

## Effects of *Saccharomyces boulardii* on fecal short-chain fatty acids and microflora in patients on long-term total enteral nutrition

Stéphane M Schneider, Fernand Girard-Pipau, Jérôme Filippi, Xavier Hébuterne, Dominique Moyses, Gustavo Calle Hinojosa, Anne Pompei, Patrick Rampal

Stéphane M Schneider, Jérôme Filippi, Xavier Hébuterne, Gustavo Calle Hinojosa, Patrick Rampal, Department of Hepato-Gastroenterology and Clinical Nutrition, Archet University Hospital, Nice, France

Fernand Girard-Pipau, Anne Pompei, Bacteriology Laboratory, Archet University Hospital, Nice, France

Dominique Moyses, Biostatistician, Paris, France

Gustavo Calle Hinojosa, Patrick Rampal, Department of Hepato-Gastroenterology, Princess Grace Hospital, Monte-Carlo, Monaco  
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Correspondence to: Dr. Stéphane M Schneider, Department of Gastroenterology and Clinical Nutrition, Archet University Hospital, BP 3079, F-06202 Nice Cedex 3, France. stephane.schneider@unice.fr  
Telephone: +33-4-92-03-61-68 Fax: +33-4-92-03-65-75

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### Abstract

**AIM:** To assess the effects of *Sb* on fecal flora and short-chain fatty acids (SCFA) in patients on long-term TEN.

**METHODS:** Ten patients (3 females, 7 males, 59±5.5 years), on TEN for a median of 13 mo (1-125), and 15 healthy volunteers (4 females, 11 males, 32±2.0 years) received *Sb* (0.5 g bid PO) for 6 d. Two stool samples were taken before, on the last 2 d and 9-10 d after treatment, for SCFA measurement and for culture and bacterial identification. Values (mean±SE) were compared using sign tests and ANOVA.

**RESULTS:** Fecal butyrate levels were lower in patients (10.1±2.9 mmol/kg) than in controls (19.2±3.9,  $P = 0.02$ ). Treatment with *Sb* increased total fecal SCFA levels in patients (150.2±27.2 vs 107.5±18.2 mmol/kg,  $P = 0.02$ ) but not in controls (129.0±28.6 vs 113.0±15.2 mmol/kg, NS). At the end of treatment with *Sb*, patients had higher fecal butyrate (16.0±4.4 vs 10.1 [2.9] mmol/kg,  $P = 0.004$ ). Total SCFAs remained high 9 d after treatment was discontinued. Before the treatment, the anaerobe to aerobe ratio was lower in patients compared to controls (2.4±2.3 vs 69.8±1.8,  $P = 0.003$ ). There were no significant changes in the fecal flora of TEN patients.

**CONCLUSION:** *Sb*-induced increase of fecal SCFA concentrations (especially butyrate) may explain the preventive effects of this yeast on TEN-induced diarrhea.

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**Key words:** Enteral nutrition; Diarrhea; *Saccharomyces*

*boulardii*; Short-chain fatty acids; Intestinal microbiota

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### INTRODUCTION

Diarrhea is the most frequent complication of enteral tube feeding, with an incidence as high as 63%<sup>[1]</sup>. Its consequences range from discomfort to life-threatening acidosis, increased morbidity and mortality, and higher financial costs for health providers<sup>[2]</sup>. One of the causes of diarrhea in tube-fed patients may be the consequences on colonic trophicity of a deficiency in luminal short-chain fatty acids (SCFAs). We have reported a major imbalance (namely a drop in the number of fecal anaerobic bacteria and an increase in the number of aerobic bacteria) in patients on long-term total enteral nutrition (TEN)<sup>[3]</sup>. The modifications of the intestinal microflora induced by a fiber-free polymeric enteral diet can be compared to those induced by broad-spectrum antibiotics such as ceftriaxone<sup>[4,5]</sup>. These effects may be synergic<sup>[6]</sup> and explain why antibiotics are a risk factor for enteral nutrition-induced diarrhea<sup>[7,8]</sup> and why enteral nutrition is a risk factor for antibiotic-induced diarrhea<sup>[9]</sup> and *Clostridium difficile* infection<sup>[10]</sup>. SCFAs, one of the most important by-products of anaerobes in the colon, are the main fuel for the colonocyte and they are involved in water and electrolyte absorption by the colonic mucosa. They have been shown to reverse fluid secretion in the ascending colon during enteral feeding<sup>[11]</sup>, and may represent the link between diarrhea and the intestinal microflora during enteral nutrition.

*Saccharomyces boulardii* (*Sb*) is a probiotic yeast that has been successfully used for years in the prevention of antibiotic-associated diarrhea<sup>[12]</sup>. *Sb* has been proven to prevent the relapse of *Clostridium difficile* infection<sup>[13]</sup>, and seems effective in preventing relapses in patients with Crohn's disease<sup>[14]</sup>. Three randomized controlled studies have reported its efficacy in the prevention of diarrhea in TEN patients from intensive care units, with a reduction in the number of patient-days with diarrhea by 25-83%<sup>[15-17]</sup>. However, these studies did not address the mechanisms of action of the probiotic. We therefore designed a prospective study to assess the effects of *Sb* on fecal SCFAs and intestinal microflora in TEN patients. Healthy volunteers (HVO) were

used as controls.

## MATERIALS AND METHODS

### Subjects

The TEN group consisted of 10 patients (3 females and 7 males), mean age  $59 \pm 5.5$  years (mean  $\pm$  SE), who had been on TEN for a median of 13 mo (range: 1-125 mo). The indication for TEN was dysphagia in six patients (three head and neck tumors, two strokes, and one resected neurinoma); three patients had upper gastro-intestinal surgery, and one patient had low oral intake due to severe depression. Two patients were receiving proton pump inhibitors. A commercially available polymeric diet, fiber-, lactose-, and gluten-free, with a concentration of 1.33 kcal/mL (Sondalis HP<sup>®</sup>, Nestlé Clinical Nutrition, Noisiel, 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 ( $n = 7$ ), jejunostomy ( $n = 2$ ), or naso-gastric ( $n = 1$ ) tubes. Enteral nutrition was total, but patients who could drink water or tea were allowed to do so. Fifteen HVO (4 females and 11 males), mean age  $32 \pm 2.0$  years ( $P < 0.05$  vs TEN patients) were also studied. All HVO consumed a regular Western diet, and none of them had a history of gastrointestinal disease. At the time of the study, all experimental and normal subjects were in a stable condition, and none had diarrhea. Energy provided by the diet was covering their maintenance needs as calculated using the Harris and Benedict formulas, and the enteral feeds were stable throughout the study. No subject had undergone colectomy, and none had taken antibiotics or laxatives for at least 2 wk prior to the study. All subjects gave their written informed consent and the study was performed according to the Declaration of Helsinki and approved by the regional Ethics Committee.

### Study design

Five hundred milligrams b.i.d. of *Sb* as lyophilized powder, provided by Laboratoires Biocodex (Montrouge, France), were administered orally (HVO) or via the feeding tube (TEN patients) for six consecutive days. Two fecal samples were collected at 1-d intervals from all subjects on the 2 d before treatment, on the last 2 d of treatment, and 9 and 10 d after treatment was discontinued. Antibiotic or laxative use during the study was an exclusion criterion.

### Stool analysis

Stool samples were taken immediately after production, and analyzed within 2 h. SCFAs were studied as follows<sup>[18]</sup>: an aliquot of 200 mg of feces was weighed. This 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 min with an orbital shaker, and centrifuged for 5 min at 3 000 r/min at room temperature. Anhydrous  $CaCl_2$  was then added in order to remove residual water, and 2  $\mu$ L of the extract was 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); it was assayed before each stool sample was analyzed. Sensitivity was 1 mmol. 500 mg of feces was 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 as follows: 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  $10^{-6}$ <sup>[19]</sup>. For anaerobic bacteria, media inoculated were as follows: Columbia agar supplemented with sheep blood spread with the dilutions  $10^{-2}$ ,  $10^{-4}$ , and  $10^{-8}$ , Columbia blood agar supplemented with kanamycin and vancomycin, *Bifidobacterium* agar, and *Bacteroides* agar (dilutions  $10^{-2}$ ,  $10^{-4}$ , and  $10^{-7}$ ), rifampicin 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  $10^{-5}$ <sup>[19]</sup>. The differences between dilutions for each medium were based on the differences between the concentrations of concerned bacteria<sup>[20]</sup>. After 24-48 h 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 50 mL/L carbon dioxide was verified for anaerobic strains. Routine identification was performed with standard methods, then with microstrips (API 20 Enterobacteries or ID 32 Anaerobies, BioMérieux, Marcy L'Etoile, France). The ratio between anaerobes and aerobes was calculated as a marker of microbial imbalance<sup>[3,21]</sup>.

### Statistical analysis

Results are expressed as mean  $\pm$  SE. All fecal bacterial counts (colony-forming units [CFU] per gram of wet feces) were transformed to logarithms ( $\log_{10}$  CFU) for statistical analysis. Values were compared at baseline using a non-parametric method. Comparisons within groups used a sign test; differences vs baseline are presented with distribution-free confidence intervals.

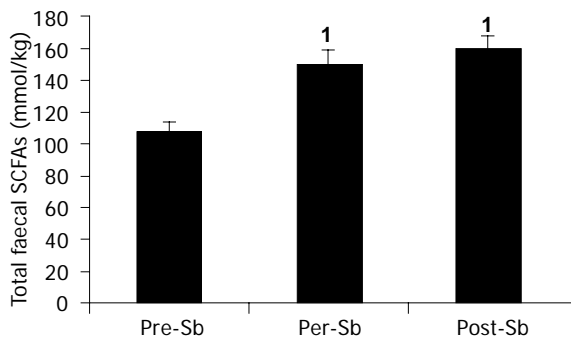
A two-way ANOVA was performed for quantitative parameters for exploratory purposes. The model included group (TEN or HVO), period of time (before, during or after treatment) and interaction group\*period of time; all two by two differences between adjusted means were calculated and tested using *t*-tests. No adjustment was performed in this exploratory context. For SFCA assessments, two values were considered in the model at each time. Statistics were performed on SAS software from SAS Institute (Cary, NC, USA). Differences were considered as statistically significant for *P* lower than 0.05. A pilot study that reported a 40% increase of total SCFA concentration after treatment with *Sb* allowed us to calculate a number needed to treat ten subjects per group.

## RESULTS

### Fecal short-chain fatty acids

Acetate, propionate, and butyrate values are represented in Table 1. For all three main SCFAs, no significant change,

either during or after treatment, was observed in HVO. In TEN patients, butyrate concentrations were increased significantly (about 60%) during treatment. SCFA values 9 d after discontinuing *Sb* were not significantly different from baseline. The sum of all SCFAs was increased significantly in TEN patients under treatment, with an average increase of 42.8 mmol/kg (95%CI: 0.0-55.2,  $P = 0.02$ ) during treatment and an average increase of 51.8 mmol/kg (95%CI: 14.4-110.6,  $P = 0.02$ ) after treatment (Figure 1). There were no changes in HVO. Minor SCFAs did not change in either group.



**Figure 1** Fecal SCFAs in TEN patients. SCFA: short-chain fatty acids; *Sb*: *Saccharomyces boulardii*;  $^1P = 0.02$  vs pre-treatment values.

ANOVA showed an overall increase for acetate and SCFA sum after treatment on change from baseline values; significant interactions were noticed for propionate due to the increase in the TEN group opposed to a decrease in the HVO group, and for butyrate corresponding to differences in after-treatment concentrations higher in the HVO group in spite of higher baseline values in this group.

### Fecal flora

Before treatment, there were significant differences in the fecal flora between HVO and TEN patients, as the latter

had higher counts of aerobic bacteria and a lower anaerobes/ aerobes ratio (Table 2). Among aerobes, the number of enterococci was higher in TEN patients ( $7.42 \pm 0.64$  log CFU/g) than in HVO ( $6.1 \pm 0.46$  log CFU/g,  $P = 0.02$ ). Among anaerobes, peptostreptococci and *Bifidobacterium* spp., present in HVO, were absent in TEN patients. *Clostridium difficile* was not identified in any subject. There were no significant changes in the numbers of colony forming units of individual species or groups of bacteria in TEN patients during treatment with *Sb*, or after treatment. The only change observed in HVO was a decrease of Gram-positive anaerobes from  $8.6 \pm 0.3$  log CFU/g to  $6.8 \pm 0.8$  during treatment ( $P = 0.035$ ).

**Table 2** Fecal bacterial populations in TEN patients and HVO

		Before treatment concentration	During treatment concentration	After treatment concentration
Total bacteria (log CFU/g)	TEN	9.64 (0.31)	9.31 (0.41)	9.54 (0.36)
	HVO	9.53 (0.16)	9.56 (0.21)	9.20 (0.19)
Anaerobes (log CFU/g)	TEN	8.25 (1.01)	7.72 (0.76)	8.63 (0.62)
	HVO	9.47 (0.16)	8.73 (0.45)	8.97 (0.25)
Aerobes (log CFU/g)	TEN	8.84 (0.26)	8.83 (0.34)	8.71 (0.32)
	HVO	7.63 (0.22)	7.63 (0.24)	7.61 (0.26)
Anaerobes/aerobes ratio	TEN	2.42 (2.28)	0.51 (2.23)	2.89 (2.43)
	HVO	69.81 (1.81)	60.13 (2.47)	23.02 (2.01)

Mean (SE). TEN, total enteral nutrition; HVO, healthy volunteers. No significant difference.

### Tolerance

There were no significant changes in the number of bowel movements and the consistency of stools during or after treatment in either HVO or TEN patients (data not shown). No fever or fungemia was reported. The only adverse effect attributed to *Sb* was a case of mild diarrhea in one patient which resolved without discontinuation of treatment.

**Table 1** Fecal SCFAs in TEN patients and HVO

		Before treatment concentration (SEM)	During treatment			After treatment		
			Concentration (SEM)	Difference with pre-treatment value (95%CI)	<i>P</i> <sup>1</sup>	Concentration (SEM)	Difference with pre-treatment value (95%CI)	<i>P</i> <sup>1</sup>
Acetate	TEN	69.9 (12.3)	97.9 (25.2)	28.0 -6.1; 36.2	0.18	110.3 (24.1)	40.4 14.4; 71.4	0.02
	HVO	71.3 (10.3)	98.4 (29.8)	27.1 -13.1; 35.6	0.79	111.5 (17.5)	40.2 -7.0; 116.0	0.42
Propionate	TEN	20.9 (3.7)	26.2 (3.8)	5.3 -0.2; 12.6	0.18	26.0 (3.4)	5.1 -3.3; 14.3	0.18
	HVO	22.9 (3.0)	18.4 (2.4)	-4.5 -11.8; 4.5	0.30	23.0 (2.7)	0.1 -3.1; 6.9	0.12
Butyrate	TEN	10.1 (2.9)	16.0 (4.4)	5.8 0.1; 12.3	0.004	12.9 (2.5)	2.7 -3.9; 12.3	0.51
	HVO	19.2 (3.9)	16.4 (3.3)	-2.7 -9.7 ; 5.6	0.61	23.3 (3.1)	4.1 -1.4 ; 16.3	0.12

Values in mmol/kg of wet feces: mean (SE); TEN: total enteral nutrition, HVO: healthy volunteers.  $^1$ Probability of sign test.

## DISCUSSION

This study shows that both the fecal concentration of butyric acid and the anaerobe/aerobe ratio were lower in patients at high risk for diarrhea due to TEN than in HVO. Despite great inter-individual variations, treatment with *Sb* increased total SCFAs and butyrate fecal concentrations in TEN patients with a long-lasting increase of total SCFA concentrations when treatment was discontinued. Treatment with *Sb* did not affect the fecal flora in TEN patients, while it decreased Gram-positive anaerobes in HVO.

*Sb* is known to interact with the intestinal flora. Yeast proteins have been shown to neutralize cholera toxin<sup>[22]</sup> and to repress *Clostridium difficile* toxins A and B<sup>[23]</sup>; *Sb* also has an antagonistic effect on the growth of pathogenic micro-organisms in the intestine<sup>[24]</sup>. In our study, increased SCFA concentrations might explain the reported prevention by *Sb* of TEN-induced diarrhea by an increased water and electrolyte absorption and by a reduction in colonic pH, even though it was not measured in the stool samples from our subjects. A lower pH inhibits the growth of *Clostridium difficile*<sup>[25,26]</sup>, a bacteria that can be acquired in up to 15% of hospitalized EN patients<sup>[10]</sup>. A viable mixed culture of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* (1 g t.i.d.) failed to prevent diarrhea in hospital inpatients receiving EN in a randomized controlled study<sup>[27]</sup>. Unlike *Sb*, lactobacilli taken orally do not increase SCFA fecal levels<sup>[28,29]</sup>, which might explain these negative results. Prebiotics can also increase SCFAs in the colon; in a recently published study, administration for 2 wk of a soluble dietary fiber (galactomannans) in 20 elderly inpatients receiving EN was shown to decrease the water content of the feces and the frequency of daily bowel movements<sup>[30]</sup>. These results were associated with an increase of fecal SCFA levels, significant for total SCFAs, acetic and propionic acids. Although we confirmed the 40% increase in total SCFAs in the pilot phase of the study that was used to determine the number of subjects, a high variability prevented some important differences from reaching statistical significance. This variability did not seem to be time- or diet-dependent, as it was comparable in volunteers on a free diet and in patients who were on a stable controlled diet. *Sb* recovery in the stools after chronic administration is known to reach a plateau by the 3<sup>rd</sup> d and to disappear 5 d after treatment is discontinued<sup>[31]</sup>. The persistence of the effects on total SCFA concentrations 9-10 d after *Sb* discontinuation is suggestive of a prolonged action of the yeast and may indicate that daily treatment may not be necessary to sustain its effects on fecal SCFA concentrations.

known to be associated with a decrease of fecal anaerobes<sup>[35]</sup>; however, age does not influence fecal SCFA levels<sup>[36]</sup>; (2) 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<sup>[37]</sup>; (3) Analysis of samples rather than 24 h-stools certainly deprives us of some information as TEN is known to modify the volume of daily stools. However, stool collection is often difficult in bed-ridden patients, especially those with neurological disorders. This is the main reason why we chose to perform this study in patients without diarrhea; (4) The correlation

between SCFAs and bacterial counts in feces is reportedly poor<sup>[38]</sup>; (5) Lastly a standard bacterial count, however thorough, may miss some information. Molecular techniques<sup>[39]</sup> may help progressing in this field.

There is growing evidence regarding the health benefits of probiotics. In EN patients; besides prevention of diarrhea, a recent study reports less post-operative infections in patients receiving EN supplemented with fiber and *Lactobacillus plantarum* 299 than in those receiving parenteral nutrition of fiber-free TEN<sup>[40]</sup>.

In conclusion, this study suggests one possible mechanism of action of the probiotic yeast *Sb*, especially for its preventive effects in enteral nutrition-induced diarrhea. It also supports its use, especially in patients who have other risk factors, such as antibiotic intake.

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