

## Original Article

# Intestinal bacterial flora in neurologically impaired patients on long-term enteral nutrition

L. SANTARPIA<sup>1</sup>, I. TORRE<sup>2</sup>, F. PENNINO<sup>2</sup>, L. ALFONSI<sup>1</sup>, R. BONIFACIO<sup>1</sup>, M. NEGRONE<sup>2</sup>, G. RIBERA D'ALCALÀ<sup>2</sup>, V. CORCIONE<sup>3</sup>, F. PASANISI<sup>1</sup>, F. CONTALDO<sup>1</sup>

<sup>1</sup>Department of Clinical and Experimental Medicine, <sup>2</sup>Department of Hygiene and Preventive Medicine, Federico II University, Naples; <sup>3</sup>Nola Civic Hospital, Naples - Italy

**ABSTRACT: Objective.** To evaluate possible effects of long-term home total enteral nutrition (TEN) on intestinal bacterial flora and its relation with some nutritional and inflammatory parameters.

**Subjects and methods.** We studied 18 dysphagic patients on long-term TEN (>6 months) and 18 healthy controls selected among the relatives living with the patients. Fourteen patients received fiber-enriched formulas and 4 standard enteral nutrition (EN) formulas. Fecal bacteria were identified, quantified as log colony-forming units (CFU)/g feces and compared with selected nutritional and inflammatory parameters.

**Results.** In patients receiving fiber-enriched formulas, the number of aerobic bacteria was higher when compared with those of their respective controls ( $p=0.02$ ). Moreover, there were significantly higher concentrations of anaerobes in patients receiving fiber-enriched formulas than in those receiving standard EN formulas ( $p=0.05$ ).

**Conclusions.** In our study, long-term TEN seemed to influence patients' intestinal bacterial flora characteristics both in patients receiving fiber-enriched and those receiving standard EN formulas. There was no relation between intestinal bacteria flora characteristics and nutritional and inflammatory parameters in long-term home TEN patients. (*Nutritional Therapy & Metabolism* 2007; 25: 89-96)

**KEY WORDS:** Intestinal bacterial flora, Enteral nutrition, Fibers

## INTRODUCTION

The human intestinal habitat contains hundreds of different species of bacteria, and the number of microbial cells within the gut lumen is about 10 times the number of eukaryotic cells in the human body (1-3). Qualitatively, among aerobes and anaerobes, over 300-400 different bacterial species have been identified (4-6). Among aerobic bacteria, the most common are *Escherichia coli*, klebsiellas, protei, staphylococci, lactobacilli and enterococci; while among anaerobes, it is bifidobacteria, bacteroides, eubacteria, *Clostridium* spp, peptostreptococci and gram-negative cocci (*Veillonella* spp.) (7-9). Bacteria living within the colon affect host homeostasis and metabolism (3) and may also interfere with some regulatory functions such as immunity and inflammation; some of them are potential pathogens and, under some circum-

stances, can be a source of infection and sepsis, for instance when the integrity of the bowel barrier is physically or functionally altered (10-13).

The relationship between diet composition and the characteristics of the intestinal bacterial flora is still an open issue: studies on this topic are available mainly in animals, showing that both elemental diets and fasting reduce intestinal bacterial flora, while slight modifications in the habitual diet are not able to produce substantial changes (14-16). But human studies have been carried out only on healthy volunteers eating elemental diets or fasting, showing modifications of both fecal volume and flora consisting in a decreased number of enterococci and in an increased number of bacteria of the family Enterobacteriaceae (17-19); only a few studies have been carried out on patients on artificial nutrition, either parenteral or enteral (20). As opposed to total par-

enteral nutrition, total enteral nutrition (TEN) appears to preserve gut mucosal structure and intestinal function (21, 22). Nevertheless, TEN seems to play a role on intestinal motility disorders such as constipation, diarrhea and bacterial translocation, perhaps through modifications of the intestinal flora. Only a few studies, however, are available on the long-term effects of TEN on intestinal bacterial flora (4, 23). Fortunately, given the difficulties involved in analyzing bacterial flora in the different intestinal segments, feces have been shown to well represent the intestinal flora. Analysis of feces is a more feasible technique and provides accurate information on intestinal flora (24).

#### AIM OF THE STUDY

The aim of this study was to evaluate the effects of long-term home TEN on intestinal bacterial flora, some hematobiochemical parameters and proinflammatory cytokines.

#### PATIENTS AND METHODS

The study population comprised 18 neurologically impaired patients with dysphagia, recruited from 2 centers for home artificial nutrition in Campania, the cities of Naples and Nola, south Italy, on long-term (>6 months) TEN and 18 age- and sex-matched healthy controls, who were selected from among relatives living with the patients.

##### Patients

Eighteen patients on long-term (range 199-2,771 days) home TEN were selected for the study. The indication for inclusion was severe dysphagia due to neurological disorders: 5 cases of amyotrophic lateral sclerosis, 4 of cerebellar ataxia, 3 of Alzheimer's Disease, 2 of post-surgery dysphagia, 1 of multiple sclerosis, 1 of neonatal cerebral disease, 1 of head trauma and 1 of Friedreich's ataxia. None of the patients had associated metabolic or gastrointestinal diseases.

Fourteen patients (6 women, 8 men, mean age  $47.6 \pm 19.1$  years, mean weight  $55.2 \pm 10.8$  kg, mean body mass index [BMI;  $\text{kg}/\text{m}^2$ ] =  $20.5 \pm 3.6$ ) were receiving a polymeric, fiber-enriched, lactose- and gluten-free, isoenergetic (1 kcal/mL) diet providing 15% protein, 55% carbohydrates and 30% fat. Four patients (3 women, 1 man, mean age  $71.8 \pm 7.3$  years, mean weight  $49.3 \pm 3.0$  kg, mean BMI  $20.4 \pm 1.9$ ) received a standard, fiber-free enteral nutrition (EN) formula (16% pro-

tein, 54% carbohydrates and 30% fat). The fibers contained in the fiber-enriched formulas were 43% soluble (inulin, arabic gum and oligofructose) and 57% insoluble (soybean polysaccharides, resistant starch and cellulose).

TEN was given through gastrostomy (n=12), jejunostomy (n=2) or nasogastric (n=4) tubes. Patients were on cyclic nocturnal or diurnal TEN. The energy provided by the diets was expected to cover their needs (about 25-30 kcal/kg per day); the total daily fiber intake for the patients receiving fiber-enriched formulas was about 12-15 g ( $0.8 \text{ g} \times 100 \text{ mL}$ ) for insoluble and 9-12 g ( $0.6 \text{ g} \times 100 \text{ mL}$ ) for soluble fibers.

At the time of the study, all patients had stable body weight and clinical condition, and none had diarrhea or gastrointestinal disorders. No patients received gastric proton pump inhibitors (PPIs) which alter gastric pH and could therefore influence intestinal bacterial flora composition. No patients received drugs influencing intestinal bacterial flora homeostasis (e.g., antibiotics).

##### Controls

The control group included 18 healthy living-together relatives, all regularly consuming a typical Mediterranean diet. Controls comprised 5 parents (3 women, 2 men), 7 offspring (4 women, 3 men), 4 spouses (2 husbands, 2 wives) and 2 brothers. Fourteen controls (8 women, 6 men, mean age  $45.1 \pm 18.1$  years, mean weight  $67.0 \pm 12.1$  kg, mean BMI  $24.2 \pm 4.2$ ) were the relatives of the patients receiving the fiber-enriched formulas and 4 (2 women, 2 men, mean age  $55.8 \pm 14.7$  years, mean weight  $68.3 \pm 6.2$  kg, mean BMI  $25.5 \pm 1.4$ ) were the relatives of the patients receiving the standard formulas.

All subjects (controls, patients and eventually relatives/tutors) gave their informed consent for participation in the study. The protocol of the study was approved by the local ethics committee, according to the principles of the Declaration of Helsinki 1975.

##### Stool analysis

Fecal bacteria was identified according to standard methods and quantified (log colony-forming units [CFU]/g feces). All fecal samples (1 per participant) were collected immediately after production, in sterile plastic hermetically sealed boxes, and immediately analyzed.

One gram of feces, taken from the center of the stool, was submitted to serial dilutions from  $10^{-1}$  to  $10^{-7}$  in brain-heart infusion broth. Then 0.1 mL of

each dilution was spread on a range of selective and nonselective media. In particular, the media inoculated and incubated at 37°C, for 24 to 48 hours, in aerobiosis were MacConkey agar no. 3, mannitol salt agar and Columbia agar supplemented with sheep blood (Oxoid). For anaerobic bacteria, the following media supplemented with sheep blood were used: Schaedler agar (dilutions 10<sup>-2</sup>, 10<sup>-4</sup> and 10<sup>-7</sup>), Schaedler Kanavanco agar, Schaedler CNA agar (dilutions 10<sup>-2</sup>, 10<sup>-4</sup> and 10<sup>-6</sup>) and selective media, such as Bacteroides Bile Esculin agar (dilutions 10<sup>-2</sup>, 10<sup>-4</sup> and 10<sup>-6</sup>), Rogosa agar (for isolation of lactobacilli), CCFA (for isolation of *Clostridium difficile*) and crystal violet agar with 10<sup>-1</sup> and 10<sup>-5</sup> dilutions. Media were incubated in an anaerobic cabinet (mixture 85% N<sub>2</sub>, 10% H<sub>2</sub> and 5% CO<sub>2</sub>) at 37°C. After 24-48 hours of incubation, colonies were subcultured and incubated in 2 different conditions: in anaerobiosis and in a CO<sub>2</sub> cabinet, to differentiate obliged from facultative anaerobes (25-30).

Routine identification was performed with standard methods, followed by microstrips: Api20A, Api20E, ApiStrep and ApiStaph (BioMerieux) (31, 32). The abbreviation "spp." refers, for a given genus, to the bacteria whose species was not identified by the technique used.

### Biochemical parameters

Routine laboratory tests (hemoglobin [Hb], lymphocyte Count [Lymph], albumin [Alb], cholesterol [Chol], cholinesterase [CHE], white blood cell count [WBC], platelets [Plt], fibrinogen, ferritin, insulin) were measured by standard procedures, and some cytokines, such as TNF- $\alpha$ , IL-1 $\beta$  and IL-6, were assayed using an enzyme-linked immunosorbent assay (ELISA). The index of insulin resistance was calculated using the HOMA (homeostasis model assessment) equation:  $HOMA = (\text{fasting insulin} \times \text{fasting glucose} \times 0.0555) / 22.5$ , where fasting insulin is measured in mU/L, and fasting glucose in mg/dL.

### Statistical analysis

Results are expressed as means  $\pm$  standard deviation, unless otherwise stated. All fecal bacterial counts (colony-forming units [CFU] per gram of wet feces) were transformed to logarithms (log<sub>10</sub> CFU) to facilitate statistical analysis. The Mann-Whitney *U*-test was used for statistical analysis. Differences were considered statistically significant for *p* values < 0.05. The data obtained were analyzed with SPSS for Windows version 12.0 (SPSS Inc, Chicago, IL, USA).

## RESULTS

### Anthropometric and biochemical parameters

The 14 patients receiving fiber-enriched formulas (Fi patients) and the corresponding 14 controls were significantly different for mean weight (55.2  $\pm$  10.8 kg vs. 67.0  $\pm$  12.1 kg, =0.01), BMI (20.5  $\pm$  3.6 vs. 24.2  $\pm$  4.2; *p*=0.02), serum Crea (0.6  $\pm$  0.2 mg/dL vs. 0.9  $\pm$  0.1 mg/dL, *p*=0.001), Alb (3.5  $\pm$  0.5 g/dL vs. 4.3  $\pm$  0.2 g/dL, *p*=0.001), Chol (149.7  $\pm$  31.4 mg/dL vs. 185.5  $\pm$  34.3 mg/dL, *p*=0.008) and CHE (8,351  $\pm$  2,943 U/L vs. 10,328  $\pm$  2,049 U/L, *p*=0.05), but not for other biochemical and inflammatory parameters (WBC, Plt, fibrinogen, ferritin, IL-6, IL-1 $\beta$  or TNF- $\alpha$ ). The 4 patients receiving standard formulas (St patients) and the corresponding 4 controls were significantly different for mean weight (49.3  $\pm$  3.0 kg vs. 68.3  $\pm$  6.2 kg, *p*=0.004) and BMI (20.4  $\pm$  1.9 vs. 25.5  $\pm$  1.4, *p*=0.006), but not for all nutritional and inflammatory parameters.

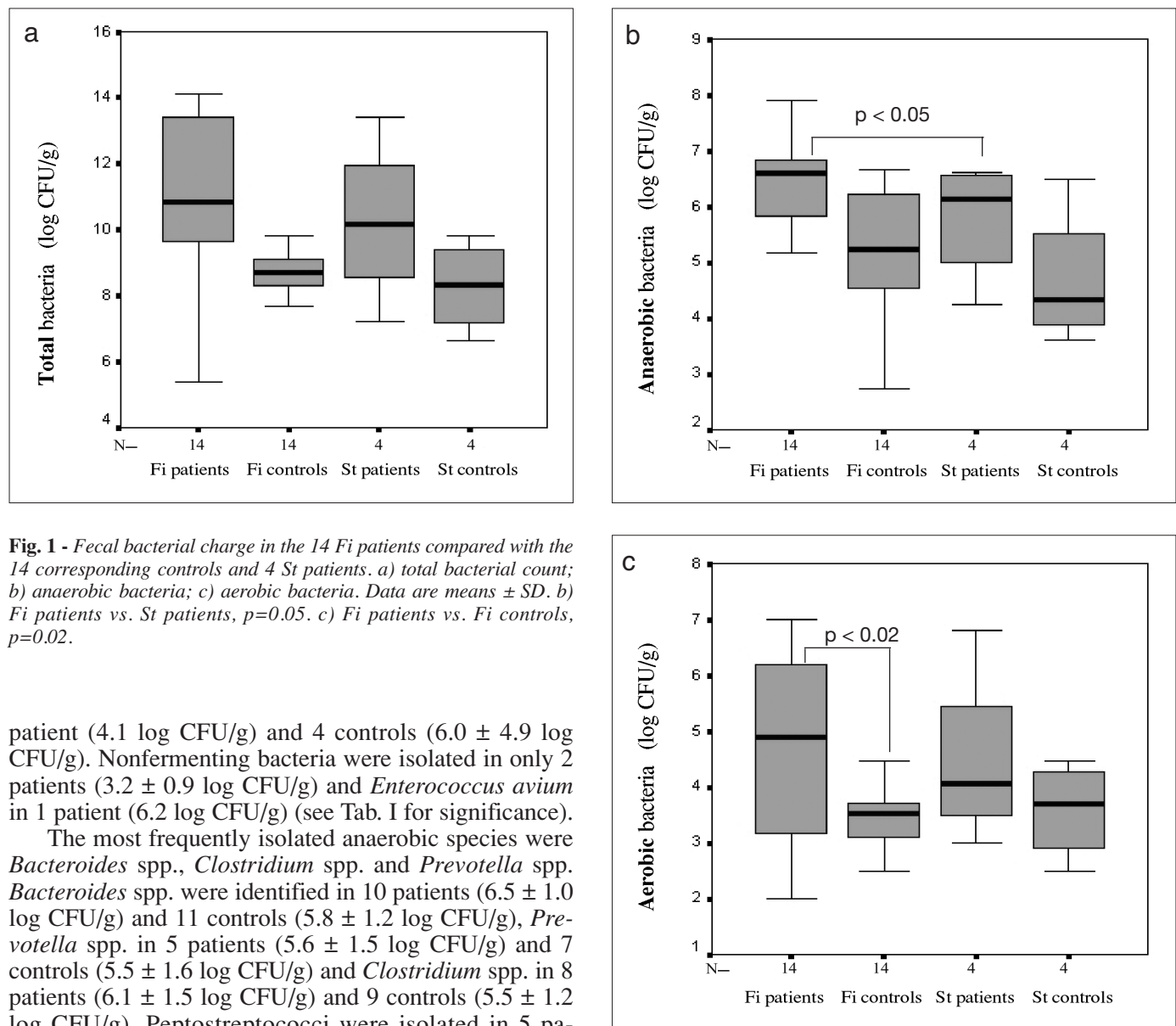
Fi patients were younger (mean age 47.6  $\pm$  19.1 vs. 71.8  $\pm$  7.3 years, *p*= 0.002) and had lower creatinine (0.6  $\pm$  0.2 g/dL vs. 0.9  $\pm$  0.05 g/dL, *p*=0.02) levels than St patients. Duration of TEN (1,300  $\pm$  832 days for Fi patients vs. 1,120  $\pm$  853 days for St patients) was not significantly different in the 2 groups.

### Intestinal flora composition

Fecal samples of all patients and controls were analyzed. Bacteria detected in the feces were enterobacteria, nonfermenting bacteria and gram-positive cocci, among aerobes; gram-positive bacilli, gram-negative bacteria and cocci among anaerobes (Tabs. I-IV).

The number of the species was 5.50  $\pm$  2.3 in Fi patients (5.53 log CFU/g mean charge) and 6.36  $\pm$  2.2 (4.63 log CFU/g mean charge) in the 14 corresponding controls; the difference was not significant. In the 4 St patients, the number of the species was 6.25  $\pm$  2.8 with a 4.77 log CFU/g mean charge; in the corresponding 4 controls, the number of species was 4.75  $\pm$  1.71 with a 4.85 log CFU/g mean charge; the difference was not significant.

In all 4 groups, the main aerobic bacteria were *E. coli*, *Staphylococcus aureus*, coagulase-negative staphylococci and *Lactobacillus* spp. In particular, with regard to the 14 Fi patients and the corresponding 14 controls, *E. coli* was isolated in 8 patients (4.6  $\pm$  1.7 log CFU/g) and in 12 controls (3.9  $\pm$  1.6 log CFU/g); coagulase-negative staphylococci in 9 patients (5.5  $\pm$  2.2 log CFU/g) and in 10 controls (3.5  $\pm$  1.4 log CFU/g), *S. aureus* in 7 patients (5.4  $\pm$  2.3 log CFU/g) and in 4 controls (3.6  $\pm$  0.6 log CFU/g) and *Lactobacillus* spp. in 1



**Fig. 1** - Fecal bacterial charge in the 14 Fi patients compared with the 14 corresponding controls and 4 St patients. a) total bacterial count; b) anaerobic bacteria; c) aerobic bacteria. Data are means  $\pm$  SD. b) Fi patients vs. St patients,  $p=0.05$ . c) Fi patients vs. Fi controls,  $p=0.02$ .

patient ( $4.1 \log \text{CFU/g}$ ) and 4 controls ( $6.0 \pm 4.9 \log \text{CFU/g}$ ). Nonfermenting bacteria were isolated in only 2 patients ( $3.2 \pm 0.9 \log \text{CFU/g}$ ) and *Enterococcus avium* in 1 patient ( $6.2 \log \text{CFU/g}$ ) (see Tab. I for significance).

The most frequently isolated anaerobic species were *Bacteroides* spp., *Clostridium* spp. and *Prevotella* spp. *Bacteroides* spp. were identified in 10 patients ( $6.5 \pm 1.0 \log \text{CFU/g}$ ) and 11 controls ( $5.8 \pm 1.2 \log \text{CFU/g}$ ), *Prevotella* spp. in 5 patients ( $5.6 \pm 1.5 \log \text{CFU/g}$ ) and 7 controls ( $5.5 \pm 1.6 \log \text{CFU/g}$ ) and *Clostridium* spp. in 8 patients ( $6.1 \pm 1.5 \log \text{CFU/g}$ ) and 9 controls ( $5.5 \pm 1.2 \log \text{CFU/g}$ ). Peptostreptococci were isolated in 5 patients ( $6.3 \pm 1.3 \log \text{CFU/g}$ ), while bifidobacteria ( $8.1 \log \text{CFU/g}$ ) and fusobacteria ( $2.3 \log \text{CFU/g}$ ) only in 1 control. *Clostridium difficile* was not isolated in either patients or controls (see Tables II-IV for significance).

Total bacteria ( $11.05 \pm 2.37$  vs.  $9.25 \pm 1.76 \log \text{CFU/g}$ ) and anaerobic count ( $6.07 \pm 1.3$  vs.  $5.4 \pm 1.6 \log \text{CFU/g}$ ) (Fig. 1a, b) were not statistically significantly different in Fi patients versus controls; while the aerobic bacteria count was higher in the 14 Fi patients than in the corresponding controls ( $5.0 \pm 1.6$  vs.  $3.8 \pm 1.0 \log \text{CFU/g}$ ,  $p=0.02$ ; Fig. 1c). As far as the 4 St patients and the respective controls, bacteria found in their feces was not statistically significantly different either for total, anaerobic or aerobic count (see Tabs. I-IV).

Concerning the type of enteral formulas (fiber-enriched vs. fiber-free), analysis of variance showed that anaerobic bacteria were significantly ( $p=0.05$ ) higher in Fi patients than in St patients, as shown in Figure 1b too.

Finally, possible relations between fecal bacterial flora and some variables such as sex, age, type of enteral formula administered, duration of TEN and some nutritional ( $\text{Alb} < 3.5$  vs.  $\text{Alb} \geq 3.5$ ) and inflammatory parameters were investigated with univariate analysis. There were no relations between intestinal bacterial flora and the parameters evaluated; the only observed difference was a significantly higher presence of anaerobes in Fi patients compared with St-patients.

**TABLE I - AEROBIC BACTERIA IN THE 14 PATIENTS RECEIVING FIBER-ENRICHED FORMULAS, 4 PATIENTS RECEIVING STANDARD FORMULAS AND CORRESPONDING CONTROLS**

Bacteria	Fi patients (n=14)	Fi controls (n=14)	St patients (n=4)	St controls (n=4)	p Value*	p Value†	p Value‡
<i>Escherichia coli</i>	4.6	3.9	5.5	3.4	ns	ns	ns
<i>Klebsiella pneumoniae</i>	-	3.5	-	-	0.01	ns	ns
<i>Klebsiella oxytoca</i>	5.6	5	4	-	ns	ns	0.05
<i>Proteus mirabilis</i>	2.3	6.5	-	-	0.02	ns	ns
<i>Enterococcus avium</i>	6.2	2	-	-	0.0001	0.0004	ns
Coagulase-negative staphylococci	5.5	3.5	3.9	4.3	0.03	ns	ns
<i>Staphylococcus aureus</i>	5.4	4	4.4	2.9	0.02	ns	ns
<i>Agrobacterium radiobacter</i>	-	-	2	-	ns	ns	ns
<i>Pseudomonas aeruginosa</i>	3.2	-	-	-	0.001	0.05	ns
<i>Pseudomonas putida</i>	-	-	4.3	-	ns	0.001	0.007
<i>Lactobacillus</i> spp.	4.1	6	-	6.1	0.005	0.002	0.004

Data are mean charges (log CFU/g).

Fi = receiving fiber-enriched formulas; ns = not significant; St = receiving standard formulas.

\*Fi patients vs. Fi controls.

†Fi patients vs. St patients.

‡St patients vs. St controls.

**TABLE II - GRAM-NEGATIVE ANAEROBIC BACTERIA IN THE 14 PATIENTS RECEIVING FIBER-ENRICHED FORMULAS, 4 PATIENTS RECEIVING STANDARD FORMULAS AND CORRESPONDING CONTROLS**

Bacteria	Fi patients (n=14)	Fi controls (n=14)	St patients (n=4)	St controls (n=4)	p Value*	p Value†	p Value‡
<i>Bacteroides caccae</i>	-	6.4	-	-	0.001	ns	ns
<i>Bacteroides capillosus</i>	6.9	6.2	-	6.8	ns	0.004	0.05
<i>Bacteroides distasonis</i>	5.1	4.5	5.3	-	ns	ns	0.05
<i>Bacteroides fragilis</i>	8.2	6.1	-	6	ns	0.001	0.05
<i>Bacteroides merdae</i>	5.9	6.3	3.5	5.4	ns	0.05	ns
<i>Bacteroides ovatus</i>	6.8	4.4	-	-	ns	0.003	ns
<i>Bacteroides uniformis</i>	6.3	5.5	-	-	ns	0.006	ns
<i>Bacteroides vulgatus</i>	6.8	6.6	6.1	5.5	ns	ns	ns
<i>Porphyromonas gingivalis</i>	6.3	6.3	4.8	3.8	ns	ns	ns
<i>Prevotella oralis</i>	6	5.5	4.3	-	ns	ns	0.05
<i>Prevotella ruminicola brevis</i>	5.5	5.4	3.6	4.1	ns	0.05	ns
<i>Prevotella ruminicola ruminicola</i>	5.6	5.8	6.5	-	ns	ns	0.007

Data are mean charges (log CFU/g).

Fi = receiving fiber-enriched formulas; ns = not significant; St = receiving standard formulas.

\*Fi patients vs. Fi controls.

†Fi patients vs. St patients.

‡St patients vs. St controls.

## DISCUSSION

Intestinal bacterial flora has several important and specific nutritional roles, in particular metabolic (fermentation of nondigestible dietary residue, saving of energy as short-chain fatty acids, vitamin K production, ions absorption, etc.), trophic and functional (control of epithelial cell proliferation and differentiation, homeostasis of the immune system), and protective ones (the

barrier effect against pathogens) (3, 12, 33, 34).

Artificial nutrition whether parenteral or enteral, is clearly not a physiological condition but sometimes is necessary in clinical practice. Some preliminary data suggest a possible negative influence of artificial nutrition, specifically enteral nutrition, on intestinal flora, such as reduction of total bacterial charge with a relatively higher number of aerobes and alteration of anaerobe/aerobe ratio (20, 35-37). In particular, Schneider et

**TABLE III** - GRAM-POSITIVE ANAEROBIC BACTERIA IN THE 14 PATIENTS RECEIVING FIBER-ENRICHED FORMULAS, 4 PATIENTS RECEIVING STANDARD FORMULAS AND CORRESPONDING CONTROLS

Bacteria	Fi patients (n=14)	Fi controls (n=14)	St patients (n=4)	St controls (n=4)	p Value*	p Value†	p Value‡
<i>Bifidobacterium adolescentis</i>	-	8.1	-	-	0.001	ns	ns
<i>Clostridium bifermentans</i>	7	4.8	-	3.3	0.02	0.002	ns
<i>Clostridium perfringens</i>	-	6.2	6.9	2.3	0.004	0.001	0.05
<i>Clostridium butyricum</i>	-	-	5.1	-	ns	0.004	0.04
<i>Clostridium clostridiformeS</i>	6.5	6	-	-	ns	0.004	ns
<i>Clostridium spp.</i>	5	5.5	4.5	4.5	ns	ns	ns
<i>Enterobacterium limosum</i>	3	-	-	-	0.05	ns	ns
<i>Eubacterium lentum</i>	6.5	7.5	-	-	ns	0.002	ns
<i>Fusobacterium varium</i>	-	2.3	-	-	ns	ns	ns

Data are mean charges (log CFU/g).

Fi = receiving fiber-enriched formulas; ns = not significant; St = receiving standard formulas.

\*Fi patients vs. Fi controls.

†Fi patients vs. St patients.

‡St patients vs. St controls.

**TABLE IV** - ANAEROBIC COCCI IN THE 14 PATIENTS RECEIVING FIBER-ENRICHED FORMULAS, 4 PATIENTS RECEIVING STANDARD FORMULAS AND CORRESPONDING CONTROLS

Bacteria	Fi patients (n=14)	Fi controls (n=14)	St patients (n=4)	St controls (n=4)	p Value*	p Value†	p Value‡
<i>Peptostreptococcus spp.</i>	6.3	-	-	-	0.001	0.04	ns
<i>Veillonella parvula</i>	7	4	-	-	ns	0.01	ns

Data are mean charges (log CFU/g).

Fi = receiving fiber-enriched formulas; ns = not significant; St = receiving standard formulas.

\*Fi patients vs. Fi controls.

†Fi patients vs. St patients.

‡St patients vs. St controls.

al (20) evaluated intestinal bacterial flora in 8 patients on TEN with fiber-free formulas, showing an increase in aerobic bacteria and a decrease in anaerobic bacteria, when compared with controls. In any case, the literature on this topic is quite small, and our study confirms the results previously obtained.

Our study involved 18 neurological, dysphagic patients on long-term TEN, free of gastrointestinal diseases, and it confirmed some differences in intestinal bacterial flora composition of TEN patients when compared with their corresponding controls. Controls were chosen among patients' families to avoid possible genetic and environmental influences on intestinal bacterial flora.

In the 14 Fi patients, aerobic bacterial charge analyzed by Mann-Whitney *U*-test, showed significantly higher levels than in controls. In particular nonfermenting aerobes such as *Pseudomonas aeruginosa* and *Pseudomonas putida*, usually absent in the intestinal

bacterial flora and potential pathogens, together with a decreased number of lactobacilli and a total absence of bifidobacteria, were detected only in the Fi patients' samples. Lactobacilli and bifidobacteria represent the major protective components of bacterial flora, and their reduction/absence in intestinal flora is a an important risk factor for the growth of pathogenic bacteria.

In addition to the type of enteral formula and the duration of TEN, some nutritional and inflammatory biochemical parameters have been considered to evaluate their influence on intestinal bacterial flora composition. In agreement with Schneider et al (20), in our study, age, sex, nutritional and inflammatory parameters did not influence fecal bacteria. Only the type of enteral formula influenced intestinal flora composition; in particular, fiber-enriched formulas seem to increase the number of protective anaerobic bacteria, compared with standard formulas.

In conclusion, this preliminary study shows the pres-

ence of potential pathogenic nonfermenting bacteria in TEN patients compared with controls, thus suggesting a possible negative influence of long-term TEN on intestinal flora. The influence of TEN on intestinal flora is more evident in Fi patients, probably due to the larger number of patients. The absence of significant differences in the intestinal flora composition in St patients compared with controls was probably due to the small number of St patients studied, which reduced the power of the statistical analysis. The results obtained in the present study should therefore be confirmed by a larger multicenter study.

A second limitation of our study is represented by the standard microbiological methods used to identify and quantify intestinal bacterial flora. Nowadays, molecular analysis is a more accurate method and allows us to identify a greater number of bacteria; unfortunately, this methodology was not yet in use in our hospital when the study started.

In any case, despite some limitations, this study confirms the few data already reported in the literature on intestinal bacterial flora in a very selected population such as that of long-term TEN patients.

A larger, multicenter study would be useful to further clarify the relationship between TEN, intestinal bacterial flora and patients' nutritional and inflammatory status.

Address for correspondence:  
Franco Contaldo, MD  
Department of Clinical and Experimental Medicine  
Federico II University, Naples  
Via Pansini, 5  
80131 Naples, Italy  
e-mail: contaldo@unina.it

## REFERENCES

1. Simon GL, Gorbach SL. Intestinal flora in health and disease. *Gastroenterology* 1984; 86: 174-93.
2. Bengmark SP. Microbial flora of the gastrointestinal tract: the role of probiotic flora. *Gut* 1998; 42: 2-7.
3. Guarner F, Malagelada JR. Gut flora in health and disease. *Lancet* 2003; 361: 512-9.
4. Stephen AM, Cummings JH. The microbial contribution to human faecal mass. *J Med Microbiol* 1980; 13: 45-56.
5. Long SS, Swenson RM. Development of anaerobic fecal flora in healthy newborn infants. *J Pediatr* 1977; 91: 298-301.
6. Deitch EA. Does the gut protect or injure patients in the ICU. In: Cerra FB, ed. *Perspectives in critical care*, vol 1. St. Louis, MO: Quality Medical Publishing, 1988; 15-32.
7. Spaeth G, Berg RD, Specian RD, et al. Food without fibers promotes bacterial translocation from the gut. *Surgery* 1990; 108: 240-7.
8. Orrhage K, Nord CE. Factors controlling the bacterial colonization of the intestine in breastfed infants. *Acta Paediatr Suppl* 1999; 88: 47-57.
9. Montemerlo H, Menendez AM, Marcenac F, et al. Enteral nutrition: reduction in the contamination risk. *Nutr Hosp* 1996; 11: 102-7.
10. Hooper LV, Wong MH, Thelin A, Hansson L, Falk PG, Gordon JI. Molecular analysis of commensal host-microbial relationships in the intestine. *Science* 2001; 291: 881-4.
11. Salminen S, Bouley C, Bouton-Rualt MC, et al. Functional food science and gastrointestinal physiology and function. *Br J Nutr* 1998; 80 (Suppl): S147-71.
12. Cummings JH, Beatty ER, Kingman SM, Bingham SA, Englyst HN. Digestion and physiological properties of resistant starch in the human large bowel. *Br J Nutr* 1996; 75: 733-47.
13. Cummings JH, Englyst HN. Fermentation in the human large intestine and the available substrates. *Am J Clin Nutr* 1987; 45 (Suppl): S1243-55.
14. Haskel Y, Udassin R, Freund HR, Zhang J, Hanani M. Liquid enteral diets induce bacterial translocation by increasing cecal flora without changing intestinal motility. *JPEN* 2001; 25: 60-4.
15. Cummings JH, Wiggins HS, Jenkins D, et al. Influence of diets high and low in animal fat on bowel habit, gastrointestinal transit, transit time, fecal microflora, bile acid and fat excretion. *J Clin Invest* 1978; 61: 953-63.
16. Mainous M, Xu DZ, Lu Q, Berg RD, Deitch EA. Oral TPN-induced bacterial translocation and impaired immune defenses are reversed by refeeding. *Surgery* 1991; 110: 277-83.
17. Crowther JS, Drasar BS, Goddard P, Hill MJ, Johnson K. The effect of a chemically defined diet on the faecal flora and faecal steroid concentration. *Gut* 1973; 14: 790-3.
18. Bounous G, Devroede GJ. Effects of an elemental diet on human fecal flora. *Gastroenterology* 1974; 66: 210-4.
19. Atterbery HR, Sutter VL, Finegold SM. Effect of a par-

- tially chemically defined diet on normal human fecal flora. *Am J Clin Nutr* 1972; 25: 1391-8.
20. Schneider SM, Le Gall P, Girard-Pipau F, et al. Total artificial nutrition is associated with major changes in the fecal flora. *Eur J Nutr* 2000; 39: 248-55.
  21. American Gastroenterological Association. American Gastroenterological Association technical review on tube feeding for enteral nutrition. *Gastroenterology* 1995; 108: 1282-301.
  22. Alveredy J, Chi HS, Sheldon GF. The effect of parenteral nutrition on gastrointestinal immunity: the importance of enteral stimulation. *Ann Surg* 1985; 202: 681-4.
  23. Cataldi-Betcher EL, Seltzer MH, Slocum BA, Jones KW. Complications occurring during enteral nutrition support: a prospective study. *JPEN* 1983; 7: 546-52.
  24. Moore WEC, Holdeman LV. Discussion of current bacteriological investigations of the relationships between intestinal flora, diet, and colon cancer. *Cancer Res* 1975; 35: 3418-20.
  25. Drasar BS, Roberts AK. Methods for the study of anaerobic microflora. In: Levett PN, ed. *Anaerobic microbiology: a practical approach*. Oxford: Oxford University Press, 1991; 183-200.
  26. Phillips E, Nash P. Culture media. In: Lennette EH, Balows A, Hausler WJ Jr, Shadomy HJ, eds. *Manual of clinical microbiology*; 4th ed. Washington DC: American Society for Microbiology, 1985; 1051-92.
  27. Summanem P, Baron EJ, Citron DM, et al. *Wadsworth anaerobic bacteriology manual*; 5th ed. Belmont, CA: Star Publishing Company, 1993.
  28. Koneman EW, Allen SD, Dowell VR Jr, Janda WM, Schreckenberger PC, Winn WC Jr, eds. *Testo atlante di microbiologia diagnostica*; 2nd ed. Rome: Delfino editore, 1995.
  29. Desport JC, Mounier M, Preux PM, et al. Evaluation of the microbial safety of a new 1.5 l enteral feeding diet reservoir system. *Clin Nutr* 2004; 23: 983-8.
  30. Macy JM, Probst I. The biology of gastrointestinal bacteroides. *Annu Rev Microbiol* 1979; 65: 390-7.
  31. Mackowiak PA. The normal microbial flora. *N Engl J Med* 1982; 307: 83-93.
  32. Franks H, Harmsen HJM, Raangs GC, et al. Variations of bacterial populations in human feces measured by fluorescent in situ hybridization with group-specific 16S rRNA-targeted oligonucleotide probes. *Appl Environ Microbiol* 1998; 64: 3336-45.
  33. Jiang HQ, Bos NA, Cebra JJ. Timing, localization and persistence of colonization by segmented filamentous bacteria in the neonatal mouse gut depend on immune status of mothers and pups. *Infect Immun* 2001; 69: 3611-7.
  34. Gordon JI, Hooper LV, McNevin MS, Wong M, Bry L. Epithelial cell growth and differentiation: Part III: promoting diversity in the intestine: conversations between the microflora, epithelium and diffuse GALT. *Am J Physiol* 1997; 273: G565-70.
  35. Bornside GH, Cohn I. Stability of normal human fecal flora during a chemically defined, low residue liquid diet. *Ann Surg* 1975; 181: 58-60.
  36. Meijer-Severs GJ, van Santen E. Short-chain fatty acids 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* 1989; 24: 1276-80.
  37. Kimura K, McCartney AL, McConnel MA, Tannock GW. Analysis of fecal populations of Bifidobacteria and Lactobacilli of their human host to the predominant strains. *Appl Environ Microbiol* 1997; 63: 3394-8.

Received: February 28, 2007

Accepted: April 30, 2007