Original Article

Gastrointestinal tolerance of a new infant milk formula in healthy infants: multicenter study conducted in Taiwan

Nancy Chen[1] MS, RD, Pedro A Alarcon[2] MD, PhD, Gail M Comer[2] MD and Randall L Tressler[2] MD

[1]*Abbott Laboratories, Taipei, Taiwan*
[2]*Abbott Laboratories, Abbott Park, Illinois, USA*

The objective of this study was to test whether the gastrointestinal tolerance of a new infant formula equalled or exceeded the tolerance of other milk-based infant formulas, and to compare the tolerance of the new formula to that of human milk. This prospective, observational, multicenter, open-label study was conducted in Taiwan. Healthy, full-term infants aged 28–98 days were enrolled on their current feeding regimen (no treatment assigned). Feeding regimens included human milk (HM), a new infant formula (NF, Similac Advance®), other marketed infant formulas (OF, mainly Enfalac® or S-26®), HM + NF and HM + OF. Data for stool frequency, stool consistency and gastrointestinal intolerance symptoms were recorded in study diaries by parents for a period of two weeks. Gastrointestinal tolerance was evaluated in 967 infants, of whom 481 (49.7%) received NF, 312 (32.2%) received OF, 101 (10.4%) received HM + NF, 41 (4.2%) received HM + OF and 32 (3.3%) received HM. Infants fed HM only had softer and more frequent stools than those who received NF only or OF only ($P < 0.001$). Infants fed NF only had softer stools than those fed OF only ($P < 0.001$), including those fed either Enfalac® or S-26® ($P < 0.001$). There were no significant differences between feeding groups for the incidence of general intolerance, spit-up or flatulence. All feeding regimens were well tolerated. We thereby concluded that NF is well tolerated in healthy infants and results in stool consistencies that more closely resemble those of infants fed human milk than those of infants fed other formulas.

Key words: human milk, infant formula, stool consistency, stool frequency, Taiwan.

Introduction

Many infants experience undesirable gastrointestinal (GI) effects, such as colic, constipation, flatulence or regurgitation. These symptoms are often thought to depend on the infant's diet, particularly in formula-fed infants, but may also be seen in infants receiving breast milk. Although most of these symptoms appear to be resolved spontaneously and may be part of the infant's normal development, perceived intolerance to infant formula is a frequently reported reason for changing formula.[1,2] Infants may be switched from one formula to another because of colic, excessive spit-up or changes in the frequency or consistency of the infants' stools.

Stool characteristics vary depending on the type of diet the infant receives. Infants fed with human milk (HM) usually have an average of three or more watery and/or semi-liquid bowel movements per day, which are yellowish in color in 90% of cases. Formula-fed infants normally have fewer bowel movements (1–2/day), which are usually brown or green in color, and generally soft but with a definite shape.

Stool characteristics may also vary depending on the type of infant formula used. Commercially available formulas differ from each other in the types and concentrations of proteins and lipids used, the concentrations of micronutrients, and in their processing. These differences may affect stool consistency and frequency as well as tolerance. Formulas that contain palm olein oil have been associated with decreased calcium absorption and harder stools.[1,3] In addition, some formulas contain animal fats, which are less-well absorbed than vegetable fats.[4] Proteins and nucleotides have both been reported to influence the population of intestinal microflora.[5,6] The two main protein sources in human and cow's milk are casein and whey. The ratio between these proteins in breast milk varies over time, passing from a ratio of 90:10 (whey:casein) when feeding starts,[7] to 60:40 or 50:50 in mature milk.[8,9]

A new infant formula has been developed to provide an appropriate blend of lipids and to simulate the whey:casein ratio and nucleotide concentrations of mature human milk.[1] It was hypothesized that the new formula would produce clinical outcomes similar to those associated with human milk. The aim of this study was to evaluate and compare the

Correspondence address: Dr Gail M. Comer, Abbott Laboratories, 200 Abbott Park Road, Department R4NH, Building AP-30-2, Abbott Park, IL 60064–6149, USA.
Tel. 847-938-3692; Fax: 847-938-8355
Email: gail.comer@abbott.com
Accepted 7 November 2001

GI tolerance of the new formula, other commercially available infant formulas and human milk in a large population of healthy infants in Taiwan.

Materials and methods

This open-label, observational, prospective study was conducted at 98 sites in Taiwan. It was conducted in accordance with the Declaration of Helsinki and Good Clinical Practices. Informed consent was obtained from each infant's parent or legal guardian prior to enrolment and was consistent with the regulations of the institutions and their ethics committees or institutional review boards. This was an observational study using marketed products; the infants in the study continued to receive whatever formula and amount of formula they were getting prior to this study and there were no specimen collections or changes in feeding regimen.

Infants considered for enrolment were aged 28–98 days, with a gestational age of 38–42 weeks, had a birthweight greater than or equal to 2500 g, were in apparent good health and were free of major congenital anomalies or systemic diseases. Mothers of infants to be enrolled had no evidence of significant diseases, such as diabetes (including gestational diabetes), tuberculosis or perinatal infections, that have proven adverse effects on fetuses. Subjects considered for enrolment were those attending routine visits to the pediatrician, and no inducements were offered for enrolment. All infant formulas and human milk were provided by the subject's parent or guardian.

In this two-week study, GI tolerance was evaluated in healthy infants who received one of the following feeding regimens: human milk (HM) only, the new infant formula (NF) only, other commercial formula (OF, Enfalac® (EF) or S-26® (SF)) only, HM supplemented with NF (HM + NF) or HM supplemented with OF (HM + OF). A summary of the main components of the study feedings is presented in Table 1. Investigators did not decide the feeding regimens. In order to be admitted to the study, infants were to have received their designated feeding for at least one week prior to enrolment and parents accepted that their infants would continue receiving that feeding, with no other milk-based feeding, for the duration of the study. The 14-day study period was considered sufficient to permit detection of any differences in gastrointestinal function between feeding groups. A longer study period may have compromised the willingness of the parents or caregivers to participate in the study and resulted in a higher dropout rate and fewer evaluable subjects.

Gastrointestinal tolerance was evaluated in terms of stool consistency and frequency and the incidence of GI intolerance symptoms, such as general intolerance, spit-up and flatulence, which were recorded in a study diary by the subject's parents/guardian. Subjects were evaluated by the investigator at the beginning (Day 0) and the end (Day 14) of the study. Each investigator was responsible for instructing parents and guardians of the subjects about recording the description of parameters related to bowel function and the incidence of GI intolerance symptoms for completion of

Table 1. Composition of major study feedings

Formula/milk	Protein (g/100 kCal)	Carbohydrate (g/100 kCal)	Fat (g/100 kCal)	Fat components (%)	Calcium (mg/100 kCal)	Phosphorus (mg/100 kCal)
Human milk (mature)	1.5	10.6	5.7	Saturated (44.2), monounsaturated (41.6), polyunsaturated (14.2)	41	21
NF	2.1	10.6	5.5	High oleic safflower oil (42), coconut oil (30), soy oil (28)	77	43
SF	2.3	10.8	5.4	Oleo, coconut oil, soy oil, safflower oil†	69	50
EF	2.2	10.4	5.5	Palm olein oil (45), coconut oil (20), soy oil (20), sunflower oil (15)	67	45

Formula content was assessed using the claims on the labels of the products and the study by Lloyd *et al.*[1] The composition of mature human milk was taken from Kleinman.[10] †Percentage of fat components not known. EF, Enfalac; NF, new formula (Similac Advance); SF, S-26.

the diary. At investigators' meetings, the investigators were given training for explaining the responsibilities to parents or guardians; the same instructions and explanations were provided for all feeding groups. At the end of the study, the investigator reviewed the diary with the parent/guardian and asked questions to verify the completeness and accuracy of the diary entries.

Statistical methods

All evaluable subjects (defined as those who complied fully with their feeding regimen during the 14-day study period) were included in outcome analyses. All enrolled subjects were included in the safety analysis. Demographic and baseline characteristics were summarized by descriptive statistics and analyzed by analysis of variance (ANOVA) by rank. Feeding regimens were classified by sex, and chi-squared analysis of feeding groups by was performed for both groups.

Stool consistency, as recorded in the study diaries, was converted to a numerical value as follows: 1 = watery (i.e., runny, mostly liquid); 2 = loose/mushy (i.e., mixed with water); 3 = soft (pasty); 4 = formed (i.e., had some shape, yet moist); 5 = hard (i.e., well-shaped, dry pellets). Stool frequency and consistency were summarized by descriptive statistics and analyzed by ANOVA by ranks. Pairwise comparisons between feeding groups were performed using the Student's *t*-test, with adjustments for multiple testing using Bonferroni's method.[11] The incidence of GI intolerance symptoms (general GI intolerance, spit-up and flatulence) was analyzed by the Cochran–Mantel–Haenzsel test on Day 14.

Pairwise comparisons were performed for all variables between the following groups: HM versus NF, HM versus OF, NF versus OF, HM versus HM + NF, and HM + NF versus HM + OF. In addition, pairwise comparisons were performed between NF (with and without HM), EF and SF (with and without HM). EF and SF were the other infant formulas consumed most frequently by the subjects (n = 84 for SF; n = 116 for EF). Because physicians were free to enrol as many subjects as feasible, no power analyses were performed; the study was designed to be strictly observational. However, the Bonferroni adjustment was used to maintain Type I error at the 0.05 level.

All subjects enrolled in the study were included in the safety analysis. Adverse events were rated for intensity and relationship to the feeding regimen and were summarized by frequency tables.

Results

Subject population

A total of 1000 infants were enrolled in the study, of which 967 were evaluable. Among evaluable subjects, 32 (3.3%) received HM only, 101 (10.4%) received HM + NF, 41 (4.2%) received HM + OF, 481 (49.7%) received NF only and 312 (32.2%) received OF only. There were no significant differences between feeding groups for any demographic and baseline variables except age (P = 0.017). Subjects in the NF only and OF only groups were older at enrolment than subjects in the other feeding groups (Table 2).

Stool consistency and frequency

There were significant differences in mean stool consistency among all feeding groups ($P < 0.001$) and for pairwise comparisons between feeding groups ($P < 0.01$). The average stool consistencies were semiliquid to soft for subjects in the HM only and NF only groups, and soft to formed for subjects in the OF group. The mean stool consistency was significantly lower (softer) in the HM group compared with the NF only and OF only groups ($P < 0.001$), and significantly lower in the NF only group compared with the OF only group ($P < 0.001$). Likewise, subjects who received HM + NF had softer stools than those who received HM + OF ($P < 0.001$), but harder stools than those who received HM only ($P < 0.01$; Fig. 1). Mean stool consistencies for infants who received either EF or SF were significantly harder than for infants who received NF ($P < 0.001$). In addition, infants who received HM + SF had significantly harder stools than infants who received HM + NF (P = 0.001; Fig. 2).

There was also a significant difference for mean stool frequency among all feeding groups ($P < 0.001$). Subjects in the HM only group had significantly more stools/day (mean 2.97) than subjects who received NF only (mean 1.43 stools/day) and subjects who received OF only (mean 1.22 stools/day; $P < 0.001$). Subjects who received HM + NF or HM + OF had more frequent stools than those who received NF or OF only, but less frequent stools than those who received HM only, although these differences were not statistically significant (Fig. 3). Likewise, subjects who received EF only or SF only had less frequent stools than those who received NF only, but these differences were not statistically significant.

Table 2. Subject demographics and weight

	HM only (n = 32)	HM + NF (n = 101)	HM + OF (n = 41)	NF only (n = 481)	OF only (n = 312)	P-value
Mean birthweight (g)	3175	3302	3249	3196	3223	0.133[a]
Mean present weight (g)	4850	4994	5051	5094	5082	0.485[a]
Mean age (weeks)	6.1	6.5	6.9	7.2	7.2	0.017[a]
Sex						0.078[b]
Male (%)	46.9	48.5	73.2	54.1	55.4	
Female (%)	53.1	51.5	26.8	45.9	44.6	

HM, human milk; NF, new formula (Similac Advance); OF, other infant formula. P-values from [a]F-test, [b]χ^2-test.

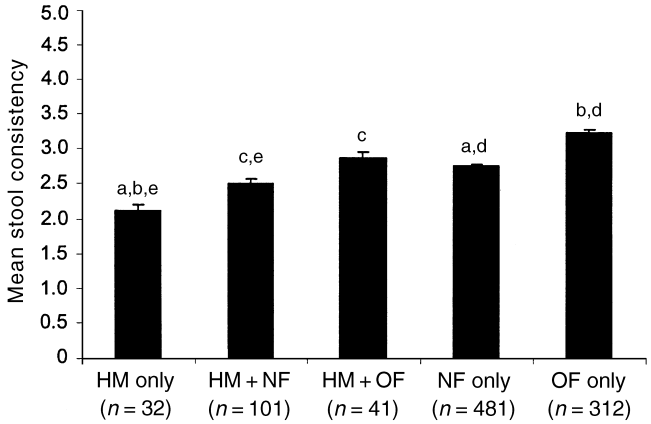

Figure 1. Mean stool consistency: pairwise comparison of feeding groups. Stool consistency was based on a scale of 1 = watery to 5 = hard. Statistical differences between feeding groups are represented by letters ('a'–'e') above the bars in the graph. When two bars have the same letter, this indicates a statistical difference between these two groups. If a bar has more than one letter, this group is statistically different from more than one other group. If a bar has no letters, there are no statistical differences between this group and any other group. The groups labelled 'a'–'d' have significant differences at the level of $P \leq 0.001$, the groups labelled 'e' differ significantly at the level of $P < 0.01$. *P*-values were calculated from the Student's *t*-test with adjustment for multiple testing by Bonferroni's method. HM, human milk; NF, new formula (Similac Advance); OF, other infant formula.

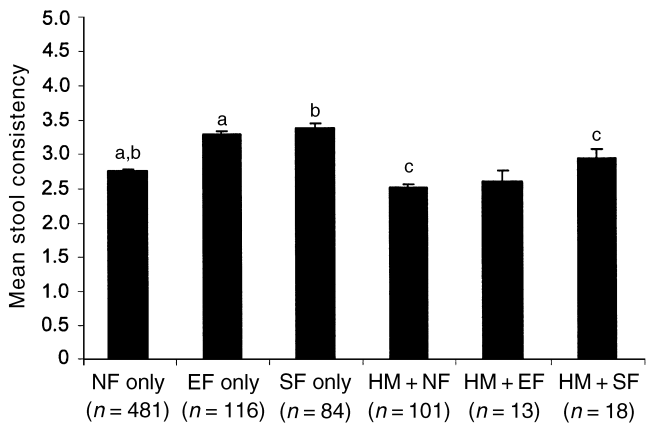

Figure 2. Mean stool consistency: pairwise comparisons of NF and other infant formulas. Stool consistency was based on a scale of 1 = watery to 5 = hard. Statistical differences between feeding groups are represented by letters ('a'–'c') above the bars in the graphs. When two bars have the same letter, this indicates a statistical difference between these two groups. If a bar has more than one letter, this group is statistically different from more than one other group. If a bar has no letters, there are no statistical differences between this group and any other group. All significant differences are at the level of $P \leq 0.001$. *P*-values were calculated from the Student's *t*-test with adjustment for multiple testing by Bonferroni's method. EF, Enfalac; NF, new formula (Similac Advance); SF, S-26.

Gastrointestinal intolerance symptoms

The overall incidence of GI intolerance symptoms was low. At the end of the study (Day 14), general intolerance was reported for only 5.9% of all subjects, spit-up for 3.3% of subjects and flatulence for 3.4% of subjects. The incidence of general intolerance was lowest in the HM and HM + NF groups (3.1% and 3.0%, respectively), but there were no statistically significant differences between feeding groups for the incidence of general intolerance, spit-up or flatulence (Table 3). In addition, there were no statistically significant differences between infants fed NF and those fed EF or SF for the incidence of any GI intolerance variables.

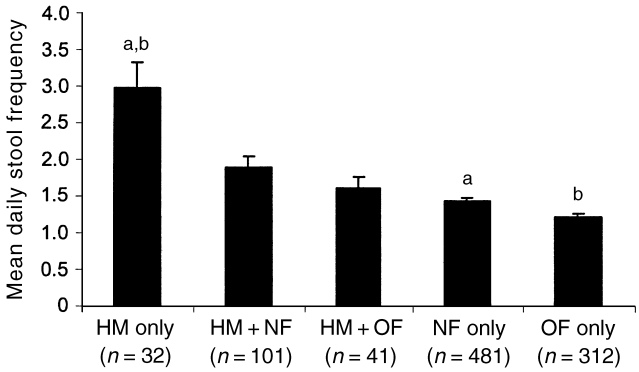

Figure 3. Mean daily stool frequency: pairwise comparison of feeding groups. Statistical differences between feeding groups are represented by letters ('a', 'b') above the bars in the graphs. When two bars have the same letter, this indicates a statistical difference between these two groups. If a bar has more than one letter, this group is statistically different from more than one other group. If a bar has no letters, there are no statistical differences between this group and any other group. All significant differences are at the level of $P \leq 0.001$. *P*-values were calculated from the Student's *t*-test with adjustment for multiple testing by Bonferroni's method. HM, human milk; NF, new formula (Similac Advance); OF, other infant formula.

Safety

The overall incidence of adverse events was low. Only 21 subjects (2.1%) experienced one or more adverse events. The most common events were rhinitis (four subjects), pain (three subjects) and flu syndrome (three subjects). There were no significant differences between feeding groups for the overall incidence of adverse events, the severity of any adverse events or the incidence of adverse events considered to be related to the study feeding. Most adverse events were mild or moderate in severity and considered unrelated to the study feeding. Only one subject (NF only group) had a serious adverse event (pyloric stenosis with vomiting), which was considered to be unrelated to the study feeding.

Seven subjects discontinued the study due to an adverse event, not necessarily related to the study formula: six in the NF only group and one in the other formula only group. Six of these events were considered to be mild in intensity, and four were considered possibly related to the study feeding.

Discussion

This open-label, observational study was designed to evaluate and compare the GI tolerance of various feeding regimens, including NF (a novel milk-based infant formula), in a large population of healthy infants in Taiwan. Feeding

Table 3. Incidence of gastrointestinal intolerance symptoms

	% HM only (n = 32)	% HM + NF (n = 101)	% HM + OF (n = 41)	% NF only (n = 481)	% OF only (n = 312)	Total % (n = 967)
Intolerance (general)	3.1 (1)	3.0 (3)	7.3 (3)	5.2 (25)	8.0 (25)	5.9 (57)
Spit-up	0.0 (0)	3.0 (3)	4.9 (2)	3.1 (15)	3.8 (12)	3.3 (32)
Flatulence	0.0 (0)	1.0 (1)	2.4 (1)	2.7 (13)	5.8 (18)	3.4 (33)

HM, human milk; NF, new formula (Similac Advance); OF, other infant formula.

groups were generally comparable with respect to subject demographic and baseline characteristics. The majority of subjects received either NF only or other infant formula only (mainly EF or SF).

Infants fed NF only had stool consistencies that more closely resembled those of infants fed HM than did the stool characteristics of infants fed OF only. In particular, stool consistency was softer in the NF group than in the EF and SF groups. Similarly, infants fed NF in combination with HM had stool consistencies that more closely resembled those of infants fed HM only than did the stool consistencies of infants fed HM + OF. These results are consistent with those observed when the same study was conducted in approximately 7000 subjects in 17 countries (Alarcon PA, Tressler RL, Mulvaney A, Lam W, Comer GM, unpubl. data, 2001).

In the international study, statistically significant differences were also observed between NF and other infant formulas for stool frequency, with subjects who received NF having more frequent stools than subjects who received other formulas. A similar trend was observed in this Taiwan study, but this was not statistically significant, probably because of the smaller numbers of infants enrolled in this study.

The specific components in an infant's formula, particularly the lipids, can affect stool characteristics.[1,3] Human milk fat has a high content of saturated fatty acids (44% fatty acids, including 23% palmitic acid) and is very well absorbed by infants. This good absorption is attributed to the fact that approximately 70% of the palmitic acid is in the *sn*-2 position. In contrast, in fats of vegetable origin, less than 15% of palmitic acid is located in the *sn*-2 position. For example, palm oil, which contains 44–48% palmitic acid, has only approximately 9% of its palmitic acid in the *sn*-2 position.[3] The new formula contains only vegetable-derived fats (high-oleic safflower, coconut and soy), whereas SF contains a different oil blend as part of its mixture of fats (Table 1). The differences in fat absorption from different formulas could account for some of the differences in stool characteristics observed for infants fed NF and SF.

The fat blend of EF contains 45% palm olein in addition to soy, coconut and high-oleic sunflower oil (20, 20 and 15%, respectively).[1] Because palm olein oil contains palmitic acid primarily not in the *sn*-2 position, this fat is poorly absorbed. Unabsorbed palmitic acid binds to calcium and forms insoluble soaps, which may be responsible for differences in GI tolerance.[12] Increased levels of insoluble soaps are also associated with harder stools. As the fat blend of NF contains no palm olein oil,[1] infants fed NF could therefore be expected to have softer stools than infants fed EF.

Hard stools sometimes cause infants to have difficulty with bowel movements and may be associated with pain or trauma. Thus, softer stools are generally preferable for the infant. The softer stool consistency observed in the NF group indicates that the outcomes for infants fed NF are similar to those associated with the consumption of HM.

The incidence of GI intolerance symptoms was very low and comparable among all feeding groups. General intolerance, spit-up and flatulence were each reported for less than 6% of infants. In the international study, subjects who received NF had a significantly lower incidence of general intolerance and spit-up than subjects who received OF (Alarcon PA, Tressler RL, Mulvaney A, Lam W, Corner GM, unpubl. data, 2001). The lack of significant differences in this study is likely due to the low overall incidence of these events and the smaller number of subjects evaluated.

In summary, the new formula is well tolerated in healthy infants and results in stool consistencies that more closely resemble those of infants fed HM than the stool consistencies of infants fed other formulas.

References

1. Lloyd B, Halter RJ, Kuchan MJ, Baggs GE, Ryan AS, Masor ML. Formula tolerance in post breastfed and exclusively formula-fed infants. Pediatrics 1999; 103: 1–6.
2. Vanderhoof JA, Murray ND, Kaufman SS, Mack DR, Antonson DL, Corkins MR, Perry D, Kruger R. Intolerance to protein hydrolysate infant formulas: an under recognized cause of gastrointestinal symptoms in infants. J Pediatr 1997; 131: 741–744.
3. Nelson SE, Rogers RR, Frantz JA, Ziegler EE. Palm olein in infant formula: absorption of fat and minerals by normal infants. Am J Clin Nutr 1996; 64: 291–296.
4. Lebenthal E, ed. Textbook of Gastroenterology and Nutrition in Infancy. New York: Raven Press, 1981.
5. Dean T. Cow's milk allergy: therapeutic options and immunological aspects. Eur J Clin Nutr 1995; 49 (Suppl.): 19–25.
6. de Boissieu D, Matarazzo P, Dupont C. Allergy to extensively hydrolyzed cow milk proteins in infants: identification and treatment with an amino acid-based formula. J Pediatr 1997; 131: 744–747.
7. Sánchez-Hidalgo VM, Flores-Huerta S, Matute G, Serrano C, Urquieta B, Espinoza R. Whey protein/casein ratio and nonprotein nitrogen in preterm human milk during the first 10 days postpartum. J Pediatr Gastroenterol Nutr 1998; 26: 64–69.
8. Ronayne De Ferrer PA, Sambucetti ME. Casein to whey protein ratio in rat and human milks: effects of maternal protein intake. J Dairy Sci 1993; 76: 1645–1653.
9. Kunz C, Lönnerdal B. Human-milk proteins: analysis of casein and casein subunits by anion-exchange chromatography, gel electrophoresis, and specific staining methods. Am J Clin Nutr 1990; 51: 37–46.

10. Kleinman RE, ed. Pediatric Nutrition Handbook, 4th edn. Elk Grove Village, IL: American Academy of Pediatric Press, 1998.
11. Milliken GA, Dallas EJ. Simultaneous inference procedures and multiple comparisons. In: Milliken GA, Dallas EJ, eds. Analysis of Messy Data, 1st edn. London: Chapman and Hall, 1992; 33–34.
12. Nelson SE, Frantz JA, Ziegler EE. Absorption of fat and calcium by infants fed a milk-based formula containing palm olein. J Am Coll Nutr 1998; 17: 327–332.