

Growth and Tolerance of Healthy Term Infants Receiving Hydrolyzed Infant Formulas Supplemented With *Lactobacillus rhamnosus* GG: Randomized, Double-Blind, Controlled Trial

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Healthy, term infants received extensively hydrolyzed casein formula (EHF; control), the same formula supplemented with *Lactobacillus rhamnosus* GG (EHF-LGG), or partially hydrolyzed whey:casein (60:40) formula supplemented with LGG (PHF-LGG), in this double-blind, randomized, controlled, parallel, prospective study. Anthropometric measures and 24-hour dietary and tolerance recalls were obtained at 30, 60, 90, 120, and 150 days of age. Blood collected in a subset of infants was analyzed for fatty acid profiles in plasma and red blood cells and for markers of allergic sensitization. Adverse events were recorded throughout the study. Growth

rates were not statistically different between EHF and PHF-LGG and between EHF and EHF-LGG from day 14 to day 30, 120, or 150. No relevant differences in formula tolerance, adverse events, or allergic and immune markers were demonstrated between groups. The extensively and partially hydrolyzed formulas supplemented with LGG support normal growth in healthy, term infants and are well tolerated and safe.

Keywords: infant formula; extensively hydrolyzed formula; partially hydrolyzed formula; LGG; infant growth; allergy; polyunsaturated fatty acids

Introduction

Supplementation of infant formula with probiotics, live microorganisms capable of colonizing the intestinal tract to beneficially influence host health,¹ may provide benefits beyond general nutrition. *Lactobacillus rhamnosus* GG (LGG), a

probiotic studied for more than 20 years,² reduces the incidence and duration of diarrhea in children.^{3,4} When added to extensively hydrolyzed (EH) formula, LGG was well tolerated and transiently colonized the intestine in healthy infants⁵; decreased the severity of atopic dermatitis (AD) in infants,^{6,7} especially those with IgE-associated AD⁸; and reduced systemic and intestinal inflammation in infants with cow milk allergy (CMA).^{6,7} LGG supplementation of atopic women during pregnancy combined with postnatal infant supplementation was associated with lower incidence of AD in infants at ages 2, 4, and 7 compared with an unsupplemented group.⁹

Several mechanisms of gut barrier reinforcement can explain the clinical effects of LGG: normalization

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of increased intestinal permeability, consequently decreasing antigen uptake¹⁰; counteraction of gut microbial imbalance¹¹; enhancement of intestinal IgA responses¹²; and alleviation of allergic inflammation⁶ notably through IL-10 induction.¹³ Additionally, specific cross talk of LGG with gut epithelial cells initiates local and systemic inflammation-related and immune-related events, especially through induction of transforming growth factor- β ,¹⁴ an important regulatory cytokine in atopic disease.¹⁵

According to the hygiene hypothesis,¹⁶ the worldwide rise in the incidence of allergic diseases is related to reduced exposure to infections early in life when the immune response phenotype is consolidated.¹⁷ Association of immune response to microbial antigens with preferential expression of Th1 cytokines¹⁸ counterbalances the typical Th2-skewed immune response at birth,¹⁹ whose continuation would lead to atopic diseases.¹⁸ The gastrointestinal system, which harbors the largest lymphoid tissue and bacterial reservoir of the body, has a key role in the maturation of the immune system to a nonatopic profile. In this context, probiotics may help correct the quantitative and qualitative microbiota imbalance observed in atopic children when compared with healthy children,²⁰ even before the onset of allergy.²¹

CMA affects 2.2% to 2.5% of infants and children up to 3 years of age.^{15,22} EH formulas are recommended for formula-fed infants diagnosed with CMA.^{23,24} Possible benefits from LGG supplementation of EH or partially hydrolyzed (PH) formula might affect the severity and/or long-term prevalence of allergies and infections during infancy and childhood.^{3,4,6-9} The primary objective of this study was to evaluate the effect of an EH or PH formula supplemented with LGG on growth and tolerance in healthy, term infants from 14 to 120 days of age. Secondary objectives were to analyze the incidence of allergies and infections as well as allergic sensitization and antibody response to routine vaccinations in a subset of infants who received study formula up to 150 days of age. In this subset, fatty acids (FAs) were analyzed to determine the effect of LGG formula supplementation on plasma and red blood cell (RBC) FA profiles.

Methods

Study Population

Eligible infants were of 38 to 42 weeks gestational age, with birth weight ≥ 2500 g and solely formula-fed at

least 24 hours prior to randomization. Exclusion criteria included history of underlying disease or congenital malformation likely to interfere with normal growth and development or participant evaluation, formula intolerance, weight at 14 days of age $< 98\%$ of birth weight, large for gestational age born from a mother diabetic at childbirth, immunodeficiency, fever at randomization, systemic antibiotic use within 7 days of randomization, systemic steroid use any time since birth, LGG-supplemented diet any time since birth (including infants breast-fed when mother consumed dietary LGG), and diarrhea within 24 hours prior to randomization. Parents or guardians provided written informed consent prior to enrollment. The research protocol and informed consent forms observing the Declaration of Helsinki (including October 1996 amendment) were approved by the institutional review board/ethics committee of each participating institution. The study complied with good clinical practices.

Design

This double-blind, randomized, controlled, parallel, prospective study was conducted at 14 sites in the United States (see Acknowledgments for participating physicians and site personnel) between June 2006 and April 2007. The objective was to evaluate growth and tolerance in healthy, term infants enrolled at 14 days of age and randomized to receive a control EH casein formula (EHF; Nutramigen LIPIL, Mead Johnson & Company, Evansville, IN), the same formula supplemented with LGG (EHF-LGG), or a partially hydrolyzed whey:casein (60:40) formula supplemented with LGG (PHF-LGG) through 120 days of age. A subset of infants continued to receive study formula through 150 days of age. All formulas were supplemented with 0.64% arachidonic acid (ARA) and 0.32% docosahexaenoic acid (DHA) of total FAs, amounts similar to worldwide human milk medians.^{25,26} EHF-LGG and PHF-LGG were supplemented with 10^8 colony forming units (CFU) of LGG per gram of formula powder. The study sponsor created a computer-generated, gender-stratified randomization schedule, provided in sealed consecutively numbered envelopes, for each study site. On infant enrollment, study formula was assigned by opening the next sequential envelope from the appropriate set at the study site. Study formulas, each designated by 2 unique codes known only to the sponsor, were dispensed to parents at

each study visit prior to completion or withdrawal. Anthropometric measures (body weight, length, and head circumference) were recorded at randomization and at 30, 60, 90, and 120 days of age for all infants. Anthropometric measures and blood samples were obtained in a subset at 150 days of age.

Parents provided information on family history of allergy in one or more first-degree relatives and completed 24-hour dietary and tolerance recalls on formula intake, stool frequency and consistency, level of fussiness and gas, and occurrence of diarrhea and constipation at all study visits subsequent to randomization. Immunizations, medications, and adverse events were recorded throughout the study. The primary outcome was weight growth rate from 14 to 120 days of age. Secondary outcomes included other anthropometric and tolerance measures, blood FA profiles, serum IgE and IgG, and adverse events.

Blood Sample Analysis

Blood samples were collected by venipuncture into EDTA vacutainer tubes. Plasma and RBCs were separated by centrifugation, frozen, and shipped on dry ice to the Retina Foundation of the Southwest (Dallas, TX), where lipids were isolated and analyzed by capillary column gas chromatography as previously described.²⁷ FA methyl esters were expressed as percentage of total FAs by weight for RBC phosphatidylethanolamine (PE), RBC phosphatidylcholine (PC), total RBCs, plasma phospholipids, and total plasma. A central clinical laboratory (North Coast Clinical Laboratory, Sandusky, OH) analyzed total serum IgE by enzyme immunoassay (EIA) and specific IgE to cow milk protein (CMP) by fluorescent EIA; IgG to diphtheria, tetanus, and *Hemophilus influenzae* type b (Hib) by EIA; and IgG to poliovirus types 1, 2, and 3 by indirect immunofluorescence. Two routine immunizations against diphtheria and tetanus (DtaP), Hib, and inactivated polio vaccine (IPV) were required for measurement of IgG response to be included in statistical analyses.

Statistical Analysis

A sample size of 51 participants per formula group was chosen to detect a clinically relevant difference of 3 g/d in weight gain from 14 to 120 days of age (80% power, 1-tailed). Analysis of variance (ANOVA) was used to assess growth rates from 14 to 30, 60, 90, 120, or 150 days of age calculated for each participant by

linear regression of weight on age. Mean weight growth rates for each LGG-supplemented formula group were compared with those for the control group using 1-tailed tests. For all secondary outcomes, overall comparisons for the 3 formula groups were 2-tailed. Unadjusted pairwise comparisons were performed if the overall test was statistically significant. All tests were conducted at $\alpha = .05$. Achieved weight, length, and head circumference; length and head circumference growth rates; and formula intake, stool frequency, and blood FAs were analyzed by ANOVA. Stool consistency, fussiness, and gas were analyzed using the Cochran-Mantel-Haenszel row means score test. Incidence of diarrhea, constipation, and adverse events were analyzed using Fisher's exact test. Serum IgG and total IgE were evaluated using the Cochran-Mantel-Haenszel row means score test; IgE to CMP was analyzed by Fisher's exact test. Post hoc analysis included adverse events classified as allergy- or infection-related. All analyses were conducted using SAS version 9.1 (Cary, NC).

Results

Participants

A total of 289 participants were randomized (EHF, 95; EHF-LGG, 95; PHF-LGG, 99). Three participants (1 per group) were randomized but consumed no study formula and were not included in subsequent analyses of the intent-to-treat population (Figure 1). A total of 210 infants completed the study (EHF, 70; EHF-LGG, 63; PHF-LGG, 77). Body weight, length, head circumference, and family history of allergy were similar for infants in all groups at enrollment (Table 1), as were race and ethnic distribution, birth anthropometric measures, and smoking in the home (data not shown).

Growth

No significant differences in growth rates were observed between groups, except for significantly higher mean daily weight gain in EHF versus the EHF-LGG group from days 14 to 60 or 90 ($P < .05$; Table 2). Mean achieved body weight, length, and head circumference were not statistically different between groups at all measured time points (data not shown). Mean achieved weight for male (Figure 2) and female (Figure 3) participants plotted on the US

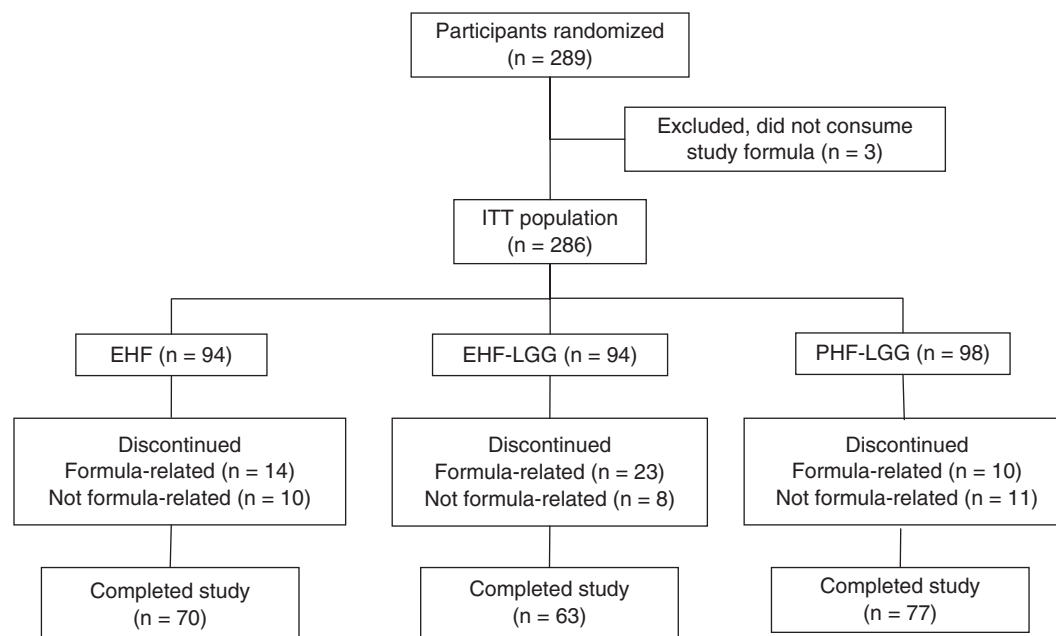


Figure 1. Flow of participants through enrollment, randomized allocation to study formula groups, and study discontinuation or completion.

Table 1. Infant Characteristics at Study Entry^a

	EHF	EHF-LGG	PHF-LGG
Total number of participants	94	94	98
Number of boys/girls	44/50	50/44	49/49
Weight (g) ^{a,b}	3698 ± 45	3720 ± 45	3673 ± 44
Length (cm) ^{a,b}	52.2 ± 0.2	52.3 ± 0.2	52.1 ± 0.2
Head circumference (cm) ^{a,b}	35.9 ± 0.1	36.0 ± 0.1	35.9 ± 0.1
Family history of allergy ^c	51 (54%)	60 (64%)	62 (63%)

Note: EHF, extensively hydrolyzed casein formula; EHF-LGG, EHF supplemented with *Lactobacillus rhamnosus* GG (LGG); PHF-LGG, partially hydrolyzed whey:casein (60:40) formula supplemented with LGG.

a. Least square means ± standard error.

b. No significant differences between groups.

c. Number of infants (%).

Centers for Disease Control (CDC) weight-for-age standard growth chart²⁸ fell between the 25th and 75th percentiles.

Tolerance

Parental assessment of formula intake was similar in all groups at all measured time points (data not shown). Stool frequency was significantly lower in the PHF-LGG at days 30, 90, and 120 (2.7 ± 0.19 , 2.0 ± 0.17 , 2.0 ± 0.16 , respectively) than in

the EHF (3.4 ± 0.20 , 2.7 ± 0.18 , 2.6 ± 0.17) or EHF-LGG (3.4 ± 0.22 , 2.6 ± 0.19 , 2.4 ± 0.17) groups ($P < .05$). Firmer stools were reported in the PHF-LGG versus the EHF-LGG group at day 90 ($P = .005$) and versus both the EHF and EHF-LGG groups at days 30 and 120 ($P < .05$). Significantly more gas was observed at day 30 in the PHF-LGG than in the EHF and EHF-LGG groups ($P < .05$). Less fussiness was reported at day 60 in the EHF versus EHF-LGG or PHF-LGG groups ($P < .05$). Parental reports of diarrhea and

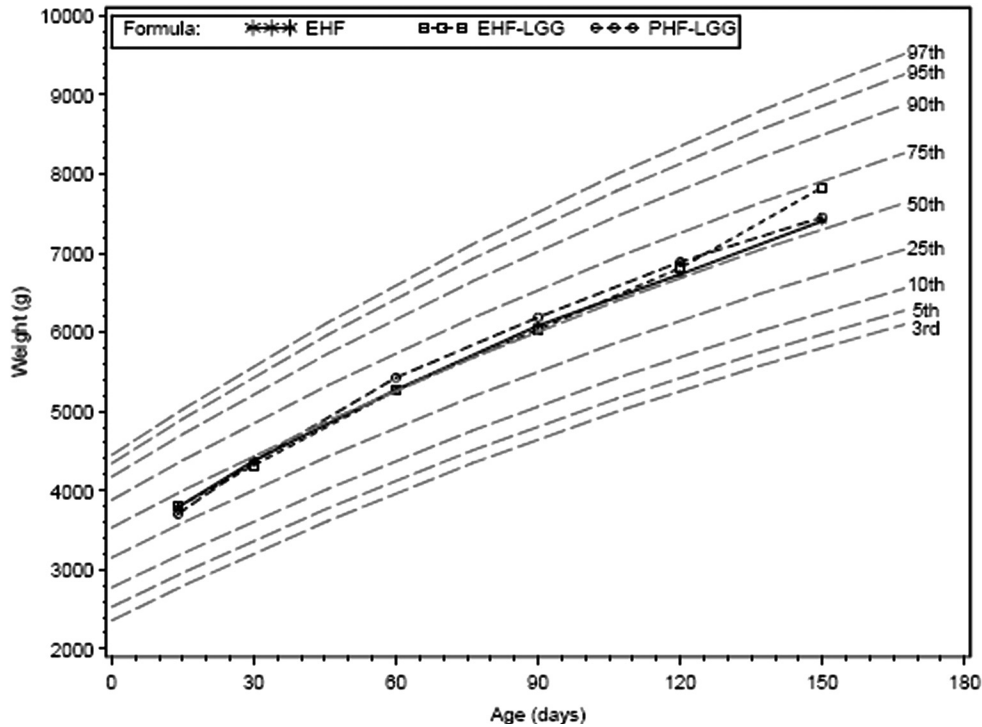


Figure 2. Mean achieved weight of male participants with Centers for Disease Control reference percentiles (3rd to 97th) from 14 to 150 days of age. EHF, stars; EHF-LGG, squares; PHF-LGG, circles. The curve from 120 to 150 days refers to a subset of participants.

Abbreviations: EHF, extensively hydrolyzed casein formula; EHF-LGG, EHF supplemented with *Lactobacillus rhamnosus* GG (LGG); PHF-LGG, partially hydrolyzed whey:casein (60:40) formula supplemented with LGG.

constipation were low (0% to 10% and 0% to 4%, respectively) and similar between groups at all measured time points.

No differences between groups were observed in study discontinuation (EHF: $n = 24$, 26%; EHF-LGG: $n = 31$, 33%; PHF-LGG: $n = 21$, 21%; $P = .19$) and discontinuation related to study formula (EHF: $n = 14$, 15%; EHF-LGG: $n = 23$, 24%; PHF-LGG: $n = 10$, 10%; $P = .12$). In the total study population, 25 participants (9%) discontinued because of formula intolerance; the most common symptoms were fussiness, vomiting, and gas (14, 10, and 10 reports, respectively). The most common reason for discontinuation unrelated to study formula was parental decision ($n = 13$, 17%).

Incidence of adverse events was similar between groups; the most common were upper respiratory infection (27%), nasal congestion (21%), gas (17%), otitis media (16%), and diaper rash (15%). The only significant difference in adverse events was excessive crying: 4 (4%) in the PHF-LGG group versus none

in the EHF and EHF-LGG (groups) ($P = .036$). Post hoc analysis demonstrated no significant group differences in incidence of adverse events categorized as infection-related (EHF, 62%; EHF-LGG, 52%; PHF-LGG, 50%) or allergy-related (EHF, 10%; EHF-LGG, 18%; PHF-LGG, 15%). Serious adverse events (EHF, $n = 3$; EHF-LGG, $n = 5$; PHF-LGG, $n = 8$) were unrelated to study formulas as assessed by study physicians, with the exception of one infant in the PHF-LGG group considered intolerant to study formula and one infant in the EHF-LGG group with gastroesophageal reflux, whose relationship with study formula was undetermined.

Fatty Acids

Blood $\omega 3$ and $\omega 6$ (polyunsaturated fatty acids) PUFAs from a subset of participants at 150 days of age generally did not differ between groups (Table 3). DHA, ARA, linoleic, and α -linolenic acids were similar between groups, with the exception of total plasma

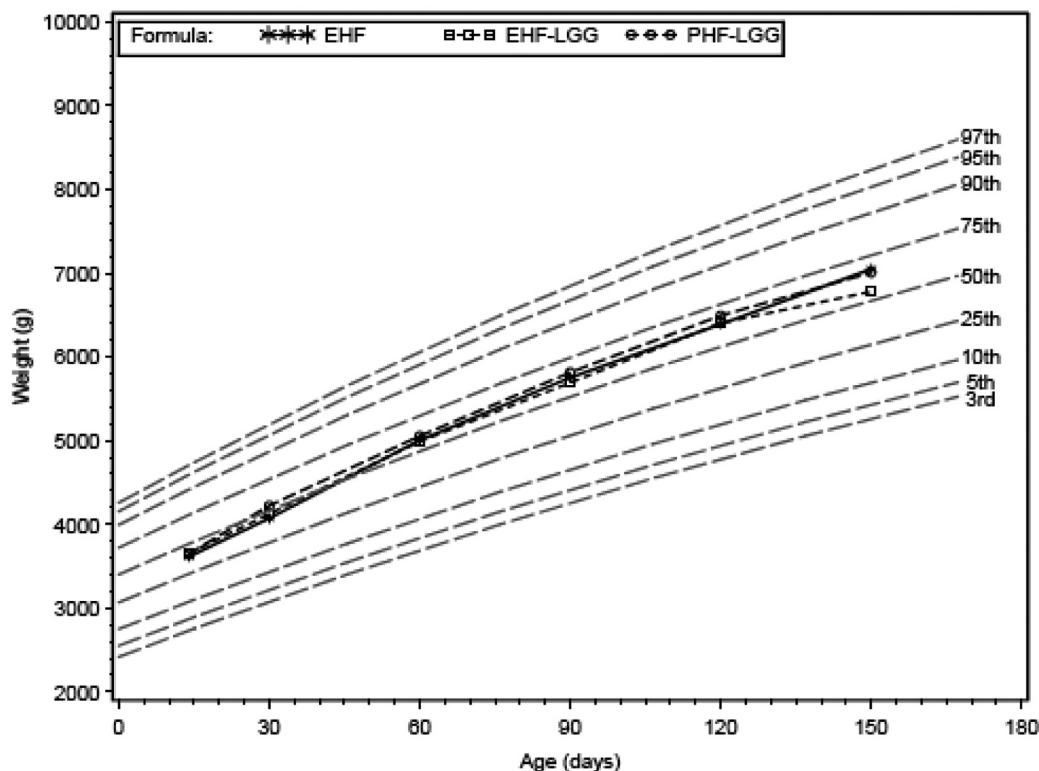


Figure 3. Mean achieved weight of female participants with Centers for Disease Control reference percentiles (3rd to 97th) from 14 to 150 days of age. EHF, stars; EHF-LGG, squares; PHF-LGG, circles. The curve from 120 to 150 days refers to a subset of participants.

Abbreviations: EHF, extensively hydrolyzed casein formula; EHF-LGG, EHF supplemented with *Lactobacillus rhamnosus* GG (LGG); PHF-LGG, partially hydrolyzed whey:casein (60:40) formula supplemented with LGG.

ARA (PHF-LGG lower than EHF or EHF-LGG; $P < .05$). In the PHF-LGG group, eicosapentaenoic acid (20:5 ω 3) was significantly lower in RBC PE, RBC PC, and plasma phospholipids as compared with the EHF or EHF-LGG ($P < .05$) groups and lower in total RBCs when compared with the EHF-LGG group ($P < .05$). With regards to RBC PC for ω 3 docosapentaenoic acid (22:5 ω 3), it was lower in the EHF or PHF-LGG groups than in the EHF-LGG group ($P < .05$); ω 6 docosapentaenoic acid (22:5 ω 6) was lower in total plasma in the EHF or EHF-LGG groups compared with the PHF-LGG group ($P < .05$). All mean values noted above demonstrating significant group differences, other than total plasma ARA, were less than 0.3% of total FAs and, thus, were not considered clinically relevant.

Allergic Sensitization and Response to Vaccinations

IgE data were available for 75 participants. No group differences were observed for total serum IgE

($P = .98$) or specific IgE to CMP ($P = .63$; data not shown). No group differences were observed for specific IgG to tetanus ($P = .53$), diphtheria ($P = .53$), Hib ($P = .24$), and polioviruses ($P \geq .71$; data not shown).

Discussion

This study demonstrated that the growth of healthy, term infants who received LGG-supplemented EH or PH formula was comparable with that of infants who received unsupplemented EH formula from 14 to 120 days of age, with no formula tolerance issues. Growth rates differed only from 14 to 60 or 90 days between the EHF and EHF-LGG groups, with daily weight gain being significantly higher in the EHF group. However, mean daily weight gain in all groups was close to the age-group standard (30 g/d).²⁹ In addition, the difference in weight growth rate from the 14- to 120-day age range between groups was less than 3 g/d and,

Table 2. Weight, Length, and Head Circumference Growth Rates From Day 14 to Days 30, 60, 90, 120, and 150^a

Day	Group	Growth Rate		
		Weight (g/d)	Length (cm/d)	Head Circumference (cm/d)
30	EHF (n = 85)	35.0 ± 1.53	0.14 ± 0.009	0.08 ± 0.005
	EHF-LGG (n = 71)	32.1 ± 1.67	0.13 ± 0.010	0.08 ± 0.005
	PHF-LGG (n = 91)	38.8 ± 1.49	0.15 ± 0.008	0.08 ± 0.005
60	EHF (n = 73)	32.5 ± 1.00 ^b	0.13 ± 0.004	0.07 ± 0.002
	EHF-LGG (n = 65)	29.8 ± 1.06	0.12 ± 0.004	0.07 ± 0.002
	PHF-LGG (n = 80)	33.7 ± 0.94	0.13 ± 0.004	0.07 ± 0.002
90	EHF (n = 72)	29.9 ± 0.83 ^b	0.11 ± 0.003	0.06 ± 0.001
	EHF-LGG (n = 65)	28.0 ± 0.87	0.11 ± 0.003	0.06 ± 0.001
	PHF-LGG (n = 78)	30.4 ± 0.78	0.11 ± 0.002	0.06 ± 0.001
120	EHF (n = 70)	27.6 ± 0.72	0.11 ± 0.002	0.05 ± 0.001
	EHF-LGG (n = 63)	26.8 ± 0.76	0.11 ± 0.002	0.05 ± 0.001
	PHF-LGG (n = 77)	28.4 ± 0.67	0.11 ± 0.002	0.05 ± 0.001
150 ^c	EHF (n = 31)	26.6 ± 1.00	0.10 ± 0.003	0.05 ± 0.001
	EHF-LGG (n = 23)	26.6 ± 1.15	0.10 ± 0.003	0.05 ± 0.002
	PHF-LGG (n = 29)	27.2 ± 1.00	0.10 ± 0.003	0.05 ± 0.001

Note: EHF, extensively hydrolyzed casein formula; EHF-LGG, EHF supplemented with *Lactobacillus rhamnosus* GG (LGG); PHF-LGG, partially hydrolyzed whey:casein (60:40) formula supplemented with LGG.

a. Least square mean ± standard error.

b. Significantly higher than the EHF-LGG group; *P* < .05, 1-tailed test.

c. Data at day 150 were from a subset of infants.

therefore, not considered nutritionally significant as a feeding-related difference.^{30(pp1-16)} Mean achieved weights further indicated normal growth, with all groups falling within the 25th and 75th reference percentiles of weight-for-age for male and female infants on CDC growth charts.²⁸ Mean daily gain and mean achieved value for length and head circumference were also similar in all groups.

In a study of healthy infants who received a LGG-supplemented nonhydrolyzed routine formula starting before 2 months of age, significantly higher growth in weight and length was observed at 6 months compared with an unsupplemented control group.³¹ Infants in the LGG group were significantly smaller in weight and length at study entry and had significantly higher formula intake than controls. Growth differences between the groups remained significant after controlling for age, birth weight, and birth length; however, increased formula intake could explain increased growth in the LGG group. Supplementation with LGG prenatally and postnatally up to 6 months of age in infants with high risk of allergies was associated with normal height and weight-for-height at 48 months.³² Although difficult to directly compare, previous studies and current results in healthy infants collectively suggest

that dietary LGG supplementation is associated with normal growth.

In this study, LGG-supplemented EH and PH formulas and the unsupplemented EH formula were equally well tolerated. Formula intake, overall incidence of gas and fussiness, and a low incidence of diarrhea and constipation were similar in all groups. Stools were firmer and less frequent in the PHF-LGG group compared with the EHF or EHF-LGG groups at some time points, leading to the conclusion that these differences were not related to LGG supplementation but rather to the degree of protein hydrolysis of the formula. Infants who received EH casein formula have also experienced looser and more frequent stools, similar to a breast-fed reference group, when compared with infants fed intact CMP-based and soy-based formulas.³³ In the current study, rates of discontinuation and adverse events were similar between groups; serious adverse event rates were low and generally deemed unrelated to study products by the study physician.

FA profiles in plasma and circulating RBCs were largely similar in infants who received EHF, EHF-LGG, or PHF-LGG. Previous studies in infants who received routine infant formula supplemented with DHA and ARA at levels similar to human milk

Table 3. Selected Polyunsaturated Fatty Acid Levels as Percentage of Total Fatty Acids in Infant Blood Lipid Fractions at 150 Days of Age^a

Fatty Acid	RBC PE (n)				RBC PC (n)				Total RBCs (n)				Plasma Phospholipids (n)				Total Plasma (n)			
	EHF		EHF–		EHF		EHF–		EHF		EHF–		EHF		EHF–		EHF		EHF–	
	(27)	(28)	LGG	PHF–	(25)	LGG	LGG	PHF–	(27)	LGG	LGG	PHF–	(27)	LGG	LGG	PHF–	(27)	LGG	LGG	PHF–
ω3 Fatty acids																				
α-linolenic	0.21	0.20	0.21	0.13	0.13	0.14	0.14	0.13	0.20	0.21	0.22	0.22	0.14	0.14	0.13	0.13	0.50	0.49	0.49	0.49
18:3ω3	(0.01)	(0.02)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.02)	(0.02)	(0.02)	(0.02)	(0.01)	(0.01)	(0.01)	(0.01)	(0.02)	(0.03)	(0.03)	(0.02)
Eicosapentaenoic	0.25	0.24	0.21 ^b	0.17	0.17	0.16	0.16	0.12 ^b	0.18	0.20	0.15 ^c	0.15 ^c	0.24	0.25	0.16 ^b	0.21	0.21	0.20	0.17	0.17
20:5ω3	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)
Docosapentaenoic	1.18	1.13	1.07	1.17	0.17	0.20 ^d	0.17	0.17	0.52	0.54	0.49	0.49	0.25	0.25	0.24	0.18	0.19	0.19	0.17	0.17
22:5ω3	(0.04)	(0.04)	(0.04)	(0.04)	(0.01)	(0.01)	(0.01)	(0.01)	(0.02)	(0.02)	(0.02)	(0.02)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)
Docosahexaenoic	10.61	10.48	10.99	3.63	3.63	3.63	3.94	3.94	5.59	5.85	5.89	5.89	5.61	5.47	5.72	3.76	3.75	3.76	3.76	3.76
22:6ω3	(0.34)	(0.36)	(0.32)	(0.16)	(0.16)	(0.17)	(0.17)	(0.15)	(0.20)	(0.22)	(0.19)	(0.19)	(0.16)	(0.17)	(0.15)	(0.12)	(0.13)	(0.13)	(0.13)	(0.11)
ω6 Fatty acids																				
Linoleic	5.22	5.34	5.23	21.47	21.47	21.25	22.01	22.01	14.54	14.86	14.57	14.57	21.34	21.83	21.73	25.70	25.88	25.42	25.42	25.42
18:2ω6	(0.20)	(0.22)	(0.20)	(0.36)	(0.36)	(0.38)	(0.34)	(0.34)	(0.53)	(0.57)	(0.50)	(0.50)	(0.35)	(0.38)	(0.34)	(0.28)	(0.30)	(0.26)	(0.26)	(0.26)
Dihomo-γ-linoleic	1.04	0.99	0.98	1.44	1.44	1.42	1.46	1.46	1.16	1.19	1.15	1.15	1.76	1.74	1.76	1.21	1.23	1.19	1.19	1.19
20:3ω6	(0.06)	(0.06)	(0.06)	(0.07)	(0.07)	(0.07)	(0.07)	(0.06)	(0.06)	(0.06)	(0.06)	(0.06)	(0.08)	(0.09)	(0.08)	(0.06)	(0.06)	(0.05)	(0.05)	(0.05)
Arachidonic	30.28	30.22	30.57	11.60	11.60	12.02	11.73	11.73	15.25	15.90	15.47	15.47	15.02	15.09	14.19	11.14	11.21	10.19 ^b	10.19 ^b	10.19 ^b
20:4ω6	(0.61)	(0.66)	(0.59)	(0.32)	(0.32)	(0.33)	(0.29)	(0.29)	(0.38)	(0.41)	(0.36)	(0.36)	(0.31)	(0.33)	(0.30)	(0.24)	(0.26)	(0.23)	(0.23)	(0.23)
Adrenic	7.83	7.84	8.27	0.40	0.40	0.53	0.44	0.44	2.87	2.90	2.97	2.97	0.37	0.37	0.36	0.28	0.29	0.27	0.27	0.27
22:4ω6	(0.23)	(0.25)	(0.22)	(0.05)	(0.05)	(0.05)	(0.05)	(0.05)	(0.13)	(0.14)	(0.12)	(0.12)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)
Docosapentaenoic	1.12	0.96	0.94	0.19	0.19	0.18	0.19	0.19	0.42	0.42	0.43	0.43	0.30	0.36	0.48	0.10	0.10	0.13 ^b	0.13 ^b	0.13 ^b
22:5ω6	(0.14)	(0.16)	(0.14)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.02)	(0.03)	(0.02)	(0.02)	(0.06)	(0.07)	(0.06)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)

Note: RBC, red blood cell; RBC PE, RBC phosphatidylethanolamine; RBC PC, RBC phosphatidylcholine; EHF, extensively hydrolyzed casein formula; EHF-LGG, EHF supplemented with *Lactobacillus rhamnosus* GG (LGG); PHF-LGG, partially hydrolyzed whey:casein (60:40) formula supplemented with LGG.

a. Least square mean ($\pm$ standard error); statistical differences reported for pairwise comparisons within fatty acid and fatty-acid fraction. Other polyunsaturated fatty acids analyzed, with mean values $<0.5\%$ and no statistical differences, are not shown.

b. Significantly different, PHF-LGG versus EHF or EHF-LGG, $P < .05$.

c. Significantly different, PHF-LGG versus EHF-LGG, $P < .05$.

d. Significantly different, EHF-LGG versus EHF or PHF-LGG, $P < .05$.

worldwide medians^{25,26} reported significantly higher levels of DHA and ARA as percentages of total RBC FAs, compared with infants fed a formula with lower or no added DHA and ARA,^{27,34} and improved visual acuity and cognitive development similar to that of breast-fed infants.³⁴⁻³⁷ In this study, mean percentages of DHA and ARA in RBC and plasma fractions from infants in all groups were similar to levels in breast-fed³⁴ or healthy, term infants fed a routine formula supplemented with 0.64% ARA and 0.32% DHA for 120 days.²⁷ A study of 15 infants with AD weaned to an EH formula or the same formula supplemented with LGG or another probiotic reported decreased proportion of α -linolenic acid and total ω 3 PUFAs and increased ω 6 to ω 3 ratio in neutral lipids in the LGG group; increased ω 6 to ω 3 ratio was also observed in serum phospholipids.³⁸ However, study formulas were not supplemented with DHA and ARA. In contrast, addition of LGG to DHA- and ARA-supplemented EH and PH formulas in the current study did not affect levels of circulating DHA and ARA.

Suggested benefits of dietary probiotic supplementation include better tolerance to allergens and increased response to vaccinations.^{39,40} Specific IgE to CMP signals sensitization to CMP and possible development of CMA¹⁵; specific IgG levels may correlate with protective efficacy of infant vaccinations.^{40,41} In the current study, total IgE and specific IgE to CMP, and IgG response to tetanus, diphtheria, Hib, and polio vaccines, analyzed as secondary outcomes in a subset of infants, were similar in all formula groups. Post hoc analysis conducted to examine the impact of LGG supplementation on incidence of allergy-related and infection-related adverse events demonstrated no significant differences between groups. This study, however, was powered as a 120-day infant growth trial. A larger sample size and extended monitoring could allow more sensitivity in antibody response measures and infection-related or allergy-related adverse events, and it might improve assessment of potential LGG influence.

LGG, a well-characterized probiotic, is available as a dietary supplement or incorporated as a food ingredient in many products internationally. In this study, both EH and PH formulas supplemented with LGG were compared with an unsupplemented EH formula; all supported normal growth in healthy, term infants and were well tolerated. Overall, plasma and RBC FA composition was unaffected by supplementation of formulas with LGG, and circulating

levels of DHA and ARA were similar to those in previous clinical reports from breast-fed infants and infants who received routine formula supplemented with DHA and ARA. Conclusions regarding the influence of LGG supplementation of EH and PH formulas on tolerance to allergens and response to vaccinations cannot be made. Further research on infant formulas supplemented with LGG in a larger population, through an extended follow-up period, is warranted.

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