

Growth and Tolerability of Healthy Term Infants Fed a New Formula Supplemented with DHA from *Schizochytrium* sp Microalgae

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Abstract

Objective: To evaluate growth and tolerability of infants fed a formula supplemented with docosahexaenoic acid (DHA) from a new strain of *Schizochytrium* sp (DHASCO[®]-B) microalgae.

Methods: Healthy term infants (n=159) with weights \geq 2500 gram were randomized on or before 14th day of life (DOL) to receive either DHASCO[®]-B or reference formula (DHASCO[®]) obtained from *Crypthecodinium cohnii* until DOL 120. Both formulas also contained arachidonic acid (ARA). Assessments included weight, length, weight/length ratio, head circumference, red blood cell (RBC) DHA and other fatty acids, metabolic panel, safety, and tolerance.

Results: The rate of mean daily weight gain (\pm SD) at DOL 120 was not significantly different between the formulas, 29.1 ± 5.92 grams/day versus 29.9 ± 7.40 grams/day for DHASCO[®] and DHASCO[®]-B, respectively and was equivalent (90% CI: -2.94 to 1.31; p=0.553). There was no significant difference between formulas in actual weight gain over time, i.e. DOL 30, 60, 90 and 120 (p=0.252), including by gender. Other anthropometric variables were also not different between the 2 groups. RBC DHA levels at DOL 120 were bioequivalent between the formulas (ratio of geometric means was 96.85%). There was no difference between formulas in any of the tolerance variables (watery stools, hard stools, gassiness, spitting up and fussiness), and no clinically meaningful difference in adverse events or laboratory values.

Conclusions: There was no significant difference in daily weight gain of healthy term infants fed a formula supplemented with DHA from *Crypthecodinium cohnii* versus the new strain of *Schizochytrium* sp microalgae. Overall weight gains were equivalent between the two formula groups. Further, RBC DHA levels were bioequivalent, and there was no difference in infant tolerability or parent satisfaction. In summary, growth, tolerability and safety profiles for the formulas were similar and typical for this age group.

Keywords: Term infants; Growth; Tolerability; Infant formula; Docosahexaenoic acid; DHA; *Crypthecodinium cohnii*; *Schizochytrium* Sp; Microalgae; Weight gain; Body length; Head circumference; Long chain polyunsaturated fatty acid; Red blood cell; RBC

Introduction

Docosahexaenoic acid (DHA) and arachidonic acid (ARA), omega-3 and omega-6 long-chain polyunsaturated fatty acids (LC-PUFA), respectively, are commonly included in infant formulas. DHA is most abundant in the gray matter of the brain and in the retina of the eye where it is an important structural component of cellular membranes enhancing membrane structure and fluidity and has a modulatory role in cell signaling [1,2]. Clinical studies in human infants have provided evidence that DHA is essential for optimal brain and retinal development that translates into benefits in both visual and cognitive performance [3-9]. During neonatal life, there is a rapid accretion of both DHA and ARA in infant brain, DHA in retina and of ARA in the whole body [10].

It is important to provide an appropriate supply of preformed DHA throughout infancy as brain development and DHA accretion continue at a very rapid pace during the first 2 years of life [11]. Both DHA and ARA occur naturally in breast milk and their amounts depend on the maternal diet, with levels that vary worldwide from 0.1 to 1.4 wt% of total fatty acids for DHA and from 0.24 to 1.0 wt% for ARA [12]. The best estimates of worldwide mean breast-milk DHA and ARA concentrations (wt% of total fatty acids) are 0.32–0.40% for DHA

and 0.47–0.56% for ARA [12]. In 2008, the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) set recommendations for commercially available infant formulas at 0.20–0.36% for DHA and 0.40–0.60% for ARA of total fatty acids [13]. The highest contents of DHA and ARA are found in US commercial term formulas at levels 0.32% DHA and 0.64% ARA [14].

In the U.S., the first commercially available DHA-containing oil for infant formulas was DHA single cell oil (DHASCO) produced from the microalgae *Crypthecodinium cohnii*. DHASCO is generally recognized as safe (GRAS) for use in infant formulas at a maximum level of 1.25% (up to 0.5% for DHA alone) of the total dietary fat [15]. Two other DHA-containing algal oils derived from distinct algal strains of *Schizochytrium* sp. (DHASCO-S[®] and DHA-O[®]) are GRAS for use in food and dietary supplements [16,17]. Recently, a

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new DHA-rich oil was obtained from a different wild-type strain of *Schizochytrium* sp., herein named DHASCO-B®. This oil is distinct from other algal oils in composition: it contains a minimum 40% DHA and up to 10% eicosapentaenoic acid (EPA) with a minor amount of docosapentaenoic acid (DPA). DHASCO-B® is intended for use in preterm and term infant formulas and follow-on formulas. DHASCO-B is a triacylglycerol, similar to the form of DHA found in breast milk. In July 2013, an independent panel of experts determined DHASCO®-B to be GRAS (with maximum 0.5% of fat as DHA in infant formula) as acknowledged by FDA Office of Food Additive Safety, Center for Food Safety and Applied Nutrition in June 2015 [18].

This 120-day clinical study compared the growth and tolerance of healthy term infants fed formula containing DHA from DHASCO®-B as 0.32% of total fatty acids versus infants fed a similar reference formula containing DHA from DHASCO® as 0.32% of total fatty acids. There were no other changes in formula composition, including use of 0.64% ARA in both formulas derived as a single cell oil (ARASCO®) from the soil fungus *Mortierella alpina*. The primary objective was to determine whether weight gain and tolerability of healthy term infants fed a commercially available term infant formula supplemented with DHASCO® was similar to that of infants fed the same formula supplemented with a new product, DHASCO®-B. A secondary objective was to determine bioequivalence between the formulas in red blood cell DHA levels, an indicator of brain levels [19].

Methods

This study was designed as a randomized, double-blinded, active-controlled, parallel two-group trial conducted at 12 U.S. clinical sites. The study design was consistent with guidelines detailed in the WHO Child Growth Standards [20]. The protocol and informed consent (IC) were approved by Chesapeake Institutional Review Board (Columbia, MD) and New England Institutional Review Board (Newton, MA) and underwent a revision to change the age of parents from ≥21 years to a minimum of 18 years and to increase enrollment from 144 to approximately 159 infants. Overall study duration was approximately 14 months. The study trial was registered with Clinicaltrials.gov (NCT02144402).

Study population

Healthy term infants (n=159) with weights ≥2500 gram were stratified by gender and randomized on or before 14th day of life (DOL) to receive either DHASCO® or DHASCO®-B formula until DOL 120. Parents and the study site investigators and staff as well as the clinical research organization and trial sponsor were blinded regarding the study formula to which individual subjects were assigned.

Infants (parents) had to meet all of the following inclusion criteria to qualify for entry into the study: full term (gestational age 37 to 42 weeks), birth weight ≥2500 grams, healthy singleton infant ≤14 days, parent (minimum 18 years of age) provided consent on behalf of the infant, parent confirmed their intention to feed their infant the assigned study formula as the sole source of nutrition for the duration of the study unless instructed otherwise by their healthcare professional, and parent voluntarily signed and dated an IC document. Infants (parents) who met any of the following exclusion criteria did not qualify for entry into the study: congenital malformation or genetic disorder that could interfere with normal growth and development, other metabolic anomalies, maternal infectious diseases, alcoholism or substance abuse, mothers who had gestational diabetes and /or were receiving insulin during pregnancy, any other adverse maternal, fetal or infant medical

history with potential to affect tolerance, growth, and/or development, and participation in another clinical study.

Interventions

At enrollment, eligible newborns were randomized 1:1 to investigational DHASCO®-B or reference DHASCO® formula and were stratified by gender into block sizes of four utilizing an interactive web response system (IWRS). Infants were fed either the investigational or reference formula. Both formulas contained DHA as 0.32% of the total fatty acids and 0.64% ARA of the total fatty acid content. Compositions of the two formulas are detailed in Table 1. Both study products were in the form of a cow's milk-based infant formula packaged in ready-to-feed (RTF) liquid formulation in 8 ounce bottles (PBM Nutritionals, a Perrigo Company, Charlottesville, VA). In the DHASCO®-B formula, the DHA source was a novel micro algal oil from a new strain of *Schizochytrium* sp. and, in DHASCO®, the source was from commercially available algal oil derived from *Cryptocodinium cohnii* (supplied by DSM Nutritional Products, Columbia, MD). ARA in both formulas was derived as a single cell oil (ARASCO®) from the soil fungus *Mortierella alpina* (DSM Nutritional Products). Both

	DHASCO®	DHASCO®-B	UNITS
Proximate Nutrients			
Arachidonic Acid	230	240	MG/L
Carbohydrate By Difference Liquids	7.2	7.2	% W/W
Docosahexaenoic Acid	120	130	MG/L
Fat	3.6	3.56	% W/W
Linoleic Acid	6330	6450	MG/L
Linoleic Acid per 100 KCAL	0.86	0.86	GM
Linolenic Acid per 100 KCAL	0.077	0.077	GM
Protein	1.46	1.46	% W/W
Vitamins and Micro-Nutrients			
Ascorbic Acid	164	181	MG/L
Biotin	84	69	MCG/L
Choline	230	208	MG/L
Cyanocobalamin	6.9	7.1	MCG/L
Folic Acid	199	186	MCG/L
Inositol	66	66	MG/L
Niacin	11917	11646	MCG/L
Pantothenic Acid	8600	8300	MCG/L
Pyridoxine	776	755	MCG/L
Riboflavin	1917	1810	MCG/L
Thiamine	1313	1282	MCG/L
Vitamin A	2844	2865	IU/L
Vitamin D3	557	605	IU/L
Vitamin E	21	21	IU/L
Vitamin K	65	65	MCG/L
Minerals			
Calcium	669	656	MG/L
Chloride	510	505	MG/L
Copper	720	704	MCG/L
Iodide	123	112	MCG/L
Iron	16	16	MG/L
Magnesium	73	72	MG/L
Manganese	177	162	MCG/L
Phosphorus	376	378	MG/L
Potassium	1117	1150	MG/L
Selenium	31	31	MCG/L
Sodium	288	286	MG/L
Zinc	10	10	MG/L

Table 1: Approximate nutrient contents of formula interventions.

study formulas met levels of nutrients for infant formulas that were recommended by the American Academy of Pediatrics Committee on Nutrition and that are regulated by the Infant Formula Act of 1980 [21] and subsequent amendments [22].

Sufficient quantities of the study formulas were provided at each study visit (DOL \leq 14, 30, 60, 90 and 120) to enable parents to feed the assigned study product *ad libitum* as the sole source of nutrition for the duration of the study. Parents were given intake and tolerance forms to complete for three 24-hour periods prior to DOL 30 and 120 visits. Parents were asked to log the time and amount of formula taken. They were also asked to respond “yes” or “no” to the following tolerance questions: (1) Were your baby’s stools watery? (2) Were your baby’s stools hard? (3) Was your baby gassy? (4) Did your baby spit up more than usual after the feedings? (5) Was your baby fussy? (6) Are you satisfied with the formula?

Outcomes

Anthropometric assessments were made at each visit that included weight, length, weight/length ratio, and head circumference. All parents consented to have a blood sample collected from their infant at DOL 120 that evaluated red blood cell (RBC) fatty acids (DHA, ARA, EPA and DPAn-6), metabolic panel (sodium, potassium, chloride, carbon dioxide [CO₂], blood urea nitrogen, glucose, creatinine, and calcium). Consecutive subjects were enrolled in the blood collection sub-study until the target number of 72 had been reached (71 of the 72 subjects were able to provide blood). The recording of adverse events (coded by MedDRA 17.0) started after the parent signed the informed consent form and the subject was given the first feeding with study formula and continued until study completion.

Statistical methods

Rate of weight gain (gram/day) was calculated from the weight measured at enrollment subtracted from the weight measured at a subsequent visit, divided by the number of days between the actual visits. The 90% confidence interval approach of the “two one-sided test” (TOST) methodology was used to assess equivalence between the formulas. Study success and equivalence were established if the 90% confidence interval for the difference between groups in the daily weight gain was contained within the interval (-3.4 to 3.4) [23]. Further, a comprehensive assessment of the effects of gender on the primary endpoint was completed. For assessment of the effects of gender, two methodological approaches were summarized; 1) gender-stratified TOSTs were calculated to assess equivalence within genders, and 2) ANOVA methods with gain as the dependent variable, and with the treatment group, site and gender as factors in the model.

Continuous variables were summarized with the mean, standard deviation, median and range and were analyzed by analysis of covariance (ANOVA) or Wilcoxon rank sum tests, as appropriate. Categorical variables were analyzed by exact tests, chi-square tests or Cochran-Mantel-Haenszel tests, as appropriate. Results were considered statistically significant at the 5% level. SAS (version 9.4) general linear model (GLM) and/or MIXED procedures were used for models and group comparisons on continuous endpoints. Categorical variables were summarized with counts and percentages and SAS FREQ procedure was used for group comparisons on categorical endpoints.

RBC fatty acid levels were calculated as weight percent of fatty acid, and as microgram of fatty acid per milliliter of red blood cells. These were analyzed using ANOVA with treatment group and site as factors

in the model. Measures of bioequivalence of the RBC DHA blood levels ($\mu\text{g/mL}$) between the two groups were conducted by examining:

- If the difference between the group means of the log-transformed data was within -0.2231 and +0.2231 ($+\ln(1.25)$), and
- If the ratio of the geometric means of the investigational formula compared to the standard control formula was within 80% and 125%.

Determination of sample size was based on the assumption there was no difference between groups in daily weight gain. The overall standard deviation in daily weight gain was assumed 6 gram/day, and therefore 55 subjects per group were required to be 80% sure that the 90% confidence interval for the difference was within the interval (-3.4 to 3.4) [23]. Originally, based on an estimated 25% attrition, enrollment of 144 infants was planned (72 per treatment group (36 male and 36 female) for the study. As attrition rose, an estimated 30% attrition rate was used to increase the sample size by 15 subjects to a total of 159. This led to approximately 79 subjects in each group with approximately 39 of each gender.

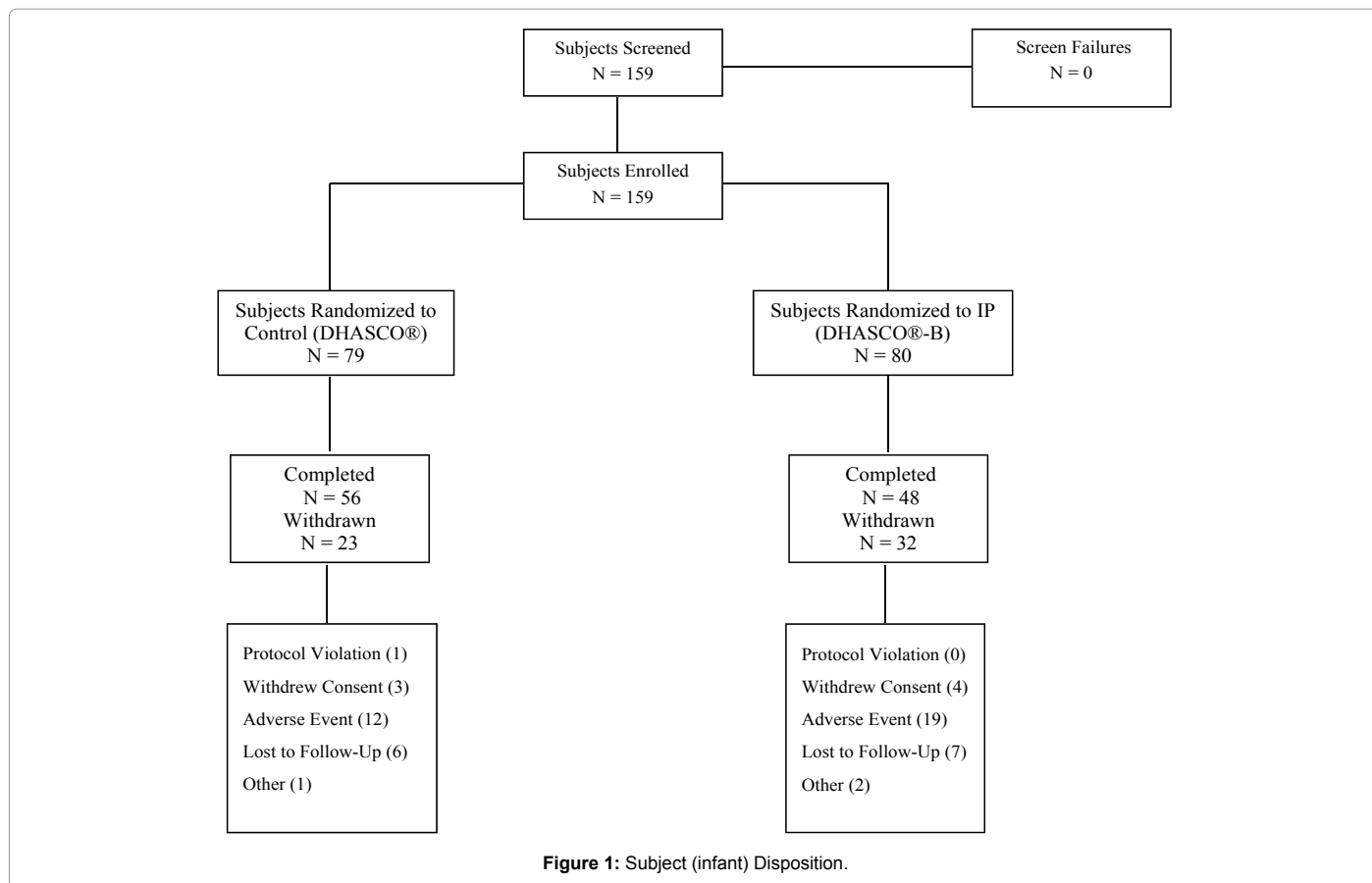
Results

Subject (infant) disposition is shown in Figure 1. There were no screen failures. Overall, 159 infants were randomized into the study (79 in the DHASCO[®] group; 80 in the DHASCO[®]-B group); 104 (65.4%) completed the study. Of the 55 discontinued infants, 31 were withdrawn by the parent or investigator due to a treatment-emergent adverse event (TEAE) or serious adverse event (SAE), 11 were discontinued by the parent or investigator for reasons other than safety, 13 were lost to follow-up, and 1 was withdrawn for protocol non-compliance. Rate of discontinuation was 36% at Visit 1, 31% at Visit 2, 18% at Visit 3 and 14% at Visit 4.

Table 2 shows that demographic and baseline characteristics were similar between the 2 treatment groups. Overall, mean age (\pm SD) of the infants at enrollment was 7.8 ± 3.88 days; the gender ratio was 81 (50.9%) female and 78 (49.1%) male; mean weight at enrollment was 3387.6 ± 428.82 g; and the majority of infants were white (81.1%). Overall, 52.2% of mothers used prenatal DHA supplements, and a total of 31 (19.5%) infants were fed breast milk prior to enrollment. Body weight, length and head circumference at enrollment were similar between the 2 groups; the overall mean (\pm SD) totals were 3387.6 grams (± 428.82), 50.6 cm (± 2.17) and 35.0 cm (± 1.48), respectively. There was greater than 75% mean compliance in both treatment groups as assessed by counting the number of bottles of formulae returned at the last visit.

Infants in the 2 groups were similar in terms of rate of weight increase from Day 14 to Days 30, 60, 90, and 120 (36.5 ± 11.54 g/day in the reference group vs. 33.5 ± 10.40 g/day in the DHASCO[®]-B group at Day 30; 32.8 ± 8.42 g/day in the reference group vs. 32.8 ± 9.81 g/day in the DHASCO[®]-B group at Day 60; 30.5 ± 7.53 g/day in the reference group vs. 31.2 ± 8.92 g/day in the DHASCO[®]-B group at Day 90; and 29.1 ± 5.92 g/day in the reference group vs. 29.9 ± 7.40 g/day in the DHASCO[®]-B group at Day 120). There was also no difference between treatment groups in actual weight gain over time, i.e. at enrollment, DOL 30, 60, 90 and 120 using repeated measures ANOVA ($p=0.252$).

The similarities in growth rate were reflected in similarities in achieved body weight (Figure 2). For the primary endpoint, the rates of mean daily weight gain (\pm SD) by DOL 120 were equivalent between the formulas (90% CI TOST: -2.94 to 1.31, within the equivalence



Characteristic	DHASCO® (n=79)	DHASCO®-B (n=80)	All Subjects (n=159)	P-Value ¹
Age (days)				
N	79	80	159	0.627
Mean ± SD	7.9 ± 3.90	7.6 ± 3.87	7.8 ± 3.88	
Range	2.0 to 14.0	0.0 to 14.0	0.0 to 14.0	
Median	7	7	7	
Gender				
Female	40 (50.6%)	41 (51.3%)	81 (50.9%)	0.938
Male	39 (49.4%)	39 (48.8%)	78 (49.1%)	
Ethnicity				
Hispanic or Latino	2 (2.5%)	11 (13.8%)	13 (8.2%)	0.01
Not Hispanic or Latino	77 (97.5%)	69 (86.3%)	146 (91.8%)	
Race ²				
American Indian or Alaskan Native	1 (1.3%)	2 (2.5%)	3 (1.9%)	1
Asian	0 (0.0%)	1 (1.3%)	1 (0.6%)	1
Black or African American	17 (21.5%)	20 (25.0%)	37 (23.3%)	0.603
Native Hawaiian or Other Pacific Islander	0 (0.0%)	1 (1.3%)	1 (0.6%)	1
White	66 (83.5%)	63 (78.8%)	129 (81.1%)	0.44
Prenatal DHA used				
No	26 (32.9%)	19 (23.8%)	45 (28.3%)	0.068
Yes	34 (43.0%)	49 (61.3%)	83 (52.2%)	
Unknown	19 (24.1%)	12 (15.0%)	31 (19.5%)	
Weight (gram)				
N	79	80	159	0.249
Mean ± SD	3430.1 ± 419.83	3345.5 ± 436.06	3387.6 ± 428.82	
Range	2495.0 to 4330.0	2322.0 to 4547.0	2322.0 to 4547.0	
Median	3362.5	3344.3	3346	

Length (cm)				
N	79	80	159	0.162
Mean ± SD	50.8 ± 2.23	50.3 ± 2.09	50.6 ± 2.17	
Range	45.2 to 54.8	43.7 to 54.6	43.7 to 54.8	
Median	50.9	50.3	50.5	
Head Circumference (cm)				
N	79	80	159	0.181
Mean ± SD	35.2 ± 1.42	34.8 ± 1.52	35.0 ± 1.48	
Range	31.2 to 37.5	29.0 to 38.1	29.0 to 38.1	
Median	35.3	35	35.1	

Table 2: Demographics and Baseline Characteristics (Safety Population). ¹Birth anthropometrics (weight, length and head circumference) were analyzed using analysis of variance (ANOVA) methods with treatment group and gender as factors in the model. Age at enrollment was analyzed using ANOVA methods with treatment group as a factor in the model. Gender, ethnicity, mother use of prenatal vitamins, and race were analyzed by exact tests, chi-square tests or Cochran-Mantel-Haenszel statistics, as appropriate, to compare the treatment groups on these demographic variables. If an endpoint was measured in duplicate, then the average of the two assessments was used as the measurement for that visit. ²The parents were permitted to choose more than one race.

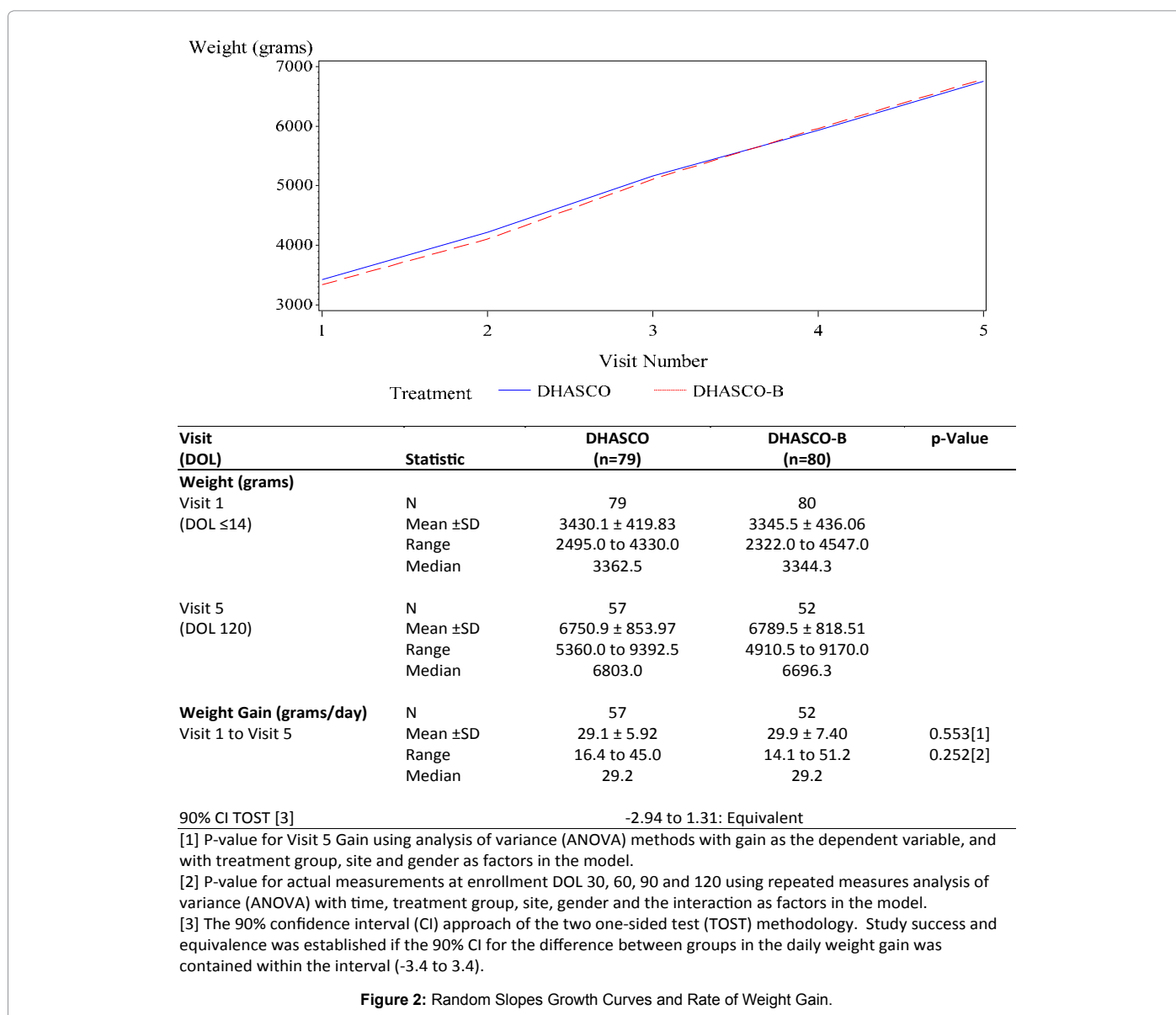


Figure 2: Random Slopes Growth Curves and Rate of Weight Gain.

interval -3.4 to 3.4). Using ANOVA methods, with daily weight gain as the dependent variable, and with treatment group, site and gender as factors in the model, there was no significant difference in rate

of weight gain between the treatment groups (29.1 ± 5.92 grams/day versus 29.9 ± 7.40 grams/day for DHASCO® and DHASCO®-B, respectively; p=0.553).

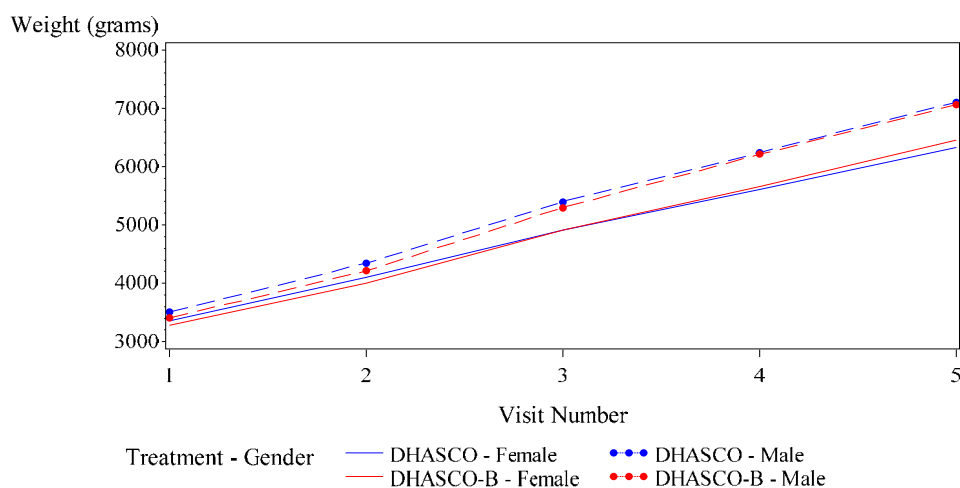
Overall, as expected, males were heavier at baseline and throughout the study (Figure 3). While both males and females had a pattern of slightly greater weight gain in the early visits with the reference formula, this trend was reversed in later visits for females in which equivalence was established between treatment groups (90% CI TOST: -3.38 to 1.76). Males, on the other hand, had a small early greater weight gain with the reference formula that resulted in nonequivalence (90% CI TOST: -3.72 to 2.32). However, in the per protocol population of subjects who completed the study, daily weight gain was equivalent for males (90% CI TOST: -3.38 to 3.08).

The mean (\pm SD) rates of daily length gain and head circumference gain by DOL 120 were not different for the reference and DHASCO[®]-B formula groups (0.1 ± 0.01 cm/day for both measures; $p=0.992$ and $p=0.223$, respectively). Actual measurements over time also showed no significant difference in length gain or head circumference gain between the formulas ($p=0.128$ and $p=0.299$, respectively). Daily ratio (weight/

length) gain by DOL 120 was not significant between the formula groups, 0.3 ± 0.09 and 0.4 ± 0.11 , for the reference and DHASCO[®]-B groups, respectively ($p=0.485$), and analysis of assessments over time also showed no difference in ratio gain between formula groups ($p=0.335$).

Tolerance evaluation showed no difference between formula groups in any of the following variables at either DOL 30 or 120: watery stools, hard stools, gassiness, spitting up or fussiness. The most common tolerance issue was gassiness (flatulence) at Day 30 (72.2% vs. 63.8% for reference vs. DHASCO[®]-B, respectively; $p=0.538$), and Day 120 (29.1% vs. 32.5%, respectively; $p=0.312$). At Day 30, 79.7% vs. 72.5% of parents (reference vs. DHASCO[®]-B, $p=0.520$) reported satisfaction; and at Day 120, 63.3% vs. 58.8% of parents (reference vs. DHASCO[®]-B, $p=1.000$) reported satisfaction with the formulas.

Fatty acid profiles in red blood cells generally reflected the



Visit (DOL)	Statistic	All Subjects (n=159)	Males (n=80)	Females (n=79)
Weight (grams)				
Visit 1 (DOL \leq 14)	N	159	80	79
	Mean \pm SD	3387.6 \pm 428.82	3459.3 \pm 390.30	3314.9 \pm 455.60
	Range	2322.0 to 4547.0	2509.0 to 4500.0	2322.0 to 4547.0
	Median	3346.0	3392.5	3250.0
Visit 5 (DOL 120)	N	109	62	47
	Mean \pm SD	6769.3 \pm 833.60	7079.8 \pm 837.31	6359.8 \pm 632.82
	Range	4910.5 to 9392.5	4910.5 to 9392.5	5200.0 to 7635.0
	Median	6760.0	6992.5	6350.0
Weight Gain (grams/day)				
Visit 1 to Visit 5	N	109	62	47
	Mean \pm SD	29.5 \pm 6.65	31.6 \pm 6.83	26.8 \pm 5.36
	Range	14.1 to 51.2	14.1 to 51.2	15.0 to 41.7
	Median	29.2	32.1	26.8
Males: 90% CI TOST			-3.72 to 2.32: Not equivalent [1]	
Females: 90% CI TOST			-3.38 to 1.76: Equivalent [1]	

[1] The 90% confidence interval (CI) approach of the two one-sided test (TOST) methodology by gender and treatment. Equivalence was established if the 90% CI for the difference between treatment groups by gender in the daily weight gain was contained within the interval (-3.4 to 3.4).

Figure 3: Random Slopes Growth Curves and Rate of Weight Gain by Gender.

composition of the oils in the two formulas (Table 3). Measures of bioequivalence of the DHA red blood cell levels between the reference (1.29 µg/mL) and DHASCO®-B (1.33 µg/mL) formulas were achieved. The difference between the group means of the log-transformed DHA data was -0.04198 (within -0.2231 and +0.2231 [+/-ln(1.25)]), and the ratio of the geometric means for DHA with the DHASCO®-B formula relative to the reference formula was 96.85% (within 80% and 125%). Total formula intake between the DHASCO®-B and reference groups did not vary significantly at DOL 30 (76.0 vs. 80.8 ounces; n=59 and n=66, respectively; p=0.256) or at DOL 120 (median: 105.5 ounces vs. 105.0 ounces; n=43 and n=47, respectively; p=0.834).

Treatment-emergent adverse events are summarized overall and for individual incidence ≥5% with either formula in Table 4. The overall incidence of TEAEs was slightly greater with DHASCO®-B than with the reference formula (72.5% and 57.0%, respectively). The

frequency of SAEs was low, 7 subjects in the DHASCO®-B group (viral infection in 4 subjects, sleep apnea in 1 subject, gastric obstruction in 1 subject and sudden infant death syndrome in 1 subject) and 2 subjects in the DHASCO® group (urinary tract infection and bronchiolitis). All SAEs were considered not related to study formula and all but one had resolved by the end of the study. The death from sudden infant death syndrome occurred approximately 3 months after the first intake of formula and was considered by the investigator as unrelated to treatment. As shown in Table 3, the incidence of infants who experienced a TEAE assessed with a definite (7.5% vs. 7.6%, respectively) or suspected (18.8% vs. 15.2%, respectively) relationship to product was similar among the 2 groups. Consistent with tolerance assessments, flatulence had the highest incidence among all subjects (21.3% DHASCO-B vs. 11.4% DHASCO, respectively). Other events were generally comparable between the formulas, and as expected gastrointestinal events were more prevalent than other TEAEs.

Microgram of Fatty Acid per ml of RBC	DHASCO® (n=33)	DHASCO®-B (n=36)	All Subjects (n=69)	P-value ¹
DHA				
Mean ± SD	56.8 ± 20.82	59.0 ± 17.43	58.0 ± 19.01	0.741
Range	14.6 to 100.1	10.5 to 84.3	10.5 to 100.1	
Median	59.5	61.4	59.5	
EPA				
Mean ± SD	1.5 ± 0.50	2.9 ± 0.93	2.3 ± 1.03	<.001
Range	0.5 to 3.0	1.0 to 5.1	0.5 to 5.1	
Median	1.5	3.1	2.0	
DPAn-6				
Mean ± SD	3.6 ± 1.37	4.8 ± 1.54	4.2 ± 1.58	0.002
Range	0.0 to 6.6	1.0 to 8.2	0.0 to 8.2	
Median	3.7	4.8	4.3	
ARA				
Mean ± SD	175.9 ± 52.46	180.6 ± 44.42	178.4 ± 48.13	0.919
Range	40.3 to 271.8	45.7 to 236.5	40.3 to 271.8	
Median	187.7	187.2	187.7	

¹P-value using analysis of variance (ANOVA) methods with the RBC data as the dependent variable, and with treatment group and site as factors in the model.

Table 3: Red Blood Cell (RBC) Fatty Acids: DHA, EPA, DPAn-6 and ARA.

	DHASCO® (n=79)	DHASCO®-B (n=80)	All Subjects (n=159)
Any Adverse Event (AE)	45 (57.0%)	58 (72.5%)	103 (64.8%)
Any Serious AE	2 (2.5%)	7 (8.8%)	9 (5.7%)
Any Related AE ¹			
Suspected	12 (15.2%)	15 (18.8%)	27 (17.0%)
Definite	6 (7.6%)	6 (7.5%)	12 (7.5%)
Individual AEs ²			
Flatulence	9 (11.4%)	17 (21.3%)	26 (16.4%)
Upper respiratory tract infection	11 (13.9%)	13 (16.3%)	24 (15.1%)
Gastroesophageal reflux disease	11 (13.9%)	12 (15.0%)	23 (14.5%)
Constipation	4 (5.1%)	7 (8.8%)	11 (6.9%)
Irritability	3 (3.8%)	7 (8.8%)	10 (6.3%)
Viral infection	2 (2.5%)	7 (8.8%)	9 (5.7%)
Abdominal pain	6 (7.6%)	5 (6.3%)	11 (6.9%)
Bronchiolitis	5 (6.3%)	3 (3.8%)	8 (5%)
Vomiting	5 (6.3%)	3 (3.8%)	8 (5%)
Cough	2 (2.5%)	5 (6.3%)	7 (4.4%)
Diarrhoea	4 (5.1%)	3 (3.8%)	7 (4.4%)

¹Suspected: there was a reasonable possibility that the investigational product caused the event. Definite: the event was temporally related to the administration of the investigational product and no other etiology explained the event.

²AEs are ranked from highest to lowest incidence for any treatment group; if a subject experienced the same event more than once, only the first occurrence was tabulated.

Table 4: Overall and Individual Incidence of Adverse Events (≥5% with either Formula).

Discussion

LCPUFAs are essential nutrients for optimal brain and retinal development and infant growth [3-9,24,25]. Studies have shown the growth of infants fed LCPUFA-supplemented formulas do not differ significantly from breast-milk feeding, and the supplemented formulas are well tolerated [10,26-29]. Further, supplementation of DHA and ARA from single cell algal and fungal sources in term infants (at recommended worldwide human milk levels) results in RBC concentrations of the LCPUFAs, which are comparable to those of breast-fed infants [19,30-32]. The present study confirmed that a new infant formula (DHASCO[®]-B) supplemented with DHA from a unique strain of *Schizochytrium* sp microalgae promoted growth according to WHO growth standards [20], and in an equivalent manner to a standard reference formula (DHASCO[®]) [15].

Both formulas (DHASCO[®]-B and DHASCO[®]) are GRAS when incorporated in infant formula at a maximum use level of 1.25% of dietary fat [15,18]. This level corresponds to a maximum of 0.5% of total fat as DHA. Assuming human infants consume about 100 to 120 kcal/kg body wt/day, of which fat comprises about 50%, an infant will consume about 50-60 kcal/kg body wt/day of fat, or about 5.5-6.7 g of fat/kg body wt/day (1 g fat=9 kcal). The DHASCO[®]-B formula maximum intake of 1.25% of daily fat for an infant would correspond to 69-83 mg DHASCO[®]-B/kg body wt/day providing 27-33 mg/kg body wt/day of DHA which is consistent with current DHA recommendations for term and preterm infants of 10-60 DHA mg/kg body weight depending on gestational age [33]. DHASCO[®]-B is intended to be used in combination with a source of arachidonic acid (ARA) when added to pre-term, term, and follow on infant formulas. The ratio of DHA to ARA typically ranges from 1:1 to 1:2.

The present study demonstrated equivalent growth profiles (gains in weight, body length and head circumference) across both formulas [20], as well as comparable tolerability. Bioequivalence of DHA levels in RBCs, representative of tissue levels, was also demonstrated with DHASCO[®]-B compared to DHASCO[®]. When analyzed by gender, females displayed equivalent weight gain across both formulas. While male infants tended to have a greater rate of weight gain in the reference group, the difference from the DHASCO[®]-B group was not clinically meaningful. These results could be confounded by the fact that males are known to have greater rates of growth in the first 4 months of life [20]. The small sample size and statistical variability due to non-completers likely contributed to the non-equivalent findings in males, as the study showed equivalence in the analysis of the male infants who completed the study per protocol.

Supplementation of term infant formula with 0.36% DHA and 0.72% ARA during the first year of life yields clear differences in total RBC lipid composition and in visual function [30]. By 17 weeks of age, DHA concentrations in RBCs from supplemented formulas will more than double, and by week 39 will be more than 3 times. In the current study, infants in both formula groups had an equivalent absorption of DHA that is consistent with other published trials [5,8].

In conclusion, this trial demonstrated that at 4 months of life (120 days), there was no difference in daily weight gain between infants fed formula supplemented with DHASCO[®]-B than those fed with standard formula containing DHASCO[®] and the overall weight gains were found to be equivalent between the two formulas. Length gain, head circumference gain and ratio (weight/length) gain were also equivalent. The RBC levels of DHA were bioequivalent between the two formula groups and consistent with commercial supplemented formulas.

Tolerability was not significantly different between formula groups, nor was parent satisfaction different between the formulas. Overall, safety profiles for the formulas were similar and typical for this age group and did not raise any concerns.

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