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Study Data-on-File Report AK54

**Comparative Gastrointestinal Tolerance of Various Infant Formulas in
Healthy Term Infants**

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Study Objective

The primary objective of this study was to assess the comparative GI tolerance of term infants to two experimental soy-based infant formulas with supplemental fructooligosaccharides (FOS) and mixed carotenoids (lutein, lycopene, and beta-carotene) versus a standard commercial soy-based formula with long history of safe use.

Subjects

Eligible subjects were singleton, healthy term infants (gestational age 37-42 weeks) between 0 and 8 days of age and participated until 35 days of age. Sixteen study centers in the US recruited subjects from the local population.

Study Formulas

The control formula was Similac® Isomil® Advance® (Abbott Nutrition, Abbott Laboratories, Columbus, OH), a commercially available soy-based powdered infant formula [20% carbohydrate (CHO) as sucrose, DHA/ARA], **CF**. The two test formulas were (a) an experimental soy-based, powdered infant formula with FOS (GTC Nutrition, Golden, CO) [20% CHO as sucrose, 2.5 FOS g/L, mixed carotenoids, DHA/ARA], **EF1**; and (b) an experimental soy-based, powdered sucrose-free infant formula with FOS [100% CHO as corn syrup, 2.5 FOS g/L, mixed carotenoids, DHA/ARA], **EF2**. All study formulas met or exceeded the levels of nutrients recommended for term infants by the Committee on Nutrition of the American Academy of Pediatrics[1] and the requirements by the Infant Formula Act of 1980 [2] and its subsequent amendments. [3]

Study Design and Methods

A randomized, double-blind, multi-center, parallel study was conducted between November 2008 and April 2009. Enrolled infants were fed CF, EF1 or EF2. There were 3 study visits; Study Day (SDAY)/Study Visit 1 (Enrollment Visit at 0-8 Days of Age), Study Visit 2 (at 14 Days of Age), and Study Visit 3 (Exit Visit at 35 Days of Age). Infants were enrolled and randomly assigned to receive one of the three study formulas after parents or legally authorized representatives (LAR) have voluntarily signed the written informed consent approved by the Independent Ethics Committee/Institutional Review Board (IEC/IRB). Parents were given sufficient amounts of the assigned formula to feed their infant until the

next study visit. Parents kept daily records of formula intake (volume and frequency), incidence of spit-up and vomiting associated with feedings, occurrence of fussiness, occurrence of gas, and infant's stool characteristics (frequency, consistency, color, and odor). Growth (weight, length, head circumference [HC]) was measured at Study Visits 1, 2, and 3. Parent(s) were given a urine collection kit and instructed on how to collect urine samples prior to Study Visits 2 and 3. Urine specific gravity were determined and assessed at Study Visits 2 and 3 using the method described by Friedman et al [4]. A limited physical examination/assessment, which included hydration status, was performed by a study physician or nurse practitioner at Study Visits 2 and 3. Parent(s) completed the Infant Feeding and Stool Patterns Questionnaire and Formula Satisfaction Questionnaire at Study Visits 2 and 3. Interval history interviews were conducted at Study Visits 2 and 3 to identify adverse events (AEs), serious adverse events (SAEs), consumption of human milk/formula other than study formula and as well as the use of medications, supplements, home remedies or other sources of nutrition. The study was approved by the IEC/IRB and was conducted in accordance with ethical principles that have their origin in the Declaration of Helsinki.

Study Variables

The primary study variable was mean rank stool consistency (MRSC) from Study Visit 1 to Study Visit 3 (at 35 days of age). The secondary variables included average number of stools per day, and percent of feedings with spit up/vomit associated with (within one hour) feeding from Study Visit 1 to Study Visit 3 (at 35 days of age). Safety variables included SAEs and AEs. Supportive variables included urine specific gravity, hydration status, and limited physical examination/assessment; predominant stool consistency, color and odor; percentages of stool consistency and color; occurrence of fussiness and occurrence of gas; and average daily study product intake (average volume, average number of feedings). Supportive variables also included weight, length and HC, and their interval gains; and parental responses to the Infant Feeding and Stool Patterns, and Formula Satisfaction Questionnaires.

Statistical Methods

The primary hypothesis tested on MRSC (the study primary variable) was:

H0: $\mu CF = \mu EF1 = \mu EF2$ vs. H1: At least one inequality, where μCF, $\mu EF1$, and $\mu EF2$ denoted the MRSC means for the group fed the CF, EF1 and EF2, respectively. All

hypotheses tested for this study used two-sided, 0.05 level tests. Tests of interactions when conducted were two-sided, 0.10 level tests. Three multiple comparisons were made evaluating (1) CF vs. EF1, (2) CF vs. EF2, and (3) EF1 vs. EF2. For MRSC, the subset of multiple comparisons: EF1 and EF2 versus CF were of primary interest. Both the evaluable (EV) group and the intent-to-treat (ITT) group analyses were performed in this study. The Center for Disease Control (CDC) reference data [5] was used to compute standardized z-scores and percentiles for anthropometric variables. Percent data that were not normal were transformed using arcsine of the square root, and/or analyzed non-parametrically. Sample size was estimated assuming standard deviations ranging from 0.6 to 0.8 of the primary variable, MRSC, in similar study populations. Using two-sided multiple comparison tests by Tukey which preserve the family wise error rate for all 3 pair-wise comparisons at 5%, the power is about 80% to detect differences between pairs of means ranging from 0.45 to 0.60 when the total sample size is 117 infants (39 per group). The software nQuery® Advisor Version 5.0 was used to calculate the sample size. Enrollment of 156 infants (52 per group) was planned to account for an assumed 25% attrition rate.

Results

Subject Disposition

A total of 195 subjects were enrolled into the study; 65, 67 and 63 subjects were in the CF, EF1 and EF2 groups, respectively. Seven subjects did not receive any study product and were excluded from the ITT analysis. The remaining 188 subjects were included in the ITT group. Two subjects in EF1 in the ITT group did not satisfy eligibility criteria and were excluded from the EV group. There were 186 subjects in the EV group at Study Visit 1. One hundred and forty-two subjects were evaluable at Study Visit 2, and 120 subjects were evaluable at Study Visit 3. There were no significant differences between the 3 study groups in gender, ethnicity, and study completion rates. Study completion rates for CF, EF1 and EF2 were 81%, 86% and 87%, respectively.

Mean Rank Stool Consistency

Mean ranked stool consistency (the primary study variable) was not significantly different (p>0.05) between any of the feeding groups from SDAY1 to 35 days of age (Table 2).

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Formula Intake and Stool Characteristics

Analyses of secondary variables revealed no significant differences in the average number of stools per day or percent of feedings with spit up/vomit associated with (within one hour) feeding among the 3 study formulas (Table 3). There were no significant differences observed among the study groups in most of the supportive intake and stool variables including formula intake volume; and predominant stool consistency, color, odor and gassiness. The only exceptions were average numbers of feeding per day, percent hard stools and percent yellow stools. The EF2 group had significantly higher average numbers of feedings per day compared to the EF1 group at SDAY1 to 14 in the EV analysis, but not in the ITT analyses. Percentages of hard stools were significantly higher in EF1 versus EF2 at SDAY1-14, but not at SDAY15-35 for both EV and ITT analyses. Percentages of yellow stools were significantly higher in EF1 versus CF at SDAY1-14, but not at SDAY15-35 for both EV and ITT analyses.

Growth

Growth as indicated by weight, length, HC and their respective gains were not significantly different among the study groups (Table 1). Growth measures were normal throughout the study, with the group means ranging between 24th and 60th percentiles on the CDC Growth Chart.

Safety Measures

Safety measures including SAEs, AEs, hydration status and urine specific gravity were not significantly different among the study formula groups (Tables 4 and 5). A total of 6 SAEs were reported in the study, 2 in each study group. All the SAEs were rated as "not related" or "probably not related" to the study product. No deaths were reported in the study. Of the 136 total AEs, 45 were reported in the CF group, 49 in the EF1 group, and 42 in the EF2 group. There were 7 parental AE reports of loose watery stools in the EF1 group compared to 2 in the EF2 group and 4 in the CF group. However, they were not significantly (p>0.05) different, and the hydration status and urine specific gravity for these subjects were normal. No significant differences were noted among the 3 study groups in physical assessment and urine specific gravity performed (supportive safety variables) at 14 days of age (Visit 2), and at 35 days of age (Visit 3) (Table 5). Only one subject had an abnormal urine specific gravity. This subject in the EF2 group had a specific gravity value of 1.0388 at 14 days of age (normal value is < 1.030 [3]) but subsequently had normal values at 35 days of age. This

subject did not have any reported SAE or diarrhea or watery stools. The subject successfully completed the study and was included in the EV analyses.

<u>Questionnaire Responses</u>

Parental responses to three questions in the Infant Feeding, Stool and Formula Satisfaction Questionnaires were significantly different. The question, "Would you want to continue using the study formula?", showed a significantly higher ($p<0.05$) positive response for EF2 versus EF1 and EF2 versus CF only in the ITT analysis. The question, "How well did study powder mix with water?" yielded a significantly higher ($p<0.05$) positive response for CF versus EF1 in both EV and ITT analyses; and for EF2 versus EF1 only in the ITT analysis. The parental response to the question on "My baby was gassy" indicated that the EF1 group was significantly less gassy compared to EF2 only at 14 days of age and not at 35 days of age in the EV analyses but not in the ITT analyses.

Conclusion

This study is the first clinical study to evaluate soy protein-based infant formulas supplemented with FOS and mixed carotenoids. This study demonstrated that the addition of FOS at 2.5g/L and mixed carotenoids to soy protein-based formulas, with or without sucrose, was safe and well tolerated in healthy term newborn infants.

References

1. Committee on Nutrition American Academy of Pediatrics. *Pediatric Nutrition Handbook. 6th* ed. Elk Grove Village, IL: American Academy of Pediatrics, 2009, pp 61-78.

2. Infant Formula Act of 1980, *Public Law* 96-359, Sept. 26, 1980.

3. Infant Formula Act Amendments, 21 Code of Federal Regulations (As Amended), [412] sec. 350a, Infant Formulas, Oct. 27, 1986.

4. Friedman JN, Goldman RD, Srivastava R, Parkin PC. Development of a clinical dehydration scale for use in children between 1 and 36 months of age. *J Pediatr* 2004;145:201-7.

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Table 1. Anthropometric Measures for Study Subjects *

	EV Group			ITT Group		
	CF	EF1	EF2	CF	EF1	EF2
Average Weight (g)						
SDAY1						
Mean ± SEM (n)	3297 ± 58 (62)	3318 ± 49 (62)	3150 ± 48 (62)	3297 ± 58 (62)	3311 ± 50 (64)	3150 ± 48 (62)
SDAY14						
Mean ± SEM (n)	3566 ± 56 (44)	3571 ± 56 (46)	3399 ± 56 (52)	3636 ± 59 (56)	3616 ± 59 (57)	3426 ± 51 (58)
SDAY35						
Mean ± SEM (n)	4307 ± 73 (37)	4302 ± 72 (39)	4190 ± 70 (44)	4339 ± 68 (50)	4373 ± 66 (54)	4213 ± 60 (54)
Weight Gain (g/day) SDAY1-35						
Mean ± SEM (n)	35.8 ± 1.9 (37)	34.0 ± 1.7 (39)	35.3 ± 1.6 (44)	35.1 ± 1.6 (50)	34.7 ± 1.4 (54)	35.6 ± 1.4 (54)
Length Gain (cm/day) SDAY1-35						
Mean ± SEM (n)	0.14 ± 0.01 (37)	0.14 ± 0.01 (39)	0.14 ± 0.01 (44)	0.13 ± 0.01 (49)	0.15 ± 0.01 (54)	0.14 ± 0.01 (54)
HC Gain (cm/day) SDAY1-35						
Mean ± SEM (n)	0.09 ± 0.00 (37)	0.10 ± 0.01 (39)	0.09 ± 0.00 (44)	0.09 ± 0.00 (50)	0.10 ± 0.01 (54)	0.10 ± 0.00 (54)

* No significant differences (p > 0.05).

Table 2. Primary Study Variable - Mean Rank Stool Consistency (MRSC) *[†]

	EV Group			ITT Group		
	CF	EF1	EF2	CF	EF1	EF2
SDAY1-14						
Mean ± SEM (n)	2.5 ± 0.1 (51)	2.6 ± 0.1 (57)	2.5 ± 0.1 (57)	2.5 ± 0.1 (55)	2.6 ± 0.1 (59)	2.5 ± 0.1 (59)
SDAY15-35						
Mean ± SEM (n)	2.6 ± 0.1 (40)	2.7 ± 0.1 (41)	2.5 ± 0.1 (46)	2.7 ± 0.1 (45)	2.7 ± 0.1 (46)	2.5 ± 0.1 (51)

* No significant differences (p > 0.05).

[†] MRSC Score: 1 = watery, 2 = loose/mushy, 3 = soft, 4 = formed, 5 = hard

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TABLE 3. Formula Intake and Tolerance, and Stool Characteristics in Study EV Group *						
Study Variables	**SDAY 1-14**			**SDAY 15-35**		
	CF	**EF1**	**EF2**	**CF**	**EF1**	**EF2**
Average Numbers of Feedings, #/d [T]	7.6 + 0.2 (47)	7.1 + 0.2 (56)	7.9 + 0.2 (56)	7.1 + 0.2 (40)	7.1 + 0.2 (40)	7.5 + 0.3 (47)
Average Formula Intake, mL/d	555 + 17 (47)	559 + 20 (56)	570 + 20 (56)	673 + 22 (40)	739 + 27 (40)	726 + 35 (47)
Spit-up/Vomit, % of feedings	22.4 + 4.0 (52)	23.5 + 3.8 (58)	21.9 + 3.7 (58)	17.4 + 4.1 (40)	17.8 + 4.1 (40)	17.5 + 3.6 (47)
Stool Frequency, # stools/d	2.9 + 0.3 (48)	3.3 + 0.3 (55)	3.1 + 0.3 (56)	2.1 + 0.2 (40)	2.7 + 0.3 (41)	2.7 + 0.3 (46)
Percent Hard Stools [‡]	3.5 + 1.7 (51)	5.6 + 2.5 (57)	1.3 + 0.9 (57)	4.0 + 2.2 (40)	1.4 + 0.6 (41)	2.8 + 1.4 (46)
Percent Yellow Stools [§]	51.2 + 5.1 (51)	65.3 + 4.8 (57)	58.3 + 4.7 (57)	40.2 + 6.5 (40)	50.9 + 6.4 (41)	53.0 + 6.1 (46)

* Value are mean ± SEM (n).   [T] Average Numbers of Feedings at SDAY1-14; EF2>EF1 (P=0.0215).
[‡] Percent of Hard Stools at SDAY1-14; EF1>EF2 (P=0.0207)
[§] Percent of Yellow Stools at SDAY1-14: EF1>CF (P=0.0314) using Arcsine Square Root Transformation.

Table 4. Serious Adverse Events Occurrence in Study Subjects *

Treatment group	Subjects with SAE	Gender	Age at Onset in Days	Type of SAE	Complaint or Diagnosis	Relationship to Product	Did subject complete the study?
CF	2	Male	6	Hospitalization	Respiratory syncytial virus positive bronchiolitis	Probably Not	No
		Male	21	Hospitalization	Rule out sepsis	Not Related	Yes
EF1	2	Male	34	Hospitalization, Medical Event Requiring Intervention	Streptococcal sepsis	Not Related	Yes
		Female	14	Hospitalization	Urinary tract infection	Probably Not	Yes
EF2	2	Male	17	Hospitalization	Fever, aseptic meningitis enterovirus positive	Not Related	Yes
		Female	9	Hospitalization	Vomiting	Probably Not	Yes

* No significant differences (p > 0.05).

Table 5.   Urine Specific Gravity for EV Subjects *

	Treatment group		
	CF	**EF1**	**EF2**
SDAY14			
Mean $\pm$ SEM (n)	1.0041 $\pm$ 0.0005 (40)	1.0038 $\pm$ 0.0004 (42)	1.0044 $\pm$ 0.0009 (42)
Normal ? [Urine specific gravity $\leq$ 1.030,** n (%)]			
Yes	40(100)	42(100)	46(98)
No	0(0)	0(0)	1(2)
SDAY35			
Mean $\pm$ SEM (n)	1.0043 $\pm$ 0.0004 (35)	1.0034 $\pm$ 0.0003 (38)	1.0039 $\pm$ 0.0004 (43)
Normal ? [Urine specific gravity $\leq$ 1.030,** n (%)]			
Yes	35(100)	38(100)	43(100)
No	0(0)	0(0)	0(0)

* No significant differences ($p > 0.05$).   ** Friedman et al [4]