, 4.67)	0.112
	Week 8	2.29 (1.00, 3.29)	2.00 (1.00, 3.29)	0.544
	Week 12	2.29 (1.00, 3.43)	2.00 (1.00, 3.86)	0.471
	Week 16	2.14 (1.00, 3.43)	2.00 (1.00, 3.00)	0.682
Colic[g,i]	Week 2	1.07 (1.00, 3.50)	1.00 (1.00, 3.17)	0.074
	Week 4	1.07 (1.00, 3.36)	1.00 (1.00, 3.67)	1.000
	Week 8	1.00 (1.00, 3.00)	1.00 (1.00, 4.00)	1.000
	Week 12	1.00 (1.00, 2.29)	1.00 (1.00, 2.29)	0.842
	Week 16	1.00 (1.00, 2.57)	1.00 (1.00, 2.00)	1.000

[a]Week 2 $n = 49$, Week 4 $n = 36$, Weeks 8, 12, and 16 $n = 27$. [b]Week 2 $n = 53$, Week 4 $n = 43$, Week 8 $n = 28$, Week 12 $n = 30$, Week 16 $n = 29$. [c]1 = watery, 2 = soft pudding-like, 3 = soft formed, 4 = dry formed, 5 = dry hard pellets. [d]1 = no stools, 2 = 1–4 stools, 3 = 5–8 stools, 4 = 9–12 stools, 5 = over 12 stools. [e]1 = green, 2 = yellow, 3 = yellow/brown, 4 = dark brown, 5 = black. [f]1 = none, 2 = slight, 3 = moderate, 4 = severe. [g]1 = none, 2 = mild, 3 = moderate, 4 = severe, 5 = very severe. [h]One patient recorded a value of 3.5, which for the purpose of this summary was recorded as 4 (severe). [i]One patient recorded a value of 2.5, which for the purpose of this summary was recorded as 3 (moderate).

Table 3. Study 1: summary of adverse events

	Neo-Syn ($N = 59$)		Neo ($N = 56$)	
Number of adverse events categorized as				
Mild	84		113	
Moderate	35		57	
Severe	4		4	
	Related	Unrelated	Related	Unrelated
Total number of adverse events	33	90	51	123
All system organ classes				
Appetite disorders	1 (2%)	0	0	0
Blood and lymphatic system disorders	0	0	0	2 (4%)
Congenital, familial, and genetic disorders	0	3 (5%)	0	7 (13%)
Ear and labyrinth disorders	0	0	0	1 (2%)
Eye disorders	0	3 (5%)	0	6 (11%)
Gastrointestinal atonic and hypomotility disorders	0	1 (2%)	0	0
Gastrointestinal disorders	21 (36%)	3 (5%)	29 (52%)	5 (9%)
General disorders and administration site conditions	1 (2%)	0	5 (9%)	3 (5%)
Infections and infestations	0	15 (25%)	0	23 (41%)
Injury, poisoning, and procedural complications	0	0	0	1 (2%)
Investigations	0	1 (2%)	0	2 (4%)
Metabolism and nutrition disorders	0	0	1 (2%)	0
Musculoskeletal and connective tissue disorders	0	2 (3%)	0	4 (7%)
Nervous system disorders	0	1 (2%)	0	0
Pregnancy, puerperium and perinatal conditions	0	5 (8%)	0	5 (9%)
Reproductive system and breast disorders	0	2 (3%)	0	0
Respiratory, thoracic, and mediastinal disorders	0	7 (12%)	0	7 (13%)
Skin and subcutaneous tissue disorders	0	8 (14%)	3 (5%)	16 (29%)
All related adverse events by preferred term				
Decreased appetite	1 (2%)	0	0	0
Abdominal pain	2 (3%)	0	0	1 (2%)
Abdominal feces	0	0	1 (2%)	0
Constipation	2 (3%)	1 (2%)	13 (23%)	2 (4%)
Diarrhea	2 (3%)	1 (2%)	1 (2%)	1 (2%)
Feces hard	0	0	1 (2%)	0
Flatulence	12 (20%)	1 (2%)	11 (20%)	0
Gastroenteritis	2 (3%)	1 (2%)	1 (2%)	2 (4%)
Gastroesophageal reflux disease	2 (3%)	2 (3%)	3 (5%)	4 (7%)
Infantile spitting up	2 (3%)	0	3 (5%)	0
Vomiting	4 (7%)	1 (2%)	4 (7%)	0
Irritability	1 (2%)	0	5 (9%)	1 (2%)
Dehydration[a]	0	0	1 (2%)	0
Eczema	0	2 (3%)	2 (4%)	2 (4%)
Skin odor abnormal	0	0	1 (2%)	0

Related = definitely, probably, or possibly related. Unrelated = unlikely or not related.

[a]Also reported as a serious adverse event (SAE), the only related SAE in the study. The investigator reported that the infant was suffering from intractable vomiting and lymphadenitis.

Table 4. Study 2: subject characteristics

M/F	Weight (kg)	Age (months)	Diagnosis SPT and/or RAST[a]	Diagnosis (SPT-mm) values	Diagnosis (RAST-kU/l) values	Other allergies in addition to CMA	Feeding at entry
M	9.6	12.4	SPT-POS	4		Egg	eHF
M	12.3	19.9	SPT-POS	10		Food allergies	AAF
M	8.3	13.8	SPT-POS	15		Peanut, egg	Breast milk
F	8.5	11.2	SPT-POS	10		Soy, cashew, peanut, eggs	Breast milk
F	11.8	26.2	SPT-POS	7		Food allergies	Rice milk
M	9.3	11.1	SPT-POS	4		Food allergies	Not on formula
M	5.9	3.3	SPT-POS	5		Egg	AAF
M	7.7	7.9	SPT-POS	10		Food allergies	AAF
F	9.4	13.4	SPT-POS	6		Oatmeal, corn, sweet potatoes	AAF
M	11.3	17.1	SPT-POS	5		Amoxicillin	Soy milk
M	8.8	12.1	SPT-POS	7		Peanut	Soy formula
M	16.3	40.2	SPT-POS	9		Food allergy	Soy milk
M	8.5	7.2	SPT-POS	3		Food allergies	AAF
F	12.2	20.9	SPT-POS	7		Food allergies	Soy milk
M	9.3	13.8	SPT-POS	5		Food allergies	Not on formula
M	8.6	9.0	SPT-POS	4		Eggs, peanut	AAF
M	6.1	3.8	SPT-POS	7		None	Soy formula
F	7.0	6.2	SPT-POS	3		None	eHF
F	15.6	46.9	SPT-POS	5	(57.50)	Soy	Not on formula
M	15.0	41.1	SPT-POS	5	(97.00)	None	Not on formula
F	7.5	9.0	SPT-POS	5		Soy allergy	eHF
M	9.1	14.4	SPT-POS	4		None	eHF
M	13.1	16.3	RAST		11.09	Peanut, egg white, soy	eHF
F	10.2	21.3	SPT-POS	20	(57.30)	Food allergies	Breast milk
M	8.66	13.7	RAST		27.80	Soy, peanut, egg, fish	Rice milk
F	9.0	13.3	SPT-POS	3.5		None	Soy formula
M	9.0	21.7	RAST		28.00	Egg, peanut	AAF
M	10.0	19.5	RAST		9.50	Egg, soy, peanut	Breast milk
M	9.6	16.8	RAST		81.40	Egg, soy, peanut	AAF
F	12.3	35.9	SPT-POS	10		None	AAF

AAF, amino acid–based formula; CMA, cow's milk allergy; eHF, extensively hydrolyzed formula; F, female; M, male; RAST, radioallergosorbent test; SPT-POS, skin prick test positive.

[a]In addition to confirmed clinical history of a reaction to cow's milk protein.

prone to high dropout rates, notably due to fact that these were mothers of healthy infants whose infants would not typically require an AAF, inexperienced in the use of an AAF, concerned by changes in stool characteristics, unable to maintain their trial motivation, and their willingness to frequently change formula. AAFs are known to lead to changes in stool color and consistency and may result in increased flatulence on transition to such a formula, the addition of synbiotics may alleviate some of these changes. Neo-Syn fed infants had more watery/soft pudding-like stools, however diarrhea adverse events were low (three in Neo-Syn and two in Neo), and the Neo group had more reported adverse events of constipation. The occurrence of softer stools with synbiotic formula compared to standard formula has been reported previously (28) and is more comparable to breastfed infants who have softer stools than those who are formula fed (29). Recently, it has been shown that eHFs high in specific amino acids, i.e., glutamate, influence dietary intake (30) and growth patterns (31) in healthy infants. However, in contrast to eHFs and other AAFs, Neo-Syn and Neo do contain glutamine instead of glutamate which might have minimal impact on differential formula acceptance in comparison to breast milk or cow-milk formulas.

Study 2 results are in line with previous clinical studies to evaluate the hypoallergenicity of an AAF in CMA infants and children (7). Values of cow's milk-specific IgE have been established for the diagnosis of CMA and these values were used in combination with confirmed clinical history and positive SPT results as part of the inclusion criteria of all subjects in Study 2.

Although DBPCFC is considered the gold standard tool for diagnosing CMA, it was considered excessively interventional and possibly traumatic to perform two DBPCFCs in this population. Similar inclusion criteria were used in previous studies to evaluate the hypoallergenicity of other AAFs currently on the market (27,32). All of the 30 subjects who completed the DBPCFC and 7-d feeding period had negative responses to both tests. There were no serious adverse events during the DBPCFC or extended 7-d feeding period reported for infants receiving Neo-Syn. The study provided 95% confidence that at least 90% of infants and children with CMA would have no reaction to Neo-Syn. The number of drop outs and formula related adverse events reported in the CMA population of Study 2 was very low compared to healthy infants in Study 1—this suggests that in the intended population group for AAFs any transitional effects of these formulas are outweighed and accepted due to the recognized ability to quickly relieve allergic symptoms.

Children with CMA have been shown to have higher fecal bacteria counts with a proportionally higher anaerobic bacteria and lower yeast count compared to healthy children (33). Animal studies have shown that the use of probiotics can reduce the severity of AD (34) and CMA in mice fed synbiotics (35). However, the varying results from clinical studies due to heterogeneity of effects and differences between bacterial strains utilized make it difficult for specific recommendations to be made regarding the use of synbiotics in allergy prevention or treatment. In a treatment setting in a subgroup of infants with IgE-mediated AD, a similar synbiotic blend comprising a specific sc-galacto-oligosaccharide/lc-fructo-oligosaccharides mixture (9:1, 0.8 g/100 ml) and *B. breve* M-16V altered the composition of the gut microbiota with a significant decrease in skin symptoms measured by SCORAD (15). In a prevention setting in a study investigating the occurrence of AD, 414 healthy infants receiving a prebiotic formula (containing a mix of neutral sc-galacto-oligosaccharide/lc-fructo-oligosaccharides) had a lower risk of developing AD at 1 y of age compared to the 416 infants receiving regular formula without oligosaccharides (14).

Past studies with AAFs have demonstrated safety and efficacy in the treatment of CMA and other food allergies. However, up until now these commercially available formulas have not added prebiotics or probiotics. CMA children have been shown to be sensitive to certain probiotics by SPT; however, this is because some probiotics contain cow's milk protein at a high enough level to elicit a response (16). The prebiotic components and probiotic strain in the Neo-Syn formula have been carefully chosen and do not contain any cow's milk protein or other known allergens. It is hoped that the addition of synbiotics to the already improved Neo formula will further promote the health of CMA infants by improving gut health and immunity. In these first clinical trials of an AAF supplemented with synbiotics, we clearly show that infant growth is comparable for the Neo and Neo-Syn formulas by week 16 demonstrating that the addition of the synbiotic had no negative effect on the previously proven ability of Neo to promote adequate growth when fed to healthy full-term infants when used as a sole source of nutrition. In addition, we also demonstrate that the addition of synbiotics to Neo had no negative effect on its proven hypoallergenicity in infants and children with CMA and multiple food allergies. Further longer term studies are underway to investigate the role of this synbiotic AAF on the natural history of CMA.

METHODS

Study 1: Growth and Tolerance

Study design. This prospective double-blind randomized controlled trial (DB-RCT) was conducted in 11 clinical sites in the United States. Healthy full-term infants aged from birth to 15 d were randomized to one of two study groups to receive either Neo (Nutricia, SHS International, Liverpool, UK) or Neo-Syn (Nutricia, SHS International).

Neo-Syn contained a synbiotic blend consisting of: *B. breve* M-16V (Morinaga Milk Industry, Tokyo, Japan) at a dose of 1.47×10^9 CFU/100 ml (10^8 CFU/g powder); a mixture of neutral fructo-oligosaccharides i.e., inulin derived oligofructose (Beneo P95 Raftilose P95) (Beneo Orafti S.A., Oreye, Belgium) and long-chain inulin (Beneo HP Raftiline HP) (Beneo Orafti S.A.) and present in a 9:1 ratio; and a specific pectin-derived acidic oligosaccharides. The total amount of oligosaccharides was 8 g/l with 6.8 g/l neutral oligosaccharides (85 weight %), and 1.2 g/l pectin-derived acidic oligosaccharides (15 weight %). Other key differences included an increased medium chain triglyceride concentration (33 g/100 g total fatty acids compared to 4 g/100 g total fatty acids in control) and an alternative source of essential fatty acids (17.5% fat as low erucic acid rapeseed oil, canola oil instead of soy oil).

Growth (weight, length, and head circumference), acceptance (dietary intake), tolerance (GI symptoms), and the occurrence of adverse events were compared between the two groups. Adverse events were reported and classified as related (definitely, probably or possibly related) or unrelated (unlikely or not related) during the study based on a relationship to study product intake according to the investigator. The caregivers were required to bring their infant to the study site on six occasions for assessment and data collection; day 1, day 14 (±2 d), day 28 (±2 d), day 56 (±4 d), day 84 (±4 d), and day 112 (±4 d). Body weight, length, and head circumference were determined and recorded at each study visit. Diaries recording volume of formula intake, stool characteristics (consistency, frequency, and color), and GI symptoms (spitting up, vomiting, flatulence, and colic) were completed by the parents/caregivers for the first 4 wk of the study and for 1 wk prior to each subsequent study visit. Blood samples were taken on days 1 and 112 for blood urea nitrogen, creatinine, sodium, potassium, chloride, and carbon dioxide analysis. The study design was approved by a central or site-specific Institutional Review Board for each clinical site, and all parents/caregivers provided written informed consent.

Study population. Full-term infants aged from birth to 15 d were considered eligible for the study if they had a documented informed consent (provided by parent/caregiver) and could receive study formula as their sole source of nutrition throughout the study duration. Infants weighing <5 lb 8 oz (2.5 kg) at birth or who had <37 wk gestation, severe concurrent illness, major congenital malformations, systemic or congenital infections, significant cardiac, respiratory, hematological, GI or other systemic diseases, or received systemic antibiotics prior to study entry were excluded.

Statistical analysis. The primary outcome measure for this trial was growth, and therefore a statistical power of 0.8 was required to be sufficient to allow for the detection of the smallest, clinically meaningful difference in growth increments between the groups. The AAP recommends that the smallest meaningful difference in infant growth increments is 3 g/d between the ages of 14 and 120 d. Given that the study population in this trial was healthy infants, daily weight increments were likely to be similar and therefore the AAP recommendation of

28 infants per study group is applicable (36); however, as this study was conducted in the United States, the growth SD used by the AAP to generate their sample size recommendation is applicable to this study. Therefore, 30 infants per arm of the study were required to detect a clinically relevant difference in weight gain. ITT analysis was performed with all subjects who were randomized. The primary endpoint was attained growth (i.e., incremental gains in weight) over the study period. Length and head circumference measures were also collected and analyzed. Both the growth velocities of the group as a whole (cross-sectional group data) and individuals (longitudinal individual data) were reviewed and analyzed. In assessing group data, comparisons of increments per unit of time were made. Due to the varying ages of the subjects recruited to the study, growth measures were converted to Z-scores using an appropriate reference population to allow for a meaningful comparison of the ability of the formulae to promote growth. In assessing the growth of individual infants, data were plotted on National Centre for Health Statistics growth charts and assessed qualitatively. Binary and categorical data were assessed using Fisher's exact test. PP, or "growth subgroup," analyses were performed, as above, but without those patients who did not solely consume the formula they were randomized to during the study. Summary statistics were presented by week for the stool characteristics and GI symptoms.

Study 2: Hypoallergenicity

Study design. The hypoallergenicity of Neo-Syn used in Study 1 was evaluated in a separate DB-RCT of 30 infants aged birth to 3 y with documented CMA at 13 sites in the United States. Study 2 was designed to assess the hypoallergenicity of the Neo-Syn using the AAP's Nutrition and Allergic Diseases Subcommittee's criteria (23). These criteria state that a formula must be tested in infants/children with proven CMA by elimination-challenge tests under double-blind placebo-controlled conditions. To prove hypoallergenicity, the tests should show no reaction to Neo-Syn in 90% of allergic individuals with at least 95% confidence (23,37).

Therefore, we evaluated Neo-Syn using a DBPCFC where infants were randomized to receive either Neo-Syn, followed by Neo or vice versa. Following the completion of the DBPCFC, all subjects underwent a 7-d postchallenge feeding period as follows:

- Subjects aged birth ≤8 mo continued feeding for 7 d with the blinded study product they were first exposed to during the DBPCFC procedure.
- Subjects aged >8 mo to 3 y were given the test product for 7 d.

Acceptance (dietary intake), hypoallergenicity (clinical symptoms), and adverse events were compared between the two groups. The caregivers were required to bring their infant to the study site on three occasions for assessment and data collection; day −7, day 0 (±2 d), and day 14 (±2 d). On day 7 (±1 d) the parents/caregivers of infants aged birth ≤8 mo were contacted by telephone and infants aged >8 mo to 3 y were asked to return to the study site. Body weight, length, and head circumference were recorded at each study visit. Diaries recording volume of formula intake, stool characteristics (consistency, frequency, and color) and clinical symptoms were completed by the parents/caregivers. The study design was approved by a central or site-specific Institutional Review Board for each clinical site, and all parents/caregivers provided written informed consent.

Study population. Infants aged from birth to 3 y old with confirmed IgE-mediated CMA were considered eligible for the study if they had a documented informed consent (provided by parent/caregiver). Infants weighing <5 lb 8 oz (2.5 kg) at birth or had <37 wk gestation, severe concurrent illness, major congenital malformations, systemic or congenital infections, significant cardiac, respiratory, hematological, GI, or other systemic diseases were excluded. Subjects aged birth to <8 mo were required to exclude all other sources of synbiotics from their diet for 2 wk prior to study entry and during the study, and must not have received systemic antibiotics prior to study entry.

Confirmation of CMA was required before study entry by either a positive DBPCFC with cow's milk, or an acute severe reaction after accidental ingestion and with a positive IgE test, or a confirmed clinical history of a reaction to cow's milk protein with cow's milk-specific IgE of >15 kU/l (infants >2 y)/>5 kU/l (infants <2 y) (38,39), or a confirmed clinical history of a reaction to cow's milk protein with a positive SPT with a resulting wheal diameter ≥3 mm (40), or no confirmed clinical history of a reaction to cow's milk protein with a positive SPT with a resulting wheal diameter ≥8 mm (infants >2 y)/≥6 mm (infants <2 y).

Analyses were described as ITT with all randomized subjects and PP with subjects who completed both DBPCFC and the 7-d feeding period.

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