Eur J Nutr 39 : 248–255 (2000)

ORIGINAL CONTRIBUTION

S. M. Schneider
P. Le Gall
F. Girard-Pipau
T. Piche
A. Pompei
J.-L. Nano
X. Hébuterne
P. Rampal

Total artificial nutrition is associated with major changes in the fecal flora

Received: 24 February 2000
Accepted: 5 September 2000

Dr. S. M. Schneider (✉) · P. Le Gall
T. Piche · J. L. Nano · X. Hébuterne
P. Rampal
Gastroentérologie et Nutrition
CHU de Nice – L'Archet
Université de Nice – Sophia Antipolis
BP 3079
06202 Nice Cedex 3, France
e-mail: schneide@unice.fr

F. Girard-Pipau · A. Pompei
Laboratoire de Bactériologie
CHU de Nice – L'Archet
Université de Nice – Sophia Antipolis
BP 3079
06202 Nice Cedex 3, France

Summary *Background* Animal studies have demonstrated dramatic changes in the intestinal flora during total enteral (TEN) or parenteral (TPN) nutrition. *Aim of the study* To assess the impact of TEN and TPN on human intestinal microflora. *Methods* Eight patients on fiber-free TEN, five patients on TPN, and ten controls were studied. Fecal bacteria were identified and numbered (logCFU/g feces), and fecal short-chain fatty acids (SCFAs) were measured in stool samples, by gas-liquid chromatography. *Results* In TEN patients, compared to controls ($P<0.01$), aerobes were increased (8.46±0.24) while anaerobes were decreased (5.79±0.84). In TPN patients, both aerobes and anaerobes were decreased compared to controls (5.64±0.27 and 5.31±1.09 respectively, $P<0.01$). Total SCFAs were lower in TPN patients than in TEN patients (48.3±16.6 vs 118.6± 24.1 mmol/kg, $P<0.05$). *Conclusions* Both TPN and TEN induce modifications in the intestinal microflora. During TPN, a homogeneous decrease occurs in both aerobic and anaerobic bacteria. TEN decreases only anaerobic bacteria, while aerobic bacteria are increased. This imbalance may play a role in the pathophysiology of TEN-induced diarrhea.

Key words Enteral nutrition – parenteral nutrition – colon flora – bacterial identification – short-chain fatty acids

Introduction

The relationship between diet and the intestinal microflora has been a much debated issue for several years. While daily changes in a normal western diet are associated with only slight modifications in the fecal flora [1], these modifications can be more significant when there are extreme changes in the diet, such as fasting or switching to an elemental diet without fiber. This has been described in animals, especially in rats studied for possible bacterial translocation from the gut [2–4]. Human studies have been conducted on healthy volunteers fed a chemically defined diet. In addition to a reduction in fecal volume, reported modifications include a decrease in the number of enterococci [5–7] and an increase in the number of enterobacteria [5, 7]. Total artificial nutrition, which does not concern healthy subjects, can thus be expected to induce changes in the intestinal microflora. The intestinal flora of patients on total artificial nutrition has been implicated in complications such as diarrhea [8] and bacterial translocation [9]. The former may be related to a decrease in the production of short-chain fatty acids (SCFAs) that are known to enhance colonic absorption of water [10].

The aim of our study was therefore to assess the impact of total enteral nutrition (TEN) and total parenteral nutrition (TPN) on fecal bacteria and fecal SCFAs, in comparison with healthy subjects, by using a thorough bacteriological analysis.

Methods

Patients

The TEN group consisted of eight patients (six females, two males), mean age 57±7 years (mean ± SEM), who had been on TEN for an average of 106±54 weeks. The indication for TEN was dysphagia (four surgeries of the larynx and esophagus, two strokes, and two esophageal cancers). A commercially available polymeric diet, fiber-, lactose-, and gluten-free, with a concentration of 1.33 kcal/mL (Sondalis HP®, Nestlé Clinical Nutrition, Sèvres, France) was used to provide 20 % protein (50 % from casein and 50 % from soy protein), 45 % carbohydrate (maltodextrin), and 31 % fat (24 % from corn oil, 22 % from colza oil, and 47 % as medium-chain triglycerides). Nutrition was given through gastrostomy (n=5), jejunostomy (n=2) or nasogastric (n=1) tubes. All patients were on cyclic nocturnal TEN that was infused with an electrical pump [11]. The TPN group consisted of five patients (three females, two males), mean age 53±11 years, who had been on TPN for an average 6±1 weeks. The indications for TPN were a post-surgical entero-cutaneous fistula in two, a steroid-resistant flare-up of Crohn's disease in two, and esophageal cancer in one. All Crohn patients had a Crohn's Disease Activity Index lower than 150 at the time of the study. TPN was the only treatment administered for Crohn's disease, except for one patient who was also on steroids. No TPN patient had a short bowel syndrome. All TPN patients received a ternary solution containing 34 % non-protein energy as lipids (long-chain triglycerides) and 66 % glucose, with a nitrogen/energy ratio of 1/183. A commercially available amino acid solution (Vamine®, Fresenius-Kabi, Sèvres, France) was used as the source for amino acids. Parenteral nutrition was infused during a 12 to 14 hour period overnight. Enteral and parenteral nutrition were total, but patients who could drink water or tea were allowed to do so. Ten healthy subjects (two females, eight males), mean age 30±1 years, were also studied. All volunteers consumed a regular western diet, and none of them had a history of gastrointestinal disease. At the time of the study, all patients were in a stable condition, and no patient had diarrhea. No pharmaco-nutrient (like glutamine or arginine) was used by any patient. The energy provided by the diets covered their needs. No subject had undergone colectomy or had taken antibiotics or laxatives for at least two weeks prior to the study. All subjects gave their informed consent and the study was performed according to the Declaration of Helsinki.

Stool analysis

Two fecal samples were collected at one-day intervals from all subjects. Samples were taken right after production, stored in a plastic box, and frozen at – 20 °C until analysis [12], which always took place before 12 hours of storage. 500 mg of feces were taken from the center of the stool, weighed and submitted to serial dilutions up to 10^{-8} in BHI broth. Then, 0.1 mL of each dilution was spread on a range of selective and non-selective media. Whenever necessary, the media were pre-reduced to allow anaerobic growth. Media inoculated and incubated at 37 °C in aerobiosis were the following: trypticase agar, Columbia agar supplemented with sheep blood, Columbia agar supplemented with nalidixic acid and colistin, and Drigalski medium (equivalent to Mac Conkey medium) at dilutions 10^{-2}, 10^{-4}, and 10^{-6} [13]. For anaerobic bacteria, media inoculated were the following: Columbia agar supplemented with sheep blood spread with the dilutions 10^{-2}, 10^{-4}, and 10^{-8}, Columbia blood agar supplemented with kanamycine and vancomycine, *Bifidobacterium* agar, and *Bacteroides* agar (dilutions 10^{-2}, 10^{-4}, and 10^{-7}), rifampicine agar (10^{-1} and 10^{-7}), and CCFA (for isolation of *Clostridium difficile*), MRS agar (for isolation of lactobacilli), *Veillonella* agar, and crystal violet agar, all spread with dilutions 10^{-1} and 10^{-5} [13]. The differences between dilutions for each medium were based on the differences known between the concentrations of concerned bacteria [12]. After 24 to 48 hours of incubation in an anaerobic cabinet, the number of colonies of each colony type growing on each of the media used were counted. The absence of growth under 5 % carbon dioxide was verified for anaerobic strains. Routine identification was performed with standard methods, then with microstrips (API 20 Enterobacteries or ID 32 Anaerobies, BioMerieux, Marcy L'Etoile, France). The use of "spp." refers, for a given genus, to the bacteria whose species was not identified by the techniques used. SCFAs were studied as follows [14]: an aliquot of 200 mg of feces was weighed. It was suspended in sterile distilled water (1.6 mL) and hexanoic acid (0.2 mL) was added. 50 % aqueous H_2SO_4 (0.4 mL) and diethyl ether (2 mL) were then added. The sample was mixed for 45 minutes with an orbital shaker, and centrifuged for 5 minutes at 3000 rpm at room temperature. Anhydrous $CaCl_2$ was then added in order to remove residual water, and 2 µL of the extract were injected in the gas-liquid chromatograph (Hewlett Packard 5890 Series II with a flame ionization detector). The standard solution was as follows: acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic, caproic, and hexanoic acids (10 mEq/L each). This standard solution was tested before each stool sample. Sensitivity was 1 mmol.

Statistical analysis

Results are expressed as mean ± SEM. All fecal bacterial counts (colony-forming units (CFU) per gram of wet feces) were transformed to logarithms ($\log_{10}$ CFU) for ease of statistical analysis. Fecal SCFA levels are expressed as mmol per kilogram of wet feces. Relations between the bacterial counts of each patient's two stool samples were assessed

by regression analysis. Logistic regression analysis was performed to determine which variables contributed independently to variation in bacterial populations and SCFA levels. Comparisons between groups were made with non-parametric Mann Whitney U tests. Statistics were performed using JMP software from the SAS Institute (Cary, NC). Differences were considered as statistically significant for p values lower than 0.05.

Results

Number of bacteria

In univariate analysis, age (for the number of anaerobes), disease (for the numbers of aerobes and total bacteria), and nutrition category (for all bacterial populations) were found to influence bacterial populations. However, multivariate analysis by logistic regression (Table 1) reveals that the nutrition category (i. e., controls, TEN patients, and TPN patients) was the only variable independently associated with variations in fecal bacterial populations. Fig. 1 shows the number of aerobic, anaerobic, and total bacteria in the three groups. The number of aerobic bacteria (Fig. 1a) was higher in the TEN group (8.46±0.24 logCFU/g) than in the control group (7.38±0.24) (p=0.007). It was markedly lower in the TPN group (5.64±0.26) (p=0.001 *vs* controls and p=0.001 *vs* TEN). Compared to controls (8.89±0.19), the number of anaerobic bacteria (Fig. 1b) was significantly lower in both TEN (5.79±0.54, p=0.001) and TPN patients (5.31±1.09, p=0.0006). The total number of bacteria (Fig. 1c) was stable between the control and TEN groups (8.98±0.17 *vs* 8.54±0.24 log CFU per gram of wet feces respectively, NS) whereas it dropped in the TPN group (6.65±0.47, p=0.0005 vs controls and p=0.002 vs TEN patients). The ratio of anaerobic to aerobic bacteria was 878 ± 641 in controls. This ratio was not significantly different in TPN patients (201 ± 192) than in controls, but it was significantly lower in TEN patients (0.42±0.35) (p=0.001 *vs* controls and p=0.03 *vs* TPN patients). Regression analysis revealed no difference between the first and second sample concerning total bacteria (r=0.65), aerobes (r=0.78), and anaerobes (r=0.91).

Table 1 Univariate and multivariate regression analysis of parameters determinant for the fecal flora populations

	Aerobes	Anaerobes	Total bacteria
Univariate analysis			
Age	NS	p=0.006	NS
Sex	NS	NS	NS
Disease	p=0.01	NS	p=0.07
Nutrition category	p=0.0001	p=0.0002	p=0.0001
Multivariate analysis			
Age	NS	NS	NS
Disease	NS	NS	NS
Nutrition category	p=0.002	p=0.003	p=0.01

NS: not statistically significant

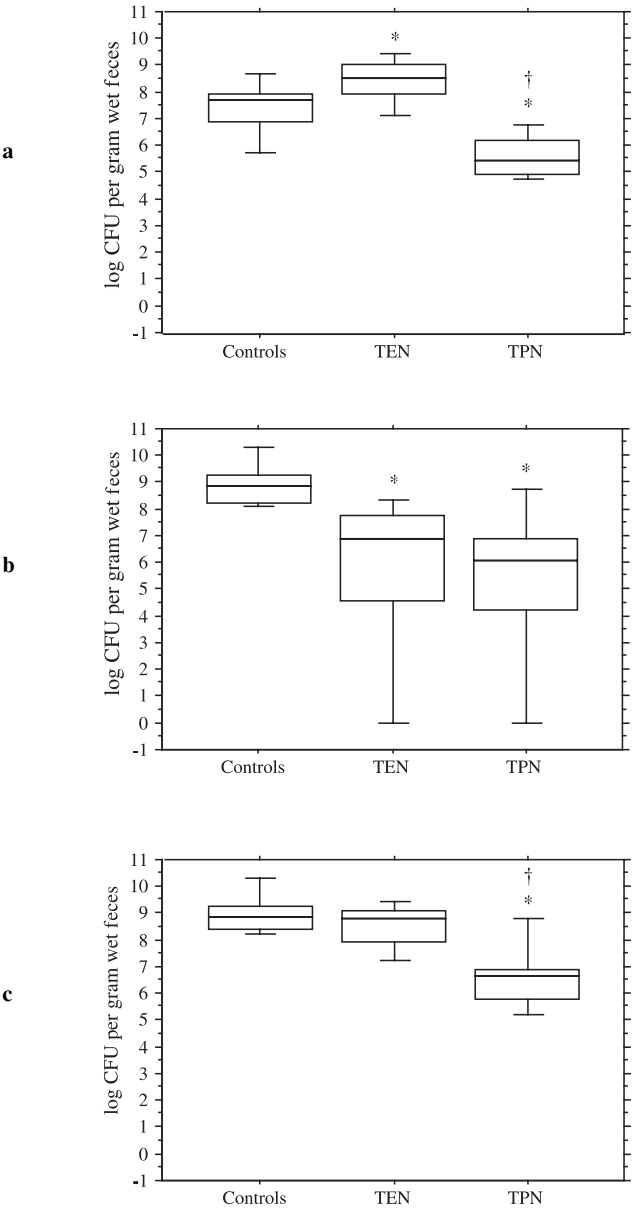

Fig. 1 Fecal bacteria during artificial nutrition. **a:** Aerobic bacteria, **b:** Anaerobic bacteria, **c:** Total bacteria. Each box represents the median and quartiles; the low and high horizontal bars represent the extreme values (comprised within 1.5-fold of the interquartile range). *CFU* colony-forming units; *TEN* total enteral nutrition; *TPN* total parenteral nutrition. *: p < 0.01 compared to the control group; †: p < 0.01 compared to the TEN group.

Bacterial species

On the whole, the median number of species was 8.5 in controls (range: 5–13), 6 in TEN patients (1–8), and 3 in TPN patients (2–6). The regression analysis found a statistical difference between the 3 groups (p=0.04). Bacteria detected in the feces are listed in Tables 2 to 5. In the three groups, the main aerobic bacteria were *E. coli*, *E. faecalis*, *Lactobacillus* spp. and, in controls, *Streptococcus* spp. No difference was observed in the average number of aerobic species detected in the three groups (medians 2.5, 3 and 2 in the control, TEN and TPN groups respectively). In contrast, a dramatic decrease was observed in the number of anaerobic species in TEN and TPN patients. The median number of anaerobic species detected were 5.5 in controls, 2 in TEN patients, and 1 in TPN patients. The regression analysis found a statistical difference between the 3 groups (p=0.04). Clostridia were the predominant anaerobic bacteria in TEN and TPN patients, and *C. difficile* was not detected in patients or in controls.

Table 2 Fecal aerobic bacteria in controls versus artificial nutrition patients (log CFU/gram of wet feces)

Bacteria	Controls	TEN	TPN
Streptococcus spp.	8.19	5.90	6.18
Enterococcus spp.	7.05	9.03	–
Enterococcus faecalis	8.10	10.12	6.84
Enterococcus durans	7.02	–	–
Enterococcus faecium	7.96	7.19	–
Enterococcus avium	6.56	–	–
Staphylococcus coagulase negative	5.30	3.00	4.70
Lactobacillus spp.	8.48	8.41	6.21
Escherichia coli	9.42	9.26	6.65
Escherichia vulneris	7.48	–	–
Klebsiella pneumoniae	8.09	–	–
Citrobacter freundii	6.85	8.60	–
Providencia rettgeri	3.70	–	–
Acinetobacter spp.	–	6.90	–

Results are mean values. *CFU* colony-forming units; *TEN* total enteral nutrition; *TPN* total parenteral nutrition; – not detected

Table 3 Fecal anaerobic cocci in controls versus artificial nutrition patients (log CFU/gram of wet feces)

Bacteria	Controls	TEN	TPN
Peptostreptococcus spp.	8.20	–	–
Peptostreptococcus prevotii	7.20	–	4.30
Peptostreptococcus anaerobius	10.15	–	–
Veillonella spp.	4.60	–	–

Results are mean values. *CFU* colony-forming units; *TEN* total enteral nutrition; *TPN* total parenteral nutrition; – not detected

Table 4 Fecal anaerobic Gram-positive bacteria in controls versus artificial nutrition patients (log CFU/gram of wet feces)

Bacteria	Controls	TEN	TPN
Eubacterium spp.	8.90	6.16	–
Eubacterium lentum	7.30	–	–
Eubacterium limosum	7.20	–	–
Bifidobacterium spp.	9.28	8.16	–
Bifidobacterium adolescentis	8.32	–	–
Clostridium spp.	7.73	7.56	10.00
Clostridium perfringens	5.30	–	5.30
Clostridium beijreinckii	5.30	–	–
Clostridium bifermentans	–	6	–
Clostridium clostridiiforme	3	–	–
Clostridium tertium	4.60	–	–
Clostridium subterminale	4.78	–	–

Results are mean values. *CFU* colony-forming units; *TEN* total enteral nutrition; *TPN* total parenteral nutrition; – not detected

Table 5 Fecal anaerobic Gram-negative bacteria in controls versus artificial nutrition patients (log CFU/gram of wet feces)

Bacteria	Controls	TEN	TPN
Bacteroides spp.	10.48	9.03	6.96
Bacteroides fragilis	8.00	6.68	–
Bacteroides eggerthii	9.75	6.90	–
Bacteroides uniformis	8.61	7.30	–
Bacteroides distasonis	8.71	5.78	–
Bacteroides ovatus	7.26	–	–
Bacteroides vulgatus	10.31	–	–
Bacteroides stercoris	7.60	7.30	–
Bacteroides thetaiotaomicron	3.00	–	–
Bacteroides merdae	9.16	5.78	6.30
Bacteroides capillosus	8.73	–	–
Prevotella spp.	7.59	–	5.78
Prevotella oralis	10.01	5.30	–
Prevotella loescheii	10.27	7.56	–
Prevotella disiens	7.60	–	–
Prevotella buccae	8.40	–	–
Porphyromonas levii	9.08	–	–

Results are mean values. *CFU* colony-forming units; *TEN* total enteral nutrition; *TPN* total parenteral nutrition; – not detected

Short-chain fatty acids

The fecal concentrations of the three main SCFAs (acetate, propionate, and butyrate) are listed in Table 6. The total SCFA concentration in feces was significantly lower in TPN patients than in TEN patients (Table 6) despite considerable interindividual variations (Fig. 2).

Table 6 Fecal short-chain fatty acids in controls versus artificial nutrition patients (mmol/kg of wet feces)

	Controls	TEN	TPN
Acetate	55.9 ± 9.2	81.1 ± 21.7	31.1 ± 10.3
Propionate	13.8 ± 2.3	18.2 ± 3.3	9.4 ± 3.9
Butyrate	17.0 ± 4.8	8.0 ± 1.9	4.9 ± 2.0
Total SCFAs	91.2 ± 16.1	118.6 ± 24.1	48.3 ± 16.6 *

Results are mean values. *TEN* total enteral nutrition; *TPN* total parenteral nutrition; *SCFAs* short-chain fatty acids
* $p < 0.05$ compared to the TEN group

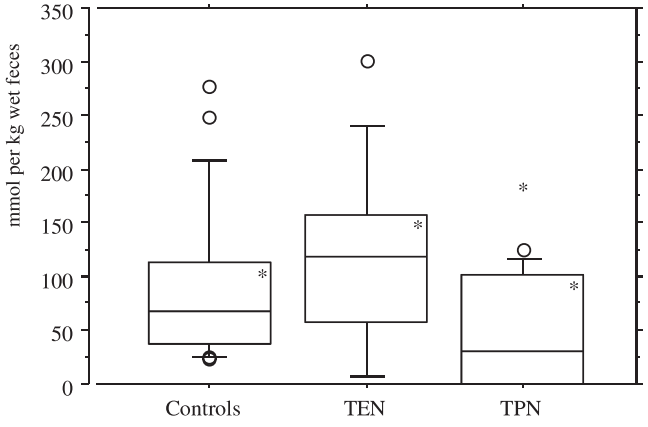

Fig. 2 Total fecal short-chain fatty acids during artificial nutrition. Each box represents the median and quartiles; the low and high horizontal bars represent the extreme values (comprised within 1.5-fold of the interquartile range). ○: outliers (i. e., outside 1.5-fold of the interquartile range); *TEN* total enteral nutrition; *TPN* total parenteral nutrition; *: $p < 0.05$ compared to the TEN group.

Discussion

Bacterial stool analysis is a long and complex procedure. Furthermore, bacterial concentrations are known to differ depending on the intestinal segment in which samples are taken. Nevertheless, analysis of feces is the most feasible technique and provides an accurate information on the intestinal microflora [15]. Analysis of samples rather than 24 h stools certainly deprives us of some information as total artificial nutrition is known to modify the volume of daily stools. However, stool collection is often difficult in bed-ridden patients, especially those with neurological disorders. Furthermore, all studies express bacterial concentrations per gram of wet feces. A major methodological issue is raised by stool freezing before analysis. Samples were pre-frozen because stools were often produced outside working hours. The different bacteria may have a differential sensitivity to this short-term freezing, but we believe that this did not modify the differences observed between the three groups. Indeed, Ballongue [16] reports minor changes in the fecal concentrations of different bacteria when analysis is performed before three days of storage (loss of between 0.5 and 1.5 log). The technique we used permitted identification of 47 different species, thus providing detailed information on the fecal microflora, as it covers more than 99 % of total bacteria [17]. Measurement of SCFA concentrations was also part of bacterial identification [18]. As colon surgery reportedly modifies fecal SCFA concentrations [19], such patients were not included in our study.

Our study findings allow us to conclude that total artificial nutrition is associated with major qualitative and quantitative changes in the fecal microflora, which is impoverished, in both TEN and TPN patients. In TEN patients, aerobic bacteria are increased while anaerobic bacteria are decreased. In TPN patients, both aerobic and anaerobic bacteria are decreased. Three out of the five TPN patients had Crohn's disease, a fact that may have affected their fecal flora. However, an increase in fecal bacterial counts has been reported in patients with terminal ileitis without ileocaecal resection [20]. We can therefore assume that the possible effects of the disease did not mask the effects of TPN *per se*. Furthermore, our results show that the patients' diseases, like age or sex, did not influence the numbers of fecal bacteria. Enteral and parenteral nutrition were exclusive in our patients, and this prevented biases that would have been induced by a concomitant oral feeding. Exclusive artificial nutrition is not a physiological condition; however, it is frequently encountered in clinical practice. Anaerobic bacteria are the dominant flora in the colon. They are responsible for colonization resistance [21], which prevents pathogens from proliferating and adhering to the colonic mucosa, and for metabolic effects (especially fermentation) [22]. Aerobic bacteria account for the major percentage of the sub-dominant flora. Previous studies have reported a ratio between anaerobes and aerobes of 1000/1 in healthy subjects [20, 23–25]. This is consistent with the ratio observed in our control group (878/1). This ratio was not significantly modified in TPN patients, and no pathogen overgrowth was observed. Modifications of the intestinal flora, such as those supposed to favor translocation, were not observed in our TPN patients. Translocation from the gut has been described in rats undergoing TPN, when compared to oral TPN and a control group. TPN-induced translocation in rats seems to be associated with an increased cecal bacterial count of both aerobes and anaerobes [26, 27]. Similar findings have been reported in rats given a TPN admixture or an elemental TEN admixture orally [3, 4, 28]. These studies underscore the differences between animal and human studies: increased intestinal permeability and mucosal atrophy under TPN, proven to date only in animals [29], might influence intestinal bacteria through their relations with the mucosa [30]. Human studies in healthy volunteers have compared normal and elemental diets. Contrasting results have been reported, from a lack of change in the intestinal flora [20, 23, 31], or

a decrease only in enterococci [6], to a pattern similar to that in our TEN patients, i. e., a decrease in total anaerobes, an increase in enterobacteria, and no effect on the total bacterial count [5, 7]. Several reasons may explain the discrepancy between certain healthy volunteer studies and ours. Time may be an important factor, as the duration of the diet was 6 and 106 weeks in our TPN and TEN patients, respectively, compared to 7 to 12 days in healthy volunteer studies. The nature of the diet itself must also be taken into account: elemental and polymeric diets, even when fiber-free, may have different fates in the colon, as they have in the small intestine [32]. Even though carbohydrates represent the main substrate for bacterial metabolism in the gut, proteins and lipids (whose composition differs in the two diets) are also important substrates that can influence colon microbiota [22]. Finally, even if the disease was not independently associated with modifications in bacterial populations in our study, we cannot completely rule out possible disease-related differences between our TEN patients and healthy volunteers fed a chemically defined diet, as stress alone can reportedly induce changes in the intestinal flora [31].

The inversion of the ratio between anaerobes and aerobes (1/2.4) that we observed in our TEN patients could be part of the pathophysiology of enteral nutrition-associated diarrhea. Indeed, diarrhea is a major problem during enteral nutrition, with an incidence as high as 60 % [33], and is responsible for an increase in morbidity and hospital costs [34]. Alongside parameters such as the route of administration, the diet osmolarity and disturbances of intestinal motility, the role of bacteria has long been suspected in TEN-induced diarrhea. No significant relation has been found between a possible contamination of the diet formula and bacterial diarrhea [8]. The modifications of the intestinal microflora that are induced by a fiber-free polymeric enteral diet can be compared to those induced by broad-spectrum antibiotics such as ceftriaxone [35, 36]. These effects may be synergic [37] and explain both why antibiotics are a risk factor for enteral nutrition-induced diarrhea [38, 39], and why enteral nutrition is a risk factor for antibiotic-induced diarrhea [40], and especially for *Clostridium difficile* infection [41].

In the colon, most SCFAs are the products of carbohydrate fermentation by the anaerobic bacteria that represent the dominant flora. Fecal SCFAs could thus have been expected to drop in both TEN and TPN patients, because the number of anaerobic species and the total number of anaerobic CFUs were low in these patients compared to controls. However, SCFAs were decreased only in TPN patients; they were unchanged in TEN patients, even in the absence of dietary fiber [42]. Although these results from our TEN patients were rather unexpected, similar findings have been reported previously [43], with no difference having been observed in acetate, propionate, and butyrate measured before and at the end of a 7 day-TEN trial in nine patients receiving a fiber-free polymeric diet. These results may have several explanations. First, SCFA concentrations are expressed per kg of wet feces, and therefore do not take into account daily SCFA production. Furthermore, fecal SCFA concentrations represent the difference between production and absorption by the colon [44]. The latter may be reduced in TEN patients for a number of reasons, such as a reduced transit time [45] or modifications of the electrolyte composition in the rectal lumen [46], neither of which were assessed in our study. Moreover, the correlation of SCFAs and bacterial counts in feces has been reported to be poor [25].

Comprehensive microbiological analysis of the feces allowed us to conclude that TPN induces, as expected and due to an "intestinal starvation", a deprivation of the fecal flora, with markedly decreased numbers of bacteria and species. TEN induces an imbalance between anaerobes and aerobes. The drop in the number of anaerobic bacteria and species might participate in the pathophysiology of TEN-induced diarrhea and might explain the increased susceptibility of these patients to antibiotic-associated diarrhea. These findings are particularly interesting at a time when the supposed superiority of TEN over TPN is being questioned by analysis of the evidence [47]. The imbalance in the intestinal flora of TEN patients calls for intervention studies. Dietary fibers may be valuable during enteral nutrition, especially by reducing diarrhea, but this goal has seldom been reached in clinical studies [39, 48–50]. Determination of the effects of dietary fiber on the intestinal flora of TEN patients may help us to better assess which fiber will be useful for them. Finally, the use of probiotics such as *Saccharomyces boulardii* has been shown to reduce the frequency of diarrhea in enteral nutrition patients [51]. This may be due to a correction of enteral nutrition-induced disorders of the intestinal flora.

Acknowledgments We are most thankful to Ms Nancy Rameau for her review of this manuscript and to Dr. Xavier Pivot for his help with statistical analysis.

References

1. Cummings JH, Wiggins HS, Jenkins DJ, Houston H, Jivraj T, Drasar BS, Hill MJ (1978) Influence of diets high and low in animal fat on bowel habit, gastrointestinal transit time, fecal microflora, bile acid, and fat excretion. J Clin Invest 61:953–963
2. Barnes EM, Burton GC (1970) The effect of hibernation on the caecal flora of the thirteen-lined ground squirrel. J Appl Bacteriol 33:505–514
3. Mainous M, Xu DZ, Lu Q, Berg RD, Deitch EA (1991) Oral TPN-induced bacterial translocation and impaired immune defenses are reversed by refeeding. Surgery 110:277–283
4. Spaeth G, Specian RD, Berg RD, Deitch EA (1990) Bulk prevents bacterial translocation induced by the oral administration of total parenteral nutrition solution. JPEN 14:442–447

5. Attebery HR, Sutter VL, Finegold SM (1972) Effect of a partially chemically defined diet on normal human fecal flora. Am J Clin Nutr 25:1391–1398
6. Bounous G, Devroede GJ (1974) Effects of an elemental diet on human fecal flora. Gastroenterology 66:210–214
7. Crowther JS, Drasar BS, Goddard P, Hill MJ, Johnson K (1973) The effect of a chemically defined diet on the faecal flora and faecal steroid concentration. Gut 14:790–793
8. Belknap DC, Davidson LJ, Flournoy DJ (1990) Microorganisms and diarrhea in enterally fed intensive care unit patients. JPEN 14:622–628
9. Pierro A, van Saene HK, Donnell SC, Hughes J, Ewan C, Nunn AJ, Lloyd DA (1996) Microbial translocation in neonates and infants receiving long-term parenteral nutrition. Arch Surg 131:176–179
10. Bowling TE, Raimundo AH, Grimble GK, Silk DB (1993) Reversal by short-chain fatty acids of colonic fluid secretion induced by enteral feeding. Lancet 342:1266–1268
11. Hébuterne X, Broussard JF, Rampal P (1995) Acute renutrition by cyclic enteral nutrition in elderly and younger patients. JAMA 273:638–648
12. Drasar BS, Roberts AK (1991) Methods for the study of anaerobic microflora. In: Levett PN (ed) Anaerobic Microbiology. A Practical Approach. Oxford University Press, Oxford, pp 183–200
13. Phillips E, Nash P (1985) Culture media. In: Lennette EH, Balows A, Hausler Jr WJ, Shadomy HJ (eds) Manual of Clinical Microbiology, 4th edn. American Society for Microbiology, Washington DC, pp 1051–1092
14. Holdeman LV, Cato EP, Moore WEC (1977) Anaerobe Laboratory Manual, Anaerobe Laboratory. Virginia Polytechnic Institute and State University, Blacksburg, VA
15. Moore WEC, Holdeman LV (1975) Discussion of current bacteriological investigations of the relationships between intestinal flora, diet, and colon cancer. Cancer Res 35:3418–3420
16. Ballongue J (1997) Technical problems related to *in vitro* study of colon flora. Scand J Gastroenterol 32 (Suppl) 14–16
17. MacFarlane GT, MacFarlane S (1997) Human colonic microbiota: ecology, physiology and metabolic potential of intestinal bacteria. Scand J Gastroenterol 32 (Suppl) 3–9
18. Guerrant GO, Lambert MA, Moss CW (1982) Analysis of short-chain acids from anaerobic bacteria by high-performance liquid chromatography. J Clin Microbiol 16:355–360
19. Scheppach W, Sachs M, Bartram P, Kasper H (1989) Faecal short-chain fatty acids after colonic surgery. Eur J Clin Nutr 43:21–25
20. Wensinck F, Custers-van Lieshout MC, Poppelaars-Kustermans PAJ, Schröder AM (1981) The faecal flora of patients with Crohn's disease. J Hyg 87:1–12
21. Wells CL, Maddaus MA, Reynolds CM, Jechorek RP, Simmons RL (1987) Role of anaerobic flora in the translocation of aerobic and facultatively anaerobic intestinal bacteria. Infect Immun 55:2689–2694
22. Cummings JH, MacFarlane GT (1997) Role of intestinal bacteria in nutrient metabolism. Clin Nutr 16:3–11
23. Bornside GH, Cohn I, Jr (1975) Stability of normal human fecal flora during a chemically defined, low residue liquid diet. Ann Surg 181:58–60
24. Simon GL, Gorbach SL (1984) Intestinal flora in health and disease. Gastroenterology 86:174–193
25. Meijer-Severs GJ, van Santen E (1989) Short-chain fatty acid and organic acid concentrations in feces of 10 human volunteers and their correlation with anaerobe cultural counts over a 15-month period. Scand J Gastroenterol 24: 1276–1280
26. Alverdy JC, Aoys E, Moss GS (1988) Total parenteral nutrition promotes bacterial translocation from the gut. Surgery 104:185–190
27. Kueppers PM, Miller TA, Chen CY, Smith GS, Rodriguez LF, Moody FG (1993) Effect of total parenteral nutrition plus morphine on bacterial translocation in rats. Ann Surg 217:286–292
28. Alverdy JC, Aoys E, Moss GS (1990) Effect of commercially available chemically defined liquid diets on the intestinal microflora and bacterial translocation from the gut. JPEN 14:1–6
29. Johnson LR, Copeland EM, Dudrick SJ, Lichtenberger LM, Castro GA (1975) Structural and hormonal alterations in the gastrointestinal tract of parenterally fed rats. Gastroenterology 68:1177–1183
30. Alexander JW (1990) Nutrition and translocation. JPEN 14:170S–174S
31. Holdeman LV, Good IJ, Moore WE (1976) Human fecal flora: variation in bacterial composition within individuals and a possible effect of emotional stress. Appl Environ Microbiol 31:359–375
32. Smith J, Arteaga C, Heymsfield S (1982) Increased ureagenesis and impaired nitrogen use during infusion of a synthetic amino acid formula. A controlled trial. N Engl J Med 306:1013–1018
33. Bliss DZ, Guenter PA, Settle RG (1992) Defining and reporting diarrhea in tube-fed patients – what a mess! Am J Clin Nutr 55:753–759
34. Eisenberg PG (1993) Causes of diarrhea in tube-fed patients: a comprehensive approach to diagnosis and management. Nutr Clin Pract 8:119–123
35. Welling GW, Meijer-Severs GJ, Helmus G, van Santen E, Tonk RH, de Vries-Hospers HG, van der Waaij D (1991) The effect of ceftriaxone on the anaerobic bacterial flora and the bacterial enzymatic activity in the intestinal tract. Infection 19:313–316
36. Meijer-Severs GJ, Van Santen E, Meijer BC (1990) Short-chain fatty acid and organic acid concentrations in feces of healthy human volunteers and their correlations with anaerobe cultural counts during systemic ceftriaxone administration. Scand J Gastroenterol 25:698–704
37. Silk DBA (1987) Towards the optimization of enteral nutrition. Clin Nutr 6:61–74
38. Keohane PP, Attrill H, Love M, Frost P, Silk DB (1984) Relation between osmolality of diet and gastrointestinal side effects in enteral nutrition. Br Med J 288:678–680
39. Guenter PA, Settle RG, Perlmutter S, Marino PL, DeSimone GA, Rolandelli RH (1991) Tube feeding-related diarrhea in acutely ill patients. JPEN 15:277–280
40. Surawicz CM, Elmer GW, Speelman P, McFarland LV, Chinn J, van Belle G (1989) Prevention of antibiotic-associated diarrhea by *Saccharomyces boulardii*: a prospective study. Gastroenterology 96:981–988
41. Bliss DZ, Johnson S, Savik K, Clabots CR, Willard K, Gerding DN (1998) Acquisition of *Clostridium difficile* and *Clostridium difficile*-associated diarrhea in hospitalized patients receiving tube feeding. Ann Intern Med 129:1012–1019
42. Kapadia SA, Raimundo AH, Grimble GK, Aimer P, Silk DB (1995) Influence of three different fiber-supplemented enteral diets on bowel function and short-chain fatty acid production. JPEN 19:63–68
43. Sobotka L, Bratova M, Slemrova M, Manak J, Vizd'a J, Zadak Z (1997) Inulin as the soluble fiber in liquid enteral nutrition. Nutrition 13:21–25
44. Jorgensen J, Holtug K, Jeppesen PB, Mortensen PB (1998) Human rectal absorption of short- and medium-chain C2-C10 fatty acids. Scand J Gastroenterol 33:590–594
45. Lewis SJ, Keaton KW (1997) Increasing butyrate concentration in the distal colon by accelerating intestinal transit. Gut 41:245–251
46. Holtug K, Hove H, Mortensen PB (1995) Stimulation of butyrate absorption in the human rectum *in vivo*. Scand J Gastroenterol 30:982–988
47. Lipman TO (1998) Grains or veins: is enteral nutrition really better than parenteral nutrition? A look at the evidence. JPEN 22:167–182
48. Hart GK, Dobb GJ (1988) Effect of a fecal bulking agent on diarrhea during enteral feeding in the critically ill. JPEN 12:465–468

49. Homann HH, Kemen M, Fuessenich C, Senkal M, Zumtobel V (1994) Reduction in diarrhea incidence by soluble fiber in patients receiving total or supplemental enteral nutrition. JPEN 18:486–490

50. Dobb GJ, Towler SC (1990) Diarrhoea during enteral feeding in the critically ill: a comparison of feeds with and without fibre. Intensive Care Med 16:252–255

51. Bleichner G, Bléhaut H, Mentec H, Moyse D (1997) *Saccharomyces boulardii* prevents diarrhea in critically ill tube-fed patients. A multicenter, randomized, double-blind placebo-controlled trial. Intensive Care Med 23:517–523