# Prebiotic ingestion does not improve gastrointestinal barrier function in burn patients

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**Abstract**

Prebiotics increase intestinal levels of health-promoting bacteria implicated in decreasing pathogen colonization, stimulating immune functions and stabilizing gut barrier functions, parameters which are altered in burn patients. We propose that regular intake of a prebiotic, oligofructose (OF), might help to improve the altered gastrointestinal (GI) permeability observed in burn patients. A randomized, double-blind, controlled clinical trial was carried out in 41 burn patients (mean burn surface area = 17.1 ± 8.2%) who ingested daily 6 g of oligofructose (OF group) or sucrose as placebo (Control group) during 15 days. Gastrointestinal permeability to sucrose and lactulose/mannitol (L/M) was evaluated on days 1 (before treatment) 3, 7, 14 and 21. A permeability test was also performed in 18 healthy subjects as controls. Thirty-one patients completed the protocol (dropout rate = 24.4%). Healthy subjects had a basal sucrose excretion of 21.3 mg (14.0–32.5 mg) and a basal L/M ratio of 0.017% (0.009–0.022%). Sucrose excretion increased 5-fold and L/M ratio 4.4-fold in burn patients on day 1 and these high levels of marker excretion decreased significantly throughout the study ($p = 0.016$ and 0.000001, respectively). No differences between the OF and Control groups were observed for sucrose excretion or L/M ratio. In conclusion, the normalization of gastrointestinal permeability is not accelerated by prebiotic intake.

*Keywords:* Burn; Intestinal permeability; Gastric permeability; Oligofructose; Prebiotic

## 1. Introduction

Critical illness is frequently associated with changes in the pattern of microbial colonization of the oropharynx and upper gastrointestinal (GI) tract. These alterations are also associated with starvation, altered intestinal motility, immunosupression and with the use of antacids and antibiotics [1]. Gastric alkalinization induced by the utilization of inhibitors of the proton pump for prophylaxis of stress ulcer, for example, has been found to predispose to gastric, intestinal and respiratory tract colonization by Gram-negative microorganisms [2]. GI colonization by *Candida*, *Pseudomonas* or *Staphylococcus* is significantly associated with concomitant pneumonia, urinary tract infection, wound infection, peritonitis and septicemia, suggesting that the upper gut acts as a reservoir for microorganisms responsible for nosocomial infections in critical patients [3]. These findings are supported by the observation that selective decontamination of the digestive tract with unabsorbable antimicrobial agents active against aerobic Gram-negative bacteria and fungi may prevent nosocomial infections and decrease mortality in intensive care units [4]. Studies in animal models have confirmed that the gut may act as a portal of entry for endotoxin and bacteria, favoring their dissemination and the subsequent development of multiple organ failure [5,6].

Disturbances of the intestinal flora are also frequently associated with alterations of the GI barrier. Such GI alterations are observed in conditions such as inflammatory bowel disease [7], celiac disease [8], rheumatoid arthritis [9], food allergies [10] or secondary to gut pathogen

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colonization [11], alcohol or non-steroidal anti-inflammatory drug intake [12,13], radiation [14], or high physical and/or psychological stress as seen in trauma victims, including burn patients [15]. GI permeability, as a reflection of GI barrier alteration, may be assessed by measuring the urinary excretion of non-metabolizable sugar markers; lactulose and mannitol are generally used to evaluate intestinal permeability [16] while sucrose is useful for gastric permeability [17]. Lactulose/mannitol (L/M) tests performed after burn injury showed a dramatic increase of intestinal permeability [15] in humans in association with wound infection and the presence of circulating endotoxin [18,19], a potentiator of intestinal barrier failure [20].

Intake of probiotics or prebiotics constitutes an alternative mechanism for regulating gut/microbiota homeostasis. Probiotics are microorganisms (essentially lactobacilli and bifidobacteria), which survive their transit along the digestive tract of the host and exert health-promoting effects, while prebiotics are non-digestible carbohydrates which selectively stimulate the growth of endogenous health-promoting bacterial populations (mostly lactobacilli and bifidobacteria), in the colon [21]. The benefits associated with the regular intake of pre and/or probiotics and resulting in high levels of ''friendly'' bacteria in the gut have been described; some of these microorganisms are relevant for critical patients as their intake is associated with a decreased risk of colonization by pathogens and/or opportunistic species [22], stimulation of both the local and systemic immune systems [23] and stabilization of the GI barrier function [12,24] including a decrease of endotoxemia [25].

The purpose of the present study was to evaluate the changes of the gastric and intestinal barrier function after burn injuries in humans by means of the lactulose/mannitol/sucrose permeability test, and to evaluate whether daily intake of oligofructose (OF), a prebiotic, improved the recovery of the GI barrier function in these patients.

## 2. Subjects and methods

### 2.1. Subjects

The study was carried out in the Burn Unit of the Public Central Emergency Hospital in Santiago, Chile. The study protocol was approved by the Ethics Committee for Research in Humans of INTA, University of Chile. Informed consent was obtained from all subjects or their relatives before inclusion in this protocol. Subjects of either sex, between 14 and 70 years of age, with 2nd and/or 3rd degree burn injuries according to the Converse Smith classification equivalent to 5–40% of the total body surface area and who were hospitalized within the first 24 h following their injury were included in the study. Patients with burns of the upper respiratory tract or who had suffered chemical or electrical burns as well as those with previous or current gastrointestinal pathologies, or with associated chronic pathologies such as diabetes, nephropathies or liver cirrhosis were excluded from the study. The Zawacki index of severity [26] was calculated and a global subjective evaluation of nutritional status was carried out in every case on the day of admission.

### 2.2. Study design

Subjects were randomly distributed into two groups, Control and Oligofructose, to receive twice daily either 3 g sucrose or of oligofructose (Raftilose 95, a gift of Orafti, Tienen, Belgium) dissolved in a glass of water or incorporated into food. Treatments were administered in double-blind fashion. Sucrose was selected as the placebo because it is rapidly hydrolyzed in the small intestine and does not reach the colon; furthermore, it is not bifidogenic. Nutrasweet® was added to the oligofructose solution to produce a level of sweetness equivalent to that of sucrose. Throughout the study, patients received the usual hospital diet (40 kcal/(kg day), 14% P), but foodstuffs with high levels of inulin (a natural prebiotic found in fruits and vegetables) as well as fermented foodstuffs containing lactic-acid bacteria were not provided. Permeability tests were carried out during the first 24 h after the burn (day 1), and on days 3, 7, 14 and 21 afterwards. Treatment began after the first permeability test and was administered during the following 15 days. GI permeability results in burn patients were compared with those obtained in a group of 18 healthy subjects (38.9% males, age 23.1 ± 4.3 years). Burn patients on enteral nutrition received ADN® (Davis Laboratory, Santiago, Chile), a high-protein formula (14% P, 51% CHO, 1 Kcal/ml, 300 mosm/L), which did not contain glutamine nor prebiotics. Infection of the burn or the urinary, respiratory or gastrointestinal tracts or systemic infections were recorded.

### 2.3. Gastrointestinal permeability evaluation

GI permeability was evaluated as previously described [27]: 150 ml of a solution containing 40 g sucrose, 7.5 g lactulose and 2 g mannitol were administered to the patients and urine was collected for 5 h in a plastic container with 10 ml of 10% thymol in isopropanol and kept at 4 °C. The volume voided was measured and aliquots were frozen at −20 °C until processed. Sugar concentrations in the urine samples were measured as previously described. Control samples of urine with known amounts of added sucrose, lactulose and mannitol were prepared and analyzed in parallel, using cellobiose and α-methyl-glucose (Sigma Chemical Co., St. Louis, MO) as internal standards. Derivatized sugars were obtained after successive incubation of 10 μL of urine with methoxyamine (Sigma Cemical Co., St. Louis, MO, USA) and *N*,*O*-bis-(trimethylsilyl)-trifluoroacetamide containing 1% trimethylchlorosilane (Alltech, Deerfield, IL) in anhydrous pyridine. Two

microliter samples dissolved in hexane were injected in the split mode on a AT1701 capillary column (Alltech, Deerfield, IL, USA) at a temperature of 200 °C in a Varian 3600 gas chromatograph equipped with a split/splitless injector and a flame ionization detector (Varian Instruments, San Fernando, CA, USA). Run to run variation of these measurements was <10%. Results are expressed as the urinary excretion of each sugar (mg) and the lactulose/mannitol ratio.

### 2.4. Statistical analysis

Comparisons of the qualitative and quantitative variables between groups on day 1 were carried out by the Chi-square and Mann–Whitney *U*-test. Urinary excretion of sucrose, lactulose and mannitol, as well as the lactulose/mannitol ratio did not follow a normal distribution and were logarithmically transformed for their posterior statistical analysis. The effects of treatment on the time course of these permeability markers during the study were analyzed by a two-way analysis of variance (ANOVA) for repeated measurements. These variables were expressed as geometric means with 95% confidence intervals (CI).

## 3. Results

Of the 61 patients admitted to the Burn Unit during the enrollment period, 41 satisfied the inclusion criteria and were recruited and randomized to the Control ($n = 20$) or the Oligofructose ($n = 21$) groups. Ten subjects, five in each study group, could not complete the study protocol (dropout = 24.4%): two patients died and eight left the hospital before the end of the study.

The characteristics on day 1 of the 31 remaining patients are described in Table 1; no differences were observed between the groups for the clinical variables and the permeability markers, except that the nutritional status assessed by global subjective evaluation was better in the Oligofructose group. On day 1, and as shown in Fig. 1, a significant correlation was observed between sucrose and lactulose excretion (Pearson $r = 0.70$, $p < 0.001$). A negative correlation was also observed at this time between the burned surface area and the total serum protein ($r = -0.63$, $p < 0.05$) and the serum albumin levels ($r = -0.52$, $p < 0.05$), but no correlations were observed between the burned surface area and the urinary excretion of sucrose, lactulose, mannitol or the lactulose/mannitol ratio.

Sucrose excretion, both in the Control and Oligofructose groups and along the study was compared with baseline values obtained in the healthy adult subjects (Fig. 2). Burns induced an important increase of sucrose urinary excretion on day 1 (127.7 mg (64.1–252.1 mg) and 94.6 mg (44.7–198.3 mg) for the Oligofructose and Control groups, respectively), which was about five times higher than in the healthy subjects (21.3 mg (14.0–32.5 mg)). This high sucrose excretion decreased rapidly and significantly during the time course of the study (two-way ANOVA, $F = 3.17$, $p = 0.016$), without returning to the baseline levels observed in the healthy subjects. However, no statistical differences between the Control and Oligofructose groups were observed in the pattern of urinary sucrose excretion along time (two-way ANOVA, $F = 1.06$, $p = 0.38$).

Fig. 3 shows the pattern of urinary lactulose/mannitol excretion ratio as a reflection of intestinal permeability on days 1, 3, 7, 14 and 21 after the burn, compared with the ratio in healthy subjects. Similarly to sucrose excretion, a dramatic alteration of intestinal permeability was detected on day 1, as indicated by a lactulose/mannitol ratio 4.4 times higher than in the healthy subjects (0.069% (0.041–0.116%) and 0.080% (0.042–0.153%) for the Oligofructose and Control groups, respectively, versus 0.017% (0.009–0.022%) for the healthy subjects). Intestinal permeability

Table 1
Characteristics on admission of the burn patients in the Control and Oligofructose groups

| | Control ($n = 15$) | Oligofructose ($n = 16$) | $p$ |
|---|---|---|---|
| Sex (M/F) | 10/5 | 12/4 | 0.42 |
| Age (year) | 41.2 ± 13.3 | 38.6 ± 20.4 | 0.35 |
| Burn surface area (%) | 18.0 ± 7.4 | 16.2 ± 9.0 | 0.45 |
| Zawacki index | 2.20 ± 0.54 | 2.07 ± 0.60 | 0.50 |
| Serum total protein (mg/dl) | 6.58 ± 1.28 | 6.56 ± 1.22 | 0.94 |
| Serum albumin (mg/dl) | 3.28 ± 0.96 | 3.50 ± 0.82 | 0.51 |
| Indirect lymphocyte count ($mm^{-3}$) | 2119 ± 1671 | 1829 ± 1137 | 0.87 |
| Urinary sucrose excretion (mg) | 94.6 (44.7–198.3) | 127.7 (64.1–252.1) | 0.9 |
| Urinary lactulose excretion (mg) | 21.5 (12.8–35.9) | 31.5 (20.3–48.9) | 0.31 |
| Urinary mannitol excretion (mg) | 71.5 (40.4–127.7) | 121.5 (90–165.7) | 0.16 |
| Lactulose/mannitol ratio (%) | 0.080 (0.042–0.153) | 0.069 (0.041–0.116) | 0.78 |
| Global subjective evaluation of | | | |
| Adequate nutrition (%) | 46.7 | 87.5 | $\chi^2 = 6.81$; $p < 0.05$ |
| Some levels of malnutrition (%) | 46.7 | 6.25 | |
| Malnutrition (%) | 6.6 | 6.25 | |

*p*-Values were calculated by using the chi-square and Mann–Whitney *U*-test for the qualitative and quantitative variables, respectively. Permeability markers were expressed as geometric means (95% CI) and the others variables as means ± S.D.

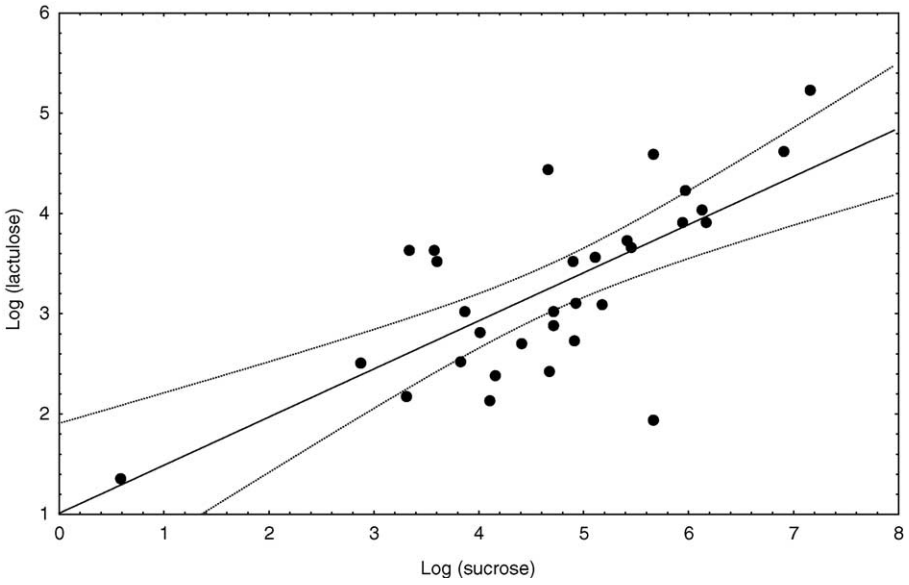


Fig. 1. Correlation between lactulose and sucrose urinary excretion in patients at day 1 after burn. (Pearson $r = 0.70$, $p < 0.001$).

significantly decreased along the study (two-way ANOVA, $F = 10.55$, $p < 0.000001$), so that on day 14, ratios were comparable to those observed in the healthy subjects. No differences in the lactulose/mannitol ratio were observed between the two experimental groups (two-way ANOVA, $F = 0.26$, $p = 0.90$).

The strong increase of the lactulose/mannitol excretion ratio induced by burns on day-1 was due to increased excretion of lactulose and a lower excretion of mannitol (Table 1). Urinary lactulose excretion decreased while mannitol excretion increased subsequently in the study (two-way ANOVA, $F = 3.98$, $p = 0.046$ and $F = 5.94$, $p = 0.00022$, for lactulose and mannitol excretion, respectively) (data not shown), which explains the normalization of the lactulose/mannitol ratio. However no differences between the Oligofructose and Control groups (two-way ANOVA, $F = 1.42$, $p = 0.23$ and $F = 2.19$, $p = 0.07$, respectively) were observed.

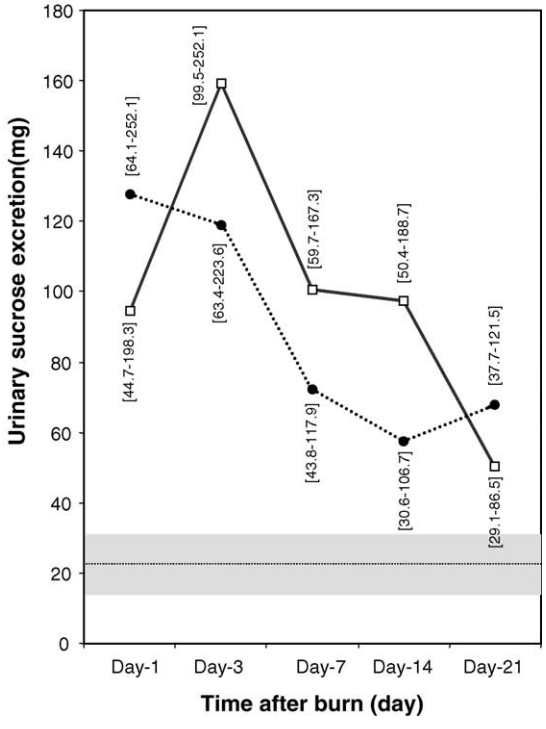


Fig. 2. Changes in urinary sucrose excretion in patients from the Control (□) or Prebiotic (●) group on days 1, 3, 7, 14 and 21 after burn.

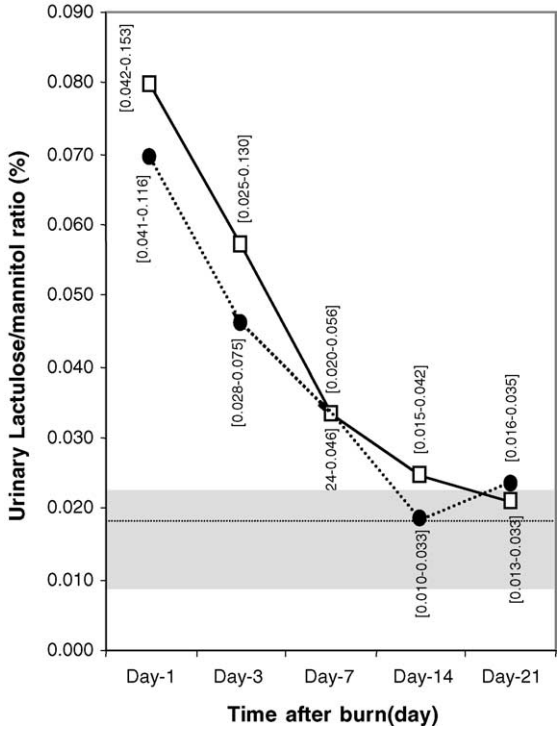


Fig. 3. Changes in urinary lactulose/mannitol ratio in patients from the Control (□) or Prebiotic (●) group on days 1, 3, 7, 14 and 21 after burn.

Table 2
Characteristics of the burn patients in the Control and Oligofructose groups

| | Control ($n = 15$) | Oligofructose ($n = 16$) | $p$ |
|---|---|---|---|
| Time of hospitalization (day) | 35.7 ± 15.8 | 40.3 ± 15.2 | 0.88 |
| Number of surgery/subject | 3.62 ± 1.50 | 3.07 ± 2.02 | 0.37 |
| Burn infections (%) | 93.3 | 87.5 | $\chi^2 = 0.30$; NS |
| Complications (%) | 20.0 | 31.2 | $\chi^2 = 0.51$; NS |
| Enteral nutrition (%) | 60.0 | 43.8 | $\chi^2 = 0.82$; NS |

$p$-Values were calculated by using the chi-square and Students $t$-test for the qualitative and quantitative variables, respectively. Results were expressed as means ± S.D. or as percentage.

As shown in Table 2, no differences were seen between the two experimental groups in relation to burn infection rates, number of complications, need for enteral nutrition, number of surgical interventions and length of hospitalization.

## 4. Discussion

Our results show that intestinal permeability increases four-fold shortly after burn injury, confirming observations by other authors [15,18]. Our results also show, for the first time to our knowledge, that not only intestinal permeability is altered by this injury but also gastric permeability, as reflected by the high urinary sucrose excretion (five-fold the basal levels observed in the healthy control subjects) on day 1 after the burn. It may be argued that this high sucrose excretion is not due to an alteration of the gastric mucosa but to slower gastric emptying in these subjects. Indeed, contrarily to intestinal permeability, which is evaluated through the differential excretion of lactulose and mannitol and is expressed as L/M ratio, gastric permeability is evaluated through the urinary excretion of a single marker, making it more sensitive to interfering variables such as gastric emptying [16,17]. However, studies carried out in an animal model of burn injury [28] and in burn patients [29] indicate that gastric emptying is not altered, supporting the idea that the high sucrose excretion observed in our study is directly related to alterations of the gastric barrier. Furthermore, we also observed a correlation between sucrose and lactulose excretions, suggesting that sucrose passes through the epithelium by using the paracellular pathway like lactulose and that the higher excretion of these markers is due to increased mucosal permeability. On the other hand, whether GI permeability correlates with burn body surface area is a debated question [15,30]; we observed that neither sucrose excretion nor the L/M ratio correlated with burn body surface area on day 1. GI barrier failure occurring after thermal injury probably results from splanchnic vasoconstriction and intestinal ischemia induced by the release of catabolic hormones such as cortisol and glucagon [30], and to the activation of the renin–angiotensin system [31,32]. This phenomenon may be associated with increased apoptosis in the gut epithelium, as it has been recently observed in burn mice [33], or with alterations of the brush border cytoskeleton [34]. Our results also showed that intestinal permeability recovered before gastric permeability; however, it is not clear why normalization is faster in the small intestine compared to the stomach. In another study, intestinal permeability remained altered two weeks following injury [20].

In this study we postulated that prebiotic ingestion may contribute to normalize the GI barrier function in burn patients. This hypothesis was based on observations that burn injury is associated with dramatic alterations of the intestinal microbiota and of GI permeability, and that increasing luminal lactobacilli and bifidobacteria through the ingestion of prebiotics or probiotics is associated with recovery of the GI barrier function. We have observed, for example, that regular intake of *Lactobacillus* GG decreased the gastric permeability alterations induced by indomethacin in healthy volunteers [12]. In relation to burn injury, a decrease of the intestinal anaerobic microbiota, including bifidobacteria, has been observed in rats, while at the same time aerobic bacteria and fungi increase. This resulted in an imbalance of the aerobic/anaerobic ratio and in a decrease of colonization resistance in these animals [36]. These changes were associated with increased bacterial translocation and endotoxinemia, histogical lesions of the mucosa and decreased amounts of intestinal sIgA and mucins. Similar alterations have been observed in burn patients [37]. Supplementation of burn rats with a bifidobacteria preparation reduced the imbalance of the aerobic/aerobic ratio, the endotoxinemia and the mucosal lesions [36]; the same preparation with bifidobacteria decreased gastrointestinal symptomatology and diarrhea in humans who suffered burns [35].

Stimulation of endogenous lactobacilli or bifidobacteria by prebiotics may also exert a protective effect against GI mucosa alterations. Lactosucrose, for example, has been shown to protect against indomethacin-induced gastric ulcerations in rats [38]; soy fiber helps maintain bowel mucosal integrity and prevents bacterial translocation in burn rats receiving enteral feeding [39], while other types of dietary fiber protect intestinal structure and function in 5-fluorouracil treated rats [40]. Although a number of studies have been carried out in animal models, data are scarce in humans. In our study, we used oligofructose, a short chain fructo-oligosaccharide whose administration is known to dose-dependently increase fecal bifidobacteria in humans [41]. However, this prebiotic did not improve GI barrier

function alterations in our patients. Neither did clinical variables such as duration of hospitalization, wound infection rate or complications improve during administration of the prebiotic. A possible explanation for this lack of effect is the use of high doses of antibiotics in all these patients, which may interfere with lactobacilli and bifidobacteria growth even after stimulation by oligofructose. In the case of probiotics, this may be overcome by the continuous administration of these exogenous, live bacteria which may compensate for the mortality induced by antibiotics; prebiotics, however, act by stimulating the growth of endogenous bacteria, and this is probably decreased when these microorganisms are affected by antibiotics. The studies cited above on animal models do not use antibiotics and this may be one reason for the prebiotic effect observed. Our results may be interpreted as suggesting that prebiotics probably are not the best option for subjects on high doses of antibiotics, and that administration of probiotics or synbiotics, a mixture of pre and probiotics, may be a better choice for these patients.

In conclusion, intestinal and gastric permeability are dramatically increased shortly after burn injury. Intestinal permeability returns to its basal levels 2 weeks after injury, while gastric permeability decreases more slowly. Regular intake of oligofructose does not induce improvements of the alterations of the gastrointestinal barrier function in these patients. Although these results are negative with respect to potential effects of prebiotics in burn patients with moderate burn surface area, more studies are necessary to evaluate their use, alone or associated with probiotics (as symbiotics) in critically ill subjects. Indeed, these almost always receive high doses of drugs, including antibiotics, and reduced supplies of food, and their gastrointestinal functions and microbiota [42] require particular care and attention. Interestingly, fiber-containing solutions for enteral nutrition are beginning to be commercially available and their early use in patients with major abdominal surgery has been shown to reduce the rates of postoperative infections in comparison with parenteral nutrition and fiber-free enteral formula [43].

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