Fructooligosaccharides and Fiber Partially Prevent the Alterations in Fecal Microbiota and Short-Chain Fatty Acid Concentrations Caused by Standard Enteral Formula in Healthy Humans[1,2]

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ABSTRACT The intestinal microbiota are important during enteral tube feeding because they exert colonization resistance and produce SCFAs. However, the effect of the enteral formula composition on major bacterial groups of the microbiota has not been clearly defined. The aim of this study was to investigate the effect of enteral formulas with and without prebiotic fructooligosaccharides (FOS) and fiber on the fecal microbiota and SCFAs. Healthy subjects ($n = 10$; 4 men, 6 women) consumed both a standard enteral formula and one containing FOS (5.1 g/L) and fiber (8.9 g/L) as a sole source of nutrition for 14 d in a randomized, double-blind, crossover trial with a 6-wk washout phase. Fecal samples were collected at the start and end of each formula phase, and were analyzed for major bacterial groups and SCFA concentrations using fluorescent in situ hybridization and GLC, respectively. Although there were reductions in total fecal bacteria due to both formula treatments, concentrations were higher after the FOS/fiber formula period compared with the standard formula period (11.2 ± 0.2 vs. 11.0 ± 0.2 $\log_{10}$ cells/g, $P = 0.005$). The FOS/fiber formula increased bifidobacteria ($P = 0.004$) and reduced clostridia ($P = 0.006$). Compared with the standard formula, the FOS/fiber formula resulted in higher concentrations of total SCFA (332.4 ± 133.8 vs. 220.1 ± 124.5 μmol/g, $P = 0.022$), acetate (219.6 ± 96.3 vs. 136.8 ± 74.5 μmol/g, $P = 0.034$) and propionate (58.4 ± 37.4 vs. 35.6 ± 25.5 μmol/g, $P = 0.02$). This study demonstrates that standard enteral formula leads to adverse alterations to the fecal microbiota and SCFA concentrations in healthy subjects, and these alterations are partially prevented by fortification of the formula with FOS and fiber. J. Nutr. 135: 1896–1902, 2005.

KEY WORDS: • *enteral nutrition* • *microbiota* • *prebiotics* • *fiber* • *SCFA*

Enteral tube feeding (ETF)[4] is a common method of nutritional support for patients in both hospital and community settings (1). Complications that can occur include an increased risk of *Clostridium difficile* colonization (2) and an abnormal secretion of water into the lumen of the ascending colon (3). There is a potential interaction between these abnormal responses and the colonic microbiota. First, the indigenous microbiota compete for nutrients (4), and some bifidobacteria produce antimicrobials, thereby exerting colonization resistance against enteropathogens (5). Second, the colonic microbiota ferment carbohydrates and proteins to produce SCFAs, which stimulate sodium and water absorption, causing a reversal of the abnormal colonic secretory response (6).

The colonic microbiota is a diverse ecosystem of >500 bacterial species (7). Its composition is dependent upon a number of factors including age (8), antibiotics (9), and disease [e.g., *C. difficile* colonization (8)] all of which are relevant to patients administered ETF. Diet itself also influences the composition and activity of the colonic microbiota. Prebiotic fructooligosaccharides (FOS) selectively stimulate the proliferation of bifidobacteria (10), and dietary fiber provides a substrate for fermentation and SCFA production (11). However, standard enteral formulas do not contain FOS or fiber. Although formulas fortified with fiber have been available for some time, formulas with both FOS and fiber have only recently been produced (12).

Despite their importance during ETF, the effects of enteral formula composition on the colonic microbiota and SCFAs are poorly understood (13). Previous studies reported conflicting results, included sample sizes so small that they precluded statistical analysis, and relied on conventional bacterial cul-

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[4] Abbreviations used: ETF, enteral tube feeding; FISH, fluorescent in situ hybridization; FOS, fructooligosaccharides; GI, gastrointestinal.

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ture (14–16). Bacterial culture has variable sensitivity and specificity (17) and has largely been superseded by genotypic analysis such as fluorescent in situ hybridization (FISH). This employs the use of fluorescently labeled oligonucleotide probes targeting the 16S rRNA of the major genera of the colonic microbiota (18).

The effect of enteral formula composition on the colonic microbiota is an important, but poorly understood, phenomenon. The aim of this study was to investigate the effect of enteral formulas with and without prebiotic FOS and fiber on the fecal microbiota and SCFA concentrations.

SUBJECTS AND METHODS

Subjects. Healthy men and women between 21 and 34 y old were recruited to a prospective, randomized, double-blind, crossover trial. Exclusion criteria included: gastrointestinal (GI) disorders, diabetes, chronic viral or inflammatory disorders, self-reported eating disorders, antibiotic prescription in the previous 3 mo, probiotic or prebiotic supplementation in the previous month, intolerance to FOS, BMI < 20 or > 30 kg/m^2, or currently following a weight-reduction diet. A sample size calculation indicated that 10 subjects were required to complete the study to detect a 1-logarithm increase in fecal bifidobacteria (90% power, 0.05 significance). Written informed consent was obtained from each subject and the study was approved by the King's College London Research Ethics Committee.

Protocol. Subjects consumed a normal diet for 14 d (baseline period), then enteral formula for 14 d (enteral formula period), and then the normal diet for a 6-wk washout phase (baseline period), and then enteral formula for 14 d (enteral formula period). During baseline periods, subjects consumed their normal diet and avoided all probiotic and prebiotic supplements. During the 14-d enteral formula periods, subjects consumed enteral formula as the sole source of nutrition with no other dietary intake except for ad libitum consumption of water and a maximum of 600 mL/d of black tea or coffee to prevent caffeine withdrawal (19). During the enteral formula periods, subjects consumed either a standard (FOS and fiber-free) enteral formula (Nutren 1.0, Nestlé Switzerland) or one supplemented with FOS and fiber (Nutren fiber, Nestlé Switzerland). The order of enteral formula consumption was assigned using a computerized random allocation program (Epistat), with both subjects and researchers unaware of enteral formula allocation.

Subjects' body weights were measured at the same time of day at the start and end of each enteral formula period. Total fecal collection was conducted on recruitment to the study and for 3 d at the end of both baseline periods and both enteral formula periods. The last sample from each fecal collection was homogenized in a stomacher (Seward Medical) and analyzed for fecal microbiota using FISH, SCFA concentrations using GLC, fecal pH using a pH electrode (BDH), and fecal water by lyophilization. In addition, subjects recorded fecal frequency and GI symptoms for 14 d during both baseline periods and both enteral formula periods.

Enteral formulas. The volume of enteral formula prescribed for each subject was based upon calculated total energy expenditure and rounded to the nearest 250 mL (1046 kJ) for convenience. Total energy expenditure was calculated by adjusting basal metabolic rate, calculated using modified Schofield equations, for occupational and nonoccupational activity using standard physical activity level tables (20). The prescription of formula was sufficient to achieve Reference Nutrient Intakes for all vitamins and minerals (20).

The 2 enteral formulas were almost identical in nutritional composition except for the content of FOS and fiber (**Table 1**). The FOS/fiber-supplemented formula contained short-chain FOS and pea fiber, which provided ~50% highly fermentable and 50% nonfermentable fiber fractions. Enteral formulas were provided in identical coded tins to ensure that both subjects and researchers were unaware of the allocation. Subjects were provided with sufficient formula to achieve their prescription and unused formula was returned for covert calculation of compliance.

Fluorescent in situ hybridization. Fecal bacteria were harvested in PBS, fixed in 4% (wt/v) paraformaldehyde and spotted onto 3-aminopropyltriethoxysilane-treated (21) 8-well slides (Milian). The fixed bacteria were serially dehydrated in ethanol and hybridized with indocarbocyanin (Cy3)-labeled oligonucleotide DNA probes (Microsynth). Probes were used to target total bacteria (22) (EUB338 5′-GCT GCC TCC CGT AGG AGT-3′), bifidobacteria (23) (Bif164 5′-CAT CCG GCA TTA CCA CCC-3′), clostridia (24) (EREC482 5′-GCT TCT TAG TCA RGT ACC G-3′, Clit135 5′-GTT ATC CGT GTG TAC AGG G-3′, Chis150 5′-TTA TGC GGT ATT AAT CTY CCT TT-3′) and bacteroides (25) (5′-CCA ATG TGG GGG ACC TT-3′). In the absence of a single probe targeting all clostridia, a mixture of the probes for the numerically predominant clusters *C. coccoides-*, *C. lituseburense* (includes *C. difficile*) *C. histolyticum-* (includes *C. perfringens*) was used (24). Hybridization was performed according to a previously published protocol (22). Briefly, bacteria were hybridized with probes that were diluted to a concentration of 4.5 mg/L in hybridization buffer [0.9 mol/L NaCl; 0.02 mol/L Tris/HCl; 0.01% (wt/v) SDS]. Hybridized bacteria were quantified on an Axioplan 2 microscope (Zeiss) equipped with an HBO-100 fluorescent lamp (Osram). Bacteria and SCFA were expressed as both the concentration/g dry feces, to standardize comparison between samples, and as relative proportions of the total, to allow comparison between feces containing different amounts of fiber.

GLC. Fecal samples for analysis of SCFAs were frozen at −80°C within 1 h of voiding (26). SCFAs were extracted from defrosted feces using an extraction buffer (1% H_3PO_4; 0.1% $HgCl_2$) containing 2,2-dimethylbutyric acid as an internal standard. Extracted SCFAs were injected splitless into a Hewlett Packard 6890 series GLC system equipped with a 530-μm i.d., 30-m fused silica capillary column with a film thickness of 1 μm (J&W Scientific). Initial oven temperature was 80°C, which increased by 10°C/min up to 145°C, and then 100°C/min up to 200°C to ensure complete elution. All chromatograms were automatically integrated on a Hewlett-Packard Chemstation program.

Fecal output and gastrointestinal symptoms. Fecal frequency was calculated from self-reported diaries during the last 7 d of both baseline periods and both enteral formula periods. Mean daily fecal weight was calculated from the 3-d total fecal collection. Fecal water was measured by lyophilization of the fecal sample at −45°C.

Subjects recorded GI symptoms for 14 d during both baseline periods and both enteral formula periods. Subjects rated the severity of stomach rumbling, stomach cramps, acid reflux, belching, nausea, vomiting, gut rumbling, gut cramps, bloating, flatulence, and other symptoms using the scale: 0 (absent), 1 (mild), 2 (moderate), or 3 (severe). These symptoms and the scale were used previously in studies of the effect of prebiotics (27,28) and of enteral formulas on subjective GI tolerance (19).

Statistical analysis. All data were analyzed using SPSS for Windows (Version 10.0). The concentrations of fecal bacteria were log transformed and the geometric mean calculated. All continuous data (log-transformed bacteria, SCFA concentrations, fecal pH, fecal output) were compared between baseline and enteral formula periods, and between enteral formula periods using a paired *t* test. Summaries

TABLE 1

Energy, macronutrient, and FOS and fiber content of standard and FOS/fiber enteral formulas[1]

	Standard formula	FOS/fiber formula
	Unit/L	
Energy, *kJ*	4184	4184
Protein, *g*	40	40.1
Fat, *g*	38	38
Carbohydrate, *g*	126.3	126.5
FOS, *g*	0	5.1
Fiber, *g*	0	8.9

[1] Values are for energy, macronutrient, and fiber content of the standard formula (Nutren 1.0, Nestlé Switzerland) and the FOS/fiber formula (Nutren fiber, Nestlé Switzerland).

TABLE 2

Fecal microbiota at baseline and during consumption of standard and FOS/fiber enteral formulas each for 14 d by 10 healthy humans[1]

	Baseline	Standard formula	*P*-value[2]	Baseline	FOS/fiber formula	*P*-value[2]	*P*-value[3]
	log$_{10}$ *cells/g dry feces*			*log*$_{10}$ *cells/g dry feces*			
Total bacteria	11.3 ± 0.1	11.0 ± 0.2	0.001	11.3 ± 0.1	11.2 ± 0.2	0.005	0.005
Bifidobacteria	9.5 ± 0.8	9.0 ± 1.5	0.183	9.7 ± 0.7	10.4 ± 0.5	0.004	0.027
Clostridia	10.6 ± 0.1	10.3 ± 0.8	0.23	10.7 ± 0.1	10.3 ± 0.4	0.006	0.961
Bacteroides	10.6 ± 0.2	10.5 ± 0.29	0.173	10.7 ± 0.2	10.7 ± 0.3	0.73	0.088
	% of total bacteria			*% of total bacteria*			
Bifidobacteria	3.3 ± 2.9	8.6 ± 10.2	0.073	5.1 ± 6.1	26.6 ± 18.0	0.003	0.003
Clostridia	19.1 ± 4.6	30.6 ± 21.9	0.118	22.7 ± 6.8	14.9 ± 8.3	0.038	0.063
Bacteroides	22.8 ± 11.0	32.9 ± 10.7	0.105	24.3 ± 7.9	37.2 ± 25.8	0.191	0.665

[1] Values are means ± SD.
[2] *P*-value for formula vs. baseline.
[3] *P*-value for standard formula vs. FOS/fiber formula.

are presented as means ± SD. The correlation between baseline concentrations of bifidobacteria and their change in concentration was calculated using a Pearson's correlation coefficient. The total incidence and severity scores for GI symptoms were calculated and compared between different diet periods using the Wilcoxon matched pairs test and summaries are presented as the median for each subject over the whole 14-d period. Differences were considered significant at $P < 0.05$.

RESULTS

Cohort. Healthy subjects ($n = 14$; 9 women, 5 men) were recruited to the study. Two women dropped out because they were unable to consume enteral formula as a sole source of nutrition, and 1 woman dropped out for personal reasons unrelated to the study; data for 1 man were not included in the analysis because he had a positive *Giardia lamblia* test during the study. Thus, 10 healthy subjects (6 women, 4 men) completed the study and were included in the analysis.

Compliance. The enteral formula prescription was 9414 ± 1393 kJ/d. Actual intake was covertly calculated from the weight of unused powdered formula returned. Intake of the standard formula (8435 ± 1364 kJ/d) was higher than the FOS/fiber formula (7770 ± 1230 kJ/d, $P = 0.025$), representing a compliance of 90 ± 16 and 83 ± 11%, respectively ($P = 0.019$). Consequently, during consumption of the FOS/fiber formula, the intake of FOS was 9.5 ± 1.5 g/d and of fiber was 16.5 ± 2.6 g/d. Although subjects lost a small amount of weight during each enteral formula period, there were no differences in weight loss between subjects consuming the standard (1.47 ± 1.43 kg) and the FOS/fiber (1.73 ± 0.92 kg) formula ($P = 0.448$).

Fecal microbiota. There were no differences in fecal microbiota or SCFA concentrations between the start and end of the first 14-d period of normal diet consumption (data not shown), demonstrating their relative stability during that phase. In addition, there were no differences in baseline concentrations of fecal microbiota or SCFAs, unless otherwise stated, nor were there any order effects.

There were lower concentrations of total fecal bacteria after consumption of both the standard ($P = 0.001$) and the FOS/fiber ($P = 0.005$) formulas compared with baseline (**Table 2**). However, concentrations of total bacteria were higher after consumption of the FOS/fiber than the standard formula ($P = 0.005$). In addition, the FOS/fiber formula increased concentrations of fecal bifidobacteria compared with both baseline ($P = 0.004$) and standard formula ($P = 0.027$). The magnitude of the bifidogenic effect was negatively correlated with the baseline concentration of bifidobacteria ($r = -0.692$, $P = 0.027$, **Fig. 1**).

Although the FOS/fiber formula lowered concentrations of fecal clostridia ($P = 0.006$), there was no difference compared with the standard formula, which is due in part to differences in baseline concentrations ($P = 0.02$, Table 2).

The standard formula did not change the relative proportions of bifidobacteria, clostridia, and bacteroides (Table 2). However, the FOS/fiber formula increased the proportion of bifidobacteria compared with both baseline ($P = 0.003$) and standard formula ($P = 0.003$), and decreased the proportion of clostridia ($P = 0.038$) compared with baseline (Table 2).

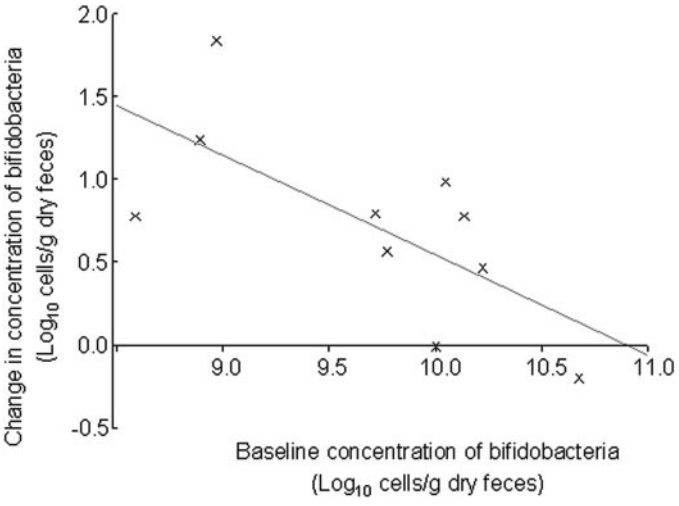

FIGURE 1 The correlation between baseline concentrations of fecal bifidobacteria (log_{10} cells/g dry feces) and their change in concentration in 10 healthy humans after consumption of the FOS/fiber enteral formula as the sole source of nutrition for 14 d ($r = -0.692$, $P = 0.027$).

Fecal SCFA and pH. Consumption of the standard formula lowered fecal concentrations of total SCFA ($P = 0.02$), acetate ($P = 0.036$), propionate ($P = 0.007$), and butyrate ($P = 0.029$) compared with baseline, whereas the FOS/fiber formula lowered concentrations of butyrate only ($P = 0.007$, **Table 3**). In addition, total SCFA ($P = 0.022$), acetate ($P = 0.034$) and propionate ($P = 0.02$) were higher after consumption of the FOS/fiber than after standard formula.

There were marked changes in the relative proportion of each SCFA following consumption of the enteral formulas (Table 3). Consumption of the standard formula resulted in an increase in the relative proportion of acetate ($P = 0.049$), isobutyrate ($P = 0.001$) and isovalerate ($P = 0.001$) and a reduction in the percentage of butyrate ($P = 0.002$) compared with baseline. However, the FOS/fiber formula resulted only in an increase in the proportion of acetate ($P = 0.004$) and a reduction in the percentage of butyrate ($P < 0.0005$) compared with baseline. Consequently, isobutyrate ($P = 0.021$), valerate ($P = 0.015$), and isovalerate ($P = 0.022$) were present in higher proportions after consumption of the standard compared with the FOS/fiber formula (Table 3).

Fecal pH increased with administration of both the standard ($P < 0.0005$) and the FOS/fiber ($P < 0.0005$) formula, although it was lower with the FOS/fiber formula than the standard formula ($P = 0.036$, Table 3).

Fecal output and gastrointestinal symptoms. Stool frequency was less than baseline during the standard formula period ($P = 0.001$), and tended to be less during the FOS/fiber formula period ($P = 0.056$, **Table 4**). Stool frequency was greater after the FOS/fiber period than the standard formula period ($P = 0.019$). Daily fecal weight was lower compared with baseline after both the standard ($P = 0.005$) and the FOS/fiber ($P = 0.034$) formula periods, although there were no differences between the 2 periods ($P = 0.149$, Table 4).

There were no differences in either the incidence or severity of stomach rumbling, stomach cramps, acid reflux, belching, vomiting, gut rumbling, gut cramps, and other symptoms between any diet period. However, the standard formula reduced the incidence ($P = 0.046$) and severity ($P = 0.041$) of flatulence compared with baseline. Although the FOS/fiber formula did not affect symptoms compared with baseline, there was a higher incidence ($P = 0.041$) and severity ($P = 0.018$) of nausea and a higher incidence ($P = 0.018$) and severity ($P = 0.008$) of flatulence compared with the standard formula. The incidence and severity of bloating tended to be greater during the FOS/fiber period than during the standard enteral formula period ($P = 0.068$, Table 4).

DISCUSSION

The colonic microbiota may protect against *C. difficile* colonization, whereas the SCFAs they produce reverse the secretion of water into the colonic lumen, 2 complications that occur during ETF. The aim of this study was to investigate the effect of enteral formulas with and without FOS and fiber on the fecal microbiota and SCFA concentrations. Healthy subjects consumed formulas with and without FOS and fiber for 14 d in a crossover feeding trial. This design was chosen in view of the small within-subject variation in microbiota and to minimize the number of subjects required while still providing statistical power.

Fecal microbiota. The reduction in total fecal bacteria after consumption of both the standard and the FOS/fiber formulas (0.3 and 0.1 $\log_{10}$, respectively) was significant due to systematic effects and the fact that this was a paired analysis. These values represent large reductions in bacterial concentrations (53 and 32%, respectively), and even larger reductions in absolute numbers of bacteria due to the simultaneous reduction in daily fecal weight. The reduction in microbiota during the standard formula period may be explained by a number of mechanisms including the absence of dietary fiber, which reduces exogenous carbohydrate available for fermen-

TABLE 3

Fecal SCFA and fecal pH at baseline and during consumption of standard and FOS/fiber enteral formulas each for 14 d by 10 healthy humans[1]

	Baseline	Standard formula	*P*-value[2]	Baseline	FOS/fiber formula	*P*-value[2]	*P*-value[3]
	μmol/g dry feces			*μmol/g dry feces*			
Total SCFA	377.6 ± 187.9	220.1 ± 124.5	0.020	471.2 ± 191.7	332.4 ± 133.8	0.100	0.022
Acetate	218.2 ± 99.4	136.8 ± 74.5	0.036	270.9 ± 112.7	219.6 ± 96.3	0.330	0.034
Propionate	72.3 ± 37.3	35.6 ± 25.5	0.007	91.8 ± 43.1	58.4 ± 37.4	0.060	0.020
Butyrate	58.7 ± 54.5	18.6 ± 12.7	0.029	72.5 ± 39.8	25.3 ± 11.3	0.007	0.056
Isobutyrate	7.7 ± 2.9	8.4 ± 5.0	0.675	10.0 ± 5.2	8.2 ± 3.2	0.263	0.863
Valerate	10.7 ± 4.0	8.5 ± 5.7	0.322	13.2 ± 5.1	8.9 ± 3.2	0.057	0.750
Isovalerate	10.0 ± 3.7	12.3 ± 6.8	0.368	12.9 ± 6.7	11.9 ± 5.0	0.663	0.859
	% of total SCFA			*% of total SCFA*			
Acetate	58.5 ± 3.9	63.6 ± 5.7	0.049	57.7 ± 4.8	66.2 ± 6.2	0.004	0.333
Propionate	18.9 ± 4.5	15.1 ± 4.9	0.094	19.2 ± 5.9	16.6 ± 6.6	0.362	0.436
Butyrate	14.0 ± 4.5	7.9 ± 1.9	0.002	15.1 ± 3.4	7.5 ± 1.7	<0.0005	0.584
Isobutyrate	2.4 ± 1.0	3.8 ± 0.6	0.001	2.2 ± 0.7	2.8 ± 1.1	0.222	0.021
Valerate	3.1 ± 0.8	3.8 ± 1.4	0.208	2.9 ± 0.7	2.8 ± 0.8	0.846	0.015
Isovalerate	3.2 ± 1.5	5.7 ± 1.1	0.001	2.8 ± 1.0	4.1 ± 1.8	0.108	0.022
pH	6.85 ± 0.42	7.80 ± 0.37	<0.0005	6.68 ± 0.33	7.59 ± 0.26	<0.0005	0.036

[1] Values are means ± SD.
[2] *P*-value for formula vs. baseline.
[3] *P*-value for standard formula vs. FOS/fiber formula.

TABLE 4

Fecal output and the incidence and severity of gastrointestinal symptoms at baseline and during consumption of standard and FOS/fiber enteral formulas each for 14 d by 10 healthy humans[1]

	Baseline	Standard formula	*P*-value[2]	Baseline	FOS/fiber formula	*P*-value[2]	*P*-value[3]
Fecal output[1]							
Frequency, *n/d*	1.0 ± 0.3	0.6 ± 0.2	0.001	1.1 ± 0.3	0.9 ± 0.3	0.056	0.019
Weight, *g/d*	132.4 ± 68.5	43.8 ± 30.1	0.005	127.5 ± 71.2	73.2 ± 37.5	0.034	0.149
Water content, %	72 ± 3.8	70.0 ± 5.4	0.338	72.4 ± 4.0	71.9 ± 5.5	0.708	0.361
Nausea							
Incidence[4]	0 (13)	1 (19)	0.473	1.5 (23)	3 (43)	0.160	0.041
Severity[5]	0 (22)	1 (21)	0.798	1.5 (32)	4.5 (65)	0.137	0.018
Bloating							
Incidence	0.5 (9)	0 (6)	0.334	0.5 (13)	1 (28)	0.340	0.068
Severity	0.5 (10)	0 (7)	0.334	0.5 (15)	1 (46)	0.168	0.068
Flatulence							
Incidence	6 (67)	2.5 (42)	0.046	3.5 (58)	10.5 (82)	0.182	0.018
Severity	6 (77)	2.5 (46)	0.041	5.5 (64)	11.5 (119)	0.097	0.008

[1] Fecal output values are means ± SD.
[2] *P*-value for formula vs. baseline.
[3] *P*-value for standard formula vs. FOS/fiber formula.
[4] Incidence values are median number of days a symptom was reported per subject (total number of days a symptom was reported for all subjects).
[5] Severity values are median total symptom score reported per subject (total symptom score reported for all subjects). Subjects used the symptom score 0 (absent); 1 (mild); 2 (moderate); 3 (severe).

tation, and an increase in GI transit time (19), which may independently reduce microbial mass (29). However, neither of these mechanisms explain the reduction in fecal bacteria observed after the FOS/fiber formula period. Although not measured in this study, an increase in fecal fiber compared with a normal diet may result in an apparent reduction in the concentration of total bacteria per gram of dry feces. The difficulty in comparing concentrations of bacteria and SCFA between fecal samples that will inevitably contain different amounts of fiber can be addressed by comparing the relative proportions of the total.

The reduction in total fecal bacteria is likely to have major effects on their ability to exert colonization resistance. Such an effect in patients administered ETF may explain in part the mechanism for the increase in *C. difficile* colonization (2). However, extrapolation of the findings from a cohort of relatively young, healthy subjects to patients administered ETF is hindered by the inherent differences in age, antibiotic prescription, and disease state.

The large increase in fecal bifidobacteria (0.7 $\log_{10}$ cells/g dry feces) was achieved by fortification of the enteral formula with only 5.1 g/L of FOS. A bifidogenic effect at low doses of FOS is advantageous because the incidence and severity of GI symptoms exhibit a dose-dependent relation (30). The negative correlation between baseline bifidobacteria and the magnitude of the bifidogenic effect confirms observations from a series of in vitro and in vivo studies (31). This relation may have important consequences in the clinical setting in which patients with the lowest concentrations of bifidobacteria, and therefore with the most to benefit from FOS supplementation, are likely to respond the most. However, whether bifidogenesis can occur in patients administered antibiotics is unclear because the addition of FOS and clindamycin to an in vitro fecal incubation reduced bifidobacteria more than clindamycin alone (32). The apparent reduction in clostridia after administration of the FOS/fiber formula is due in part to higher baseline concentrations compared with the standard formula. In view of this, the effect of a FOS/fiber formula in subjects with high concentrations of clostridia, such as patients with *C. difficile*–associated diarrhea (8), warrants investigation.

Fecal SCFA and pH. The reduction in total fecal SCFA, acetate, propionate, and butyrate after administration of the standard formula can be explained by a reduction in colonic fermentation capacity due to the reduction of both total bacteria and fermentable substrate (e.g., FOS and fiber). This is supported by the observation that the FOS/fiber formula did not reduce any of the SCFAs, except for butyrate. The reduction in fecal butyrate could reflect both a reduced production and an increased absorption of colonic butyrate. A reduced butyrate production could be due to the concomitant reduction in clostridia, which are major producers of colonic butyrate (33), whereas increased butyrate absorption and oxidation may occur during high fiber intake (34,35), suggesting that butyrate concentrations at the colonocyte may not be affected.

Although the reduction in fecal SCFAs after administration of the standard formula is likely to reflect the reduction in fermentable substrate, the possibility that this was exacerbated by an increased absorption of SCFAs, particularly of butyrate, cannot be ruled out. This is of particular note because standard formulas increase GI transit time (19), allowing greater opportunity for colonic absorption of SCFAs (36). Because a major research goal is the design of an enteral formula that allows maximal delivery of SCFAs to the cecum to reverse colonic water secretion (6), methods of characterizing SCFA production such as isotopic dilution (37) should be more widely adopted.

Interestingly, standard formula administration resulted in higher proportions of fecal isobutyrate and isovalerate compared with both the baseline and the FOS/fiber formula periods. These branched-chain SCFAs are produced from the fermentation of proteins whose supply would be maintained even in the absence of FOS and fiber (38).

Fecal output and gastrointestinal symptoms. The reduction in fecal weight after consumption of both enteral formulas confirms previous studies in healthy subjects (19,39). The

relation between fecal weight and fiber intake is described by the formula: mean fecal weight (g/d) = 38 + (5.3 × fiber intake) (40). This suggests that in the absence of fiber, fecal weight should be ~38 g/d, which closely corresponds to the 43.8 g/d observed during the standard formula period. This reduction in fecal weight is likely to be due to both the corresponding reduction in colonic microbiota, which may contribute up to 54% of fecal dry weight (41), and the absence of fiber to contribute to fecal bulk (42). Despite a fiber intake of 16.5 g/d during the FOS/fiber formula period, fecal weight was only 73.2 g/d. This supports the findings of a previous report attributing the lack of bulking effect to the small particle size of fibers in enteral formulas (19).

Importantly, neither enteral formula resulted in an increase in any GI symptoms compared with habitual diet. The reduction in incidence and severity of flatulence during the standard formula period is indicative of a reduction in colonic fermentation. Flatus volume is dramatically reduced when healthy subjects consume a standard formula for just 2 d (43). The increased incidence and severity of flatulence during the FOS/fiber period compared with the standard formula period confirms previous reports in both healthy subjects (44) and patients administered ETF (45).

There was an increased incidence of nausea during the FOS/fiber period compared with the standard formula period (median 3 vs. 1 d, respectively, $P = 0.041$). Fiber was shown to slow gastric emptying in some (46,47) but not all studies (48), whereas a fiber-supplemented enteral formula was shown to reduce nausea (19). In addition, enteral formulas supplemented with FOS alone can cause the same (44) or increased (49) nausea compared with standard formulas. These contrasting results highlight the need for objective symptom measures.

This study demonstrated that the consumption of standard enteral formula results in potentially adverse changes to the fecal microbiota, SCFA concentrations, and pH in healthy subjects. These alterations may diminish colonization resistance and reduce the absorption of water in the colonic lumen, both of which are involved in the pathogenesis of diarrhea in patients administered ETF. Supplementation of the formula with FOS and fiber partially prevents some of these adverse changes, without causing an increase in GI symptoms compared with a normal diet. Whether supplementation of an enteral formula with FOS and fiber in patients administered ETF would have similar beneficial effects on the microbiota and SCFAs and reduce the incidence of diarrhea warrants further investigation.

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