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ARTICLE

Effect of synbiotic supplementation in children and adolescents with cystic fibrosis: a randomized controlled clinical trial

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Abstract

Background/objectives Cystic fibrosis (CF) is characterized by excessive activation of immune processes. The aim of this study was to evaluate the effect of synbiotic supplementation on the inflammatory response in children/adolescents with CF.

Subjects/methods A randomized, placebo-controlled, double-blind, clinical-trial was conducted with control group (CG, $n = 17$), placebo-CF-group (PCFG, $n = 19$), synbiotic CF-group (SCFG, $n = 22$), PCFG negative ($n = 8$) and positive ($n = 11$) bacteriology, and SCFG negative ($n = 12$) and positive ($n = 10$) bacteriology. Markers of lung function (FEV_1), nutritional status [body mass index-for age (BMI/A), height-for-age (H/A), weight-for-age (W/A), upper-arm fat area (UFA), upper-arm muscle area (UMA), body fat (%BF)], and inflammation [interleukin (IL)-12, tumor necrosis factor-alpha (TNF-α), IL-10, IL-6, IL-1β, IL-8, myeloperoxidase (MPO), nitric oxide metabolites (NOx)] were evaluated before and after 90-day of supplementation with a synbiotic.

Results No significance difference was found between the baseline and end evaluations of FEV_1 and nutricional status markers. A significant interaction (time vs. group) was found for IL-12 ($p = 0.010$) and myeloperoxidase ($p = 0.036$) between PCFG and SCFG, however, the difference was not maintained after assessing the groups individually. NOx diminished significantly after supplementation in the SCFG ($p = 0.030$). In the SCFG with positive bacteriology, reductions were found in IL-6 ($p = 0.033$) and IL-8 ($p = 0.009$) after supplementation.

Conclusions Synbiotic supplementation shown promise at diminishing the pro-inflammatory markers IL-6, IL-8 in the SCFG with positive bacteriology and NOx in the SCFG in children/adolescents with CF.

Introduction

A massive continuous influx of neutrophils occurs during inflammation of the airways in cystic fibrosis (CF) [1], as a consequence there is the release of chemokines [2].

When an imbalance occurs in the intestinal microbiota, can lead to the pro-inflammatory cytokines release. This, in turn, leads to the secretion of chloride/water ion by the intestinal epithelium, which can cause inflammation, tissue damage, and diarrhea [3].

The prebiotic fructooligosaccharides (FOS) has been associated with the improvement in symptoms and the composition of intestinal microbiota [4] and, in irritable bowel syndrome, FOS regulates the immune system [5]. Probiotic bacteria, such as *Lactobacillus (L.) paracasei*, *L. rhamnosus*, and *L. acidophilus*, have been associated with antimicrobial activity [6], reduction in tissue damage caused by tumor necrosis factor-alpha (TNF-α) [7], reduction in the

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production of interleukin (IL)-8 [8], the induction in the production of IL-10[8] and immunomodulation of dendritic cells [8]. *Bifidobacterium (B) lactis* has been associated with an improvement in the function of the intestinal barrier and the attenuation of inflammation [9].

Prebiotics and probiotics can contribute to the treatment of CF once the patients are exposed to broad-spectrum antibiotics such as trobamycin and colistin [10] and, have increased intestinal permeability and abnormal microbiota [11].

The aim of this study was to evaluate the effect of synbiotic supplementation (FOS, *L. paracasei*, *L. rhamnosus*, *L. acidophilus*, and *B. lactis*) on markers of the systemic inflammatory response in children and adolescents with CF.

Subjects and methods

Study design

A randomized, placebo-controlled, double-blind trial was conducted between October/2014–June/2016 at the Joana de Gusmão Children's Hospital, Florianópolis/SC/Brazil. Approval by the Human Research Ethics Committees at the Federal University of Santa Catarina (#48959715.2.1001.0121), and is registered—Brazilian Registry of Clinical Trials (RBR-5BYRSC). The statement of informed consent was sign for the legal guardians of the children and adolescents.

Subjects

The non-probabilistic, convenience sample was composed of 72 children and adolescents allocated to a control group (CG, $n = 17$, mean age: 8.55 ± 3.03 years), placebo CF group (PCFG, $n = 19$, mean age: 10.74 ± 2.54 years) and synbiotic CF group (SCFG, $n = 22$, mean age: 9.59 ± 2.79 years). The patients with CF were also divided into a negative bacteriology group (absence of pathogenic microorganisms for CF) and positive bacteriology group [presence of one or more pathogenic microorganisms: *Pseudomonas (P) aeruginosa, Staphylococcus (S) aureus*, and *Burkholderia (B) cepacia*] according to the supplement ingested. The inclusion criteria for the CG were ≥ 1– ≤ 16 years of age and an absence of CF. The individuals in the PCFG and SCFG needed to have a diagnosis of CF (chloride sweat test ≥60 mmol/L) and be clinically stable for at least 30 days prior to the data collection process [12]. The exclusion criteria for all groups were described previously [12]. The individuals in the PCFG and SCFG were evaluated at baseline and after 90-day period, allocated to the different groups through a block randomization process and stratified by sex and age (Research-Randomizer-Form v4.0-Lancaster/Pennsylvania/USA).

Assessment of dietary intake

The dietary intake was determined using a validated semi-quantitative food frequency questionnaire according to the age group [13, 14] and analyzed using the food composition table (USDA/Washington/DC/USA) [15]. Daily supplementation and enzyme as part of the protocol [16, 17] was taken into account and the supplement intake was determined on the basis of patient reports.

Dietary supplementation

Synbiotic supplementation consisted of a combination of FOS (5.5 g/day) and four probiotic strains (10^8–10^9 CFU/day each strain): *L. paracasei*, *L. rhamnosus*, *L. acidophilus*, and *B. lactis* (Lactofos®-SKL-Functional-Nutrition-São Paulo/SP/Brazil). Maltodextrin (Maison-de-La-Santé®-Florianópolis/SC/Brazil) was the placebo. The synbiotic and placebo were offered in six-gram packets in the form of powder. The habitual diet was maintained, but with restrictions regarding the consumption of foods containing prebiotics and probiotics in the 90-day period. Tolerance to the synbiotic was assessed based on the occurrence of gastrointestinal symptoms (nausea, vomiting, excessive flatulence, abdominal distension, intestinal gurgling, and abdominal pain) and fecal consistency.

Assessment of clinical and bacteriological, lung function, and disease severity

Clinical evaluation was obtained through the medical records of patients during the study. The information collected was about steatorrhea and diarrhea presence. A bacteriological determination and the lung function [assessed by Forced Expiratory Volume in 1 s (FEV_1)] were described previously [12]. Disease severity was determined based on the Shwachman-Kulczycki (S-K score) [12].

Assessment of nutritional status

Anthropometric data were collected following the criteria of the World Health Organization (WHO) [18]. Weight was determined with a digital scale (BK50F-Balmak®-Santa-Bárbara-d'Oeste/SP/Brazil) and height was measured using a stadiometer (Alturaexata®-Belo-Horizonte/MG/Brazil). Nutritional status was classified based on the WHO [18] criteria using z-scores for height-for-age (z-H/A), weight-for-age (z-W/A) and body mass index-for-age (z-BMI/A). Triceps, biceps, sub-scapular, and supra-iliac skin folds thickness (TSF, BSF, SSSF, and SISF, respectively) were measured using the Lange skinfold caliper®-Beta Technology Incorporated (Santa-Cruz/California/USA) [18]. The percentage of body fat (%BF) was determined based on the

Table 1 Demographic, clinical, nutritional, and inflammatory markers of the control group (CG), cystic fibrosis group (CFG): CFG placebo and CFG supplemented with symbiotic

Variables	CG	CFG ($n = 41$)		*p*-value
	($n = 17$)	Placebo CFG ($n = 19$)	Synbiotic CFG ($n = 22$)	
Demographic/clinical				
Age (years)[a,b]	8.55 ± 3.03	10.74 ± 2.54	9.59 ± 2.79	0.071
Sex (*n*,% Male)	6 (35.3)	10 (52.6)	12 (54.5)	—
S-K score (points)[a,c]	NA	81.88 ± 16.02	82.78 ± 8.70	0.885
FEV_1 (%)[d,e]	NA	74.44 (51.20–97.01)	88.86 (58.55–97.00)	0.855
Energy intake[d,e]				
Energy (kcal)	NA	2727.09 (2336.47–3476.16)	2586.46 (1844.21–3094.71)	0.214
Nutritional status[b]				
z-BMI/A[a]	0.22 ± 1.36[f]	−1.21 ± 1.12[g]	−0.58 ± 1.22[f,g]	< **0.001**
z-H/A[a]	−0.32 ± 1.14[f]	−1.03 ± 1.23[g]	−0.78 ± 1.34[g,h]	**0.005**
z-W/A[a]	−0.03 ± 1.24[f]	−1.89 ± 0.92[g]	−0.67 ± 1.09[f,h]	**0.003**
z-UFA[a]	−0.33 ± 1.12[f]	−0.73 ± 0.89[g]	−0.51 ± 1.02[g,h]	**0.007**
z-UMA (z-score)[a]	−0.32 ± 1.63[f]	−1.69 ± 1.14[g]	−1.25 ± 0.87[f,g]	**0.004**
BF (%)[a]	23.50 ± 7.05[f]	14.26 ± 4.88[g]	15.98 ± 5.87[g,h]	< **0.001**
Inflammatory markers				
NOx (μmol/L)[a,b]	34.16 ± 8.84	39.37 ± 17.26	43.64 ± 17.38	0.178
hs-CRP (mg/L)[d,i]	0.27 (0.18–0.39)[f]	2.15 (1.80–24.70)[g]	1.30 (1.00–3.55)[g]	< **0.001**
TNF-α (pg/mL)[d,i]	0.53 (0.47–0.73)[f]	0.47 (0.29–1.37)[g]	0.41 (0.00–1.16)[g]	0.505
Lymphocytes (cells/$mm^3 \times 10^3$)[a,b]	2.934 ± 1.646	3.041 ± 0.955	3.267 ± 0.981	0.708
Monocytes (cells/mm^3)[a,b]	381.00 (297.50–475.25)[f]	724.00 (553.00–1175.00)[g]	733.00 (576.50–855.00)[g]	< **0.001**

S-K score Shwachman-Kulczycki score, *NA* not applicable, *FEV_1* forced expiratory volume in 1 s, *Nutritional status in z-escore:BMI/A* body mass index-for age, *H/A* height-for-age, *W/A* weight-for-age, *UFA* upper-arm fat area, *UMA* upper-arm muscle area, and *%BF* body fat percentage, *NOx* nitric oxide metabolites, *hs-CRP* high-sensitivity C-reactive protein, *TNF-α* tumor necrosis factor-alpha

[a] Values expressed as mean ± standard deviation

[b] ANOVA test and post-hoc Bonferroni test

[c] *t*-student test

[d] Values expressed as median (interquartile range 25°–75° percentile)

[e] Mann–Whitney test

[i] Kruskal–Wallis test and post-hoc Mann–Whitney test ($p < 0.05$)

[f,g,h] Same letter has no difference

Bold text indicates a statistically significance with a p-value less than 0.05

sum of the four skinfold thickness measures (BSF, TSF, SSSF, and SISF) adjusted for sex and age group. Mid-up arm circumference (AC) was measured with a non-elastic tape for the calculation of upper-arm muscle area (UMA). The method proposed by Frisancho [19] was used for the determination of upper-arm fat area (UFA) and UMA; these measures were also expressed in z-scores (Siscres-Growth-and-Development-Program-Campinas/SP/Brazil).

Analysis of inflammatory markers

Serum concentrations of IL-12, TNF-α, IL-10, IL-6, and IL-8 were determined using flow cytometry using commercial Cytometric Bead Array inflammatory cytokine kit in a FACS Canto II flow cytometer both from BD-Biosciences®-San Diego/CA/USA, with the aid of the Cell Quest-Pro software program. Standard curves for each cytokine were plotted and the concentrations of each test sample were calculated using the FCAP array program v.1.0.2 (BD-Biosciences®-Osasco/SP/Brazil). MPO activity was evaluated using the colorimetric method ELISA (Organon-Tecknica®-Roseland/New-Jersey/USA) [20] and nitric oxide metabolites (NOx) was determined using the Griess reaction [21]. The concentration of high-sensitivity C-reactive protein was determined based on nephelometry using the CardioPhase®-hs-CRP reagent and BN® II equipment (Siemens-Healthcare-Diagnostics- Tarrytown/New-York/USA). The white blood cell count was analyzed using a semi-automated method with the aid of the Heco 5

Table 2 Lung function, nutritional status, and inflammatory markers in patients with cystic fibrosis (CF) supplemented with placebo (CF) group or synbiotic (CF) group

Variables	Placebo CF group (PCFG) ($n = 19$)			Synbiotic CF group (SCFG) ($n = 22$)			
	Before	After	p-value	Before	After	p-value	Interaction time vs. group[a] (p-value)
Lung function[b]							
FEV_1 (%)	73.58 ± 22.28	77.39 ± 21.76	0.427	80.25 ± 23.87	75.99 ± 20.78	0.306	0.696
Nutritional status[b]							
z-BMI/A[b]	−1.21 ± 1.12	−1.20 ± 1.29	0.943	−0.58 ± 1.22	−0.68 ± 1.13	0.120	0.589
z-H/A[b]	−1.03 ± 1.23	−0.87 ± 1.43	0.449	−0.78 ± 1.34	−0.66 ± 1.24	0.497	0.380
z-W/A[b]	−1.98 ± 1.29	−1.79 ± 1.31	0.515	−0.67 ± 1.10	−0.88 ± 1.22	0.276	0.443
z-UFA[b]	−0.73 ± 0.89	−0.74 ± 0.99	0.980	−0.51 ± 1.02	−0.59 ± 0.90	0.374	0.544
z-UMA[b]	−1.70 ± 1.11	−1.34 ± 1.12	0.073	−1.25 ± 0.86	−1.21 ± 1.28	0.813	0.425
BF (%)[b]	14.41 ± 4.79	14.78 ± 4.18	0.624	15.98 ± 5.87	16.05 ± 4.97	0.419	0.644
Inflammatory markers[b,c]							
IL-12 (pg/mL)	0.68 ± 0.83	−0.04 ± 0.45	0.842	0.32 ± 0.54	0.49 ± 0.90	0.250	**0.010**
TNF-α (pg/mL)	−0.03 ± 0.70	−0.22 ± 0.91	0.916	−0.02 ± 0.78	−0.40 ± 1.33	0.125	0.759
IL-10 (pg/mL)	0.32 ± 0.50	0.18 ± 0.50	0.495	0.35 ± 0.56	0.13 ± 0.74	0.056	0.561
IL-6 (pg/mL)	1.04 ± 0.55	0.77 ± 0.77	0.158	0.71 ± 0.49	0.48 ± 0.45	0.074	0.540
IL-1β (pg/mL)	0.43 ± 0.65	−0.28 ± 1.02	0.446	0.23 ± 0.59	0.02 ± 0.84	0.321	0.486
IL-8 (pg/mL)	1.57 ± 0.38	1.46 ± 0.52	0.187	1.54 ± 0.25	1.41 ± 0.37	0.053	0.583
MPO (mU/mL)	6.65 ± 0.29	6.47 ± 0.40	0.321	6.56 ± 0.19	6.52 ± 0.17	0.532	**0.036**
NOx (μmol/L)	3.68 ± 0.40	3.36 ± 0.67	0.319	3.71 ± 0.38	3.44 ± 0.38	**0.030**	0.400

Values expressed as mean ± standard deviation

FEV_1 forced expiratory volume in 1 s, *Nutritional status in z-score:BMI/A* body mass index-for age, *H/A* height-for-age, *W/A* weight-for-age, *UFA* upper-arm fat area, *UMA* upper-arm muscle area, and *%BF* body fat percentage, *IL-12* interleukin 12, *TNF-α* tumor necrosis factor-alpha, *IL-10* interleukin-10, *IL-6* interleukin 6, *IL-1β* interleukin-1beta, *IL-8* interleukin 8, *MPO* myeloperoxidase, *NOx* nitric oxide metabolites

[a]Repeated-measures generalized linear model with adjustment for age, sex and energy intake with evaluation of the interaction between type of supplement (placebo or synbiotic) and supplementation time (After–Before) ($p < 0.05$)

[b]Repeated-measures generalized linear model with adjustment for age and sex

[c]Values were normalized by $\log_{10}$ transformation

Bold text indicates a statistically significance with a p-value less than 0.05

Plus equipment (Radim-Company®-Pomezia/Rome/Italy) [22].

Statistical analysis

The Shapiro–Wilk test was used to determine the normality of the data. Differences among the CG, PCFG and SCFG were analyzed using either analysis of variance or the Kruskal–Wallis test followed by Bonferroni and Mann–Whitney post-hoc tests, respectively. Data with non-Gaussian distribution were submitted to logarithmic normalization. Sphericity and homogeneity of the data were evaluated using Mauchly's test and Levene's test, respectively. Changes in markers of lung function, nutritional status, and inflammation following supplementation (placebo or synbiotic) and over time (final-baseline) were analyzed using a repeated-measures generalized linear model to determine the possible effect of an interaction between the intra-subject and inter-subjects factor on the dependent variable. The analyses were adjusted for sex, age, and energy intake. Due to missing data, intention-to-treat analysis included a principal analysis using multiple imputations. Five sets of imputed data were created using the following prediction variables: sex, age, FEV_1, H/A, BMI/I, BF, UMA, UFA, MPO, NOx, IL-12, TNF-α, IL-10, IL-6, IL-1β, and IL-8. The effect size was calculated using Cohen's d (d) adjusted for use in repeated measurements and for the removal of bias from small samples in the estimates of standardized effects [23]. Effect sizes were assumed as trivial (< 0.20), small (between 0.20 and 0.49), medium (between 0.50 and 0.79) or large (> 0.80) [23]. Cohen's d effect size was calculated using an effect size calculator (http://www.sportsci.org/index.html). Statistical analysis: SPSS v16.0 for Windows™ and p-value < 0.05 was considered indicative of statistical significance.

Table 3 Lung function, nutritional status, and inflammatory markers in patients with cystic fibrosis supplemented with placebo (CFG supplemented with placebo) according to the absence or presence of pathogenic microorganisms

Variables	Placebo CFG negative bacteriology ($n = 8$)			Placebo CFG positive bacteriology ($n = 11$)		
	Before	After	*p*-value	Before	After	*p*-value
Lung function[a]						
FEV_1 (%)	87.78 ± 36.89	90.00 ± 27.73	0.709	77.96 ± 23.83	68.75 ± 29.75	0.296
Nutritional status[a]						
z-BMI/A[a]	−1.36 ± 0.97	−1.02 ± 0.93	0.565	−1.05 ± 1.58	−1.26 ± 1.55	0.425
z-H/A[a]	−1.32 ± 1.42	−1.27 ± 1.41	0.132	−0.51 ± 1.28	−0.44 ± 1.31	0.416
z-W/A[a]	−2.20 ± 1.30	−1.82 ± 1.19	0.073	−1.97 ± 1.23	−1.74 ± 1.44	0.643
z-UFA[a]	−0.87 ± 0.86	−0.81 ± 0.88	0.852	−0.28 ± 0.86	−0.61 ± 0.82	0.071
z-UMA[a]	−1.79 ± 1.22	−1.47 ± 1.16	0.278	−1.36 ± 1.26	−1.29 ± 1.17	0.843
BF (%)[a]	13.54 ± 4.63	14.42 ± 4.35	0.995	15.86 ± 5.25	15.36 ± 4.88	0.616
Inflammatory markers[a,b]						
IL-12 (pg/mL)	0.28 ± 0.78	0.14 ± 0.33	0.781	0.82 ± 1.05	0.01 ± 0.38	0.765
TNF-α (pg/mL)	−0.84 ± 0.78	−0.38 ± 0.39	0.466	0.89 ± 0.04	0.01 ± 0.93	0.677
IL-10 (pg/mL)	0.31 ± 0.26	0.12 ± 0.33	0.892	0.36 ± 0.55	0.25 ± 0.48	0.828
IL-6 (pg/mL)	1.04 ± 0.74	0.62 ± 0.65	0.133	1.05 ± 0.33	0.91 ± 0.74	0.357
IL-1β (pg/mL)	0.06 ± 0.65	−0.38 ± 0.86	0.940	0.92 ± 0.04	−0.13 ± 1.40	0.904
IL-8 (pg/mL)	1.57 ± 0.43	1.55 ± 0.50	0.999	1.57 ± 0.26	1.38 ± 0.39	0.188
MPO (mU/mL)	6.62 ± 0.27	6.49 ± 0.27	0.466	6.69 ± 0.23	6.45 ± 0.29	0.730
NOx (μmol/L)	3.64 ± 0.41	3.40 ± 0.29	0.279	3.71 ± 0.42	3.32 ± 0.79	0.462

Values expressed as mean ± standard deviation

FEV_1 forced expiratory volume in 1 s, *Nutritional status in z-score*: *BMI/A* body mass index-for age, *H/A* height-for-age, *W/A* weight-for-age, *UFA* upper-arm fat area, *UMA* upper-arm muscle area, and *%BF* body fat percentage, *IL-12* interleukin 12, *TNF-α* tumor necrosis factor-alpha, *IL-10* interleukin-10, *IL-6* interleukin 6, *IL-1β* interleukin-1beta, *IL-8* interleukin 8, *MPO* myeloperoxidase, *NOx* nitric oxide metabolites

[a]Repeated-measures generalized linear model with adjustment for age, sex, and energy intake. Significance of $p < 0.05$

[b]Values were normalized by $\log_{10}$ transformation

Results

Characterization of sample

During the study all participants were clinically stable, had no steatorrhea and/or maldigestion, and were using enzymes according to the recommendations of the Clinical Practice Guidelines [17]. Regarding pathogenic bacteria, 57.9% of the patients in the PCFG and 45.5% in the SCFG were colonized by one or more bacteria. The most prevalent were *S. aureus* (47.7%) and *B. cepacea* (21.1%) in the PCFG as well as *S. aureus* (18.2%), and *P. aeruginosa* (22.7%) in the SCFG.

The male sex predominated in the PCFG and SCFG and the female sex predominated in the CG (64.7%). Both groups of patients had high mean S-K scores (PCFG: 81.88 ± 16.02%, SCFG: 82.78 ± 8.70%). General physical status was classified as either excellent or good in 75% of the PCFG and 88.9% of the SCFG and there were no classifications of severe CF. PCFG and SCFG had mean FEV_1 values ≥ 70% in the classification of pulmonary obstruction ($p = 0.885$) (Table 1).

The energy consumption between PCFG and SCFG were similar, with no significant difference ($p = 0.214$) (Table 1).

Regarding the markers of nutritional status, z-BMI/A, z-H/A, z-W/A, z-UFA, z-UMA, and %BF differed significantly between the CG and patient groups ($p < 0.05$), with no significant difference between the PCFG and SCFG. The patient groups differed significantly from one another only with regard to z-W/A (Table 1).

Significant differences in the inflammatory response markers CRP and monocytes were found when the PCFG and SCFG when compared to CG ($p < 0.001$) (Table 1).

Effect of synbiotic on markers of lung function and nutritional status

No significant difference and interactions were found between group (PCFG and SCFG) and time (final-baseline) of FEV_1 (Table 2). Likewise, when the groups were subdivided into negative and positive bacteriology (Tables 3 and 4).

Regarding nutritional status, increases were found in % BF (SCFG: 15.98 ± 5.87% vs. 16.05 ± 4.97%; SCFG with

Table 4 Lung function, nutritional status, and inflammatory markers in patients with cystic fibrosis supplemented with synbiotic (CFG supplemented with synbiotic) according to the absence or presence of pathogenic microorganisms

Variáveis	Synbiotic CFG negative bacteriology ($n = 12$)			Synbiotic CFG positive bacteriology ($n = 10$)		
	Before	After	*p*-value	Before	After	*p*-value
Lung function[a]						
FEV_1 (%)	83.36 ± 16.70	77.18 ± 19.93	0.618	75.60 ± 33.25	74.21 ± 23.83	0.303
Nutritional status[a]						
z-BMI/A[a]	−0.19 ± 1.25	−0.49 ± 1.15	0.102	−0.85 ± 1.27	−0.76 ± 1.25	0.427
z-H/A[a]	−1.02 ± 1.24	−0.91 ± 1.18	0.316	−0.44 ± 1.36	−0.49 ± 1.31	0.189
z-W/A[a]	−0.95 ± 1.04	−1.09 ± 1.05	0.237	−0.15 ± 1.11	−0.52 ± 1.47	0.484
z-UFA[a]	−0.28 ± 1.00	−0.48 ± 0.72	0.683	−0.80 ± 0.01	−0.80 ± 0.89	0.616
z-UMA[a]	−1.18 ± 0.77	−1.07 ± 1.49	0.942	−1.33 ± 1.00	−1.37 ± 1.01	0.889
BF (%)[a]	17.09 ± 5.89	16.63 ± 5.43	0.450	14.66 ± 5.87	15.35 ± 4.58	0.729
Inflammatory markers[a,b]						
IL-12 (pg/mL)	0.19 ± 0.14	0.71 ± 1.09	0.192	0.49 ± 0.70	0.22 ± 0.55	0.909
TNF-α (pg/mL)	−0.41 ± 0.34	−0.80 ± 1.05	0.266	−0.19 ± 0.92	−0.22 ± 1.06	0.847
IL-10 (pg/mL)	0.34 ± 0.30	−0.00 ± 0.37	0.085	0.50 ± 0.53	0.37 ± 0.57	0.458
IL-6 (pg/mL)	0.51 ± 0.40	0.52 ± 0.45	0.473	0.91 ± 0.52	0.45 ± 0.49	**0.033**
IL-1β (pg/mL)	0.04 ± 0.17	0.00 ± 0.46	0.985	0.13 ± 0.86	0.79 ± 0.56	0.200
IL-8 (pg/mL)	1.59 ± 0.28	1.50 ± 0.40	0.925	1.47 ± 0.23	1.35 ± 0.18	**0.009**
MPO (mU/mL)	6.50 ± 0.17	6.54 ± 0.12	0.756	6.56 ± 0.18	6.52 ± 0.18	0.314
NOx (µmol/L)	3.74 ± 0.38	3.47 ± 0.53	0.070	3.86 ± 0.46	3.54 ± 0.42	0.542

Values expressed as mean ± standard deviation

FEV_1 forced expiratory volume in 1 s, *Nutritional status in z-score*: *BMI/A* body mass index-for age, *H/A* height-for age, *W/A* weight-for age, *UFA* upper-arm fat area, *UMA* upper-arm muscle area, and *%BF* body fat percentage, *IL-12* interleukin 12, *TNF-α* tumor necrosis factor-alpha, *IL-10* interleukin-10, *IL-6* interleukin 6, *IL-1β* interleukin-1beta, *IL-8* interleukin 8, *MPO* myeloperoxidase, *NOx* nitric oxide metabolites

[a]Repeated-measures generalized linear model with adjustment for age, sex and energy intake. Significance of $p < 0.05$

[b]Values were normalized by $\log_{10}$ transformation

Bold text indicates a statistically significance with a p-value less than 0.05

positive bacteriology: 14.66 ± 5.87% vs. 15.35 ± 4.58%), UMA (SCFG: −1.25 ± 0.86 z-score vs. −1.21 ± 1.28 z-score; SCFG with negative bacteriology: −1.18 ± 0.77 z-score vs. −1.07 ± 1.49 z-score), and z-BMI/A (SCFG with positive bacteriology: −0.85 ± 1.27 z-score vs. −0.76 ± 1.25 z-score), but the difference did not achieve statistical significance (Table 4).

Effect of synbiotic on inflammatory markers

In the evaluation of supplement and time, a statistically significant interaction was found with regard to inflammatory markers IL-12 ($p = 0.010$) and MPO ($p = 0.036$). When analyzing the effect of each supplement individually no significant differences and an important effect size were found. The NOx diminished significantly after supplementation with the synbiotic in the SCFG ($p = 0.030$, $d = 0.79$) (Table 2).

The pro-inflammatory cytokines IL-6 ($d = 0.42$), IL-1β ($d = 0.39$), and IL-8 ($d = 0.35$) diminished following supplementation in the SCFG, but the differences did not achieve statistical significance (Table 2). In the individual analysis of the effect of the synbiotic in the SCFG with positive bacteriology, significant decrease were found for IL-6 (0.91 ± 0.52 pg/mL vs. 0.45 ± 0.49 pg/mL, $d = 0.72$) and IL-8 (1.47 ± 0.23 pg/mL vs. 1.35 ± 0.18 pg/mL, $d = 0.58$) (Table 4).

In the SCFG negative and positive bacteriology, the NOx decrease were not significant, however, it had a medium effect size ($p = 0.070$, $d = 0.74$; $p = 0.542$, $d = 0.75$, respectively) (Table 4).

Discussion

The results regarding the characterization of the sample corroborate previous studies that evaluated pulmonary function, nutritional status, and inflammatory markers of CF patients in relation to non-CF controls. In general, this population presents a compromise of the pulmonary function and consequently of the nutritional state, already having been shown a relation between both factors [24–27].

Regarding the inflammatory process, in the present study, patients with CF also showed an increase in the concentration of pro-inflammatory markers [12].

Studies addressing the use of synbiotic or probiotic supplementation in CF are scarce, offer conflicting results and employ different methodological designs, with differences in the age of the participants, markers evaluated and analysis techniques. However, the use of these supplements in the prevention or treatment of pulmonary exacerbation and lowered the frequency of hospital admissions of CF has been proposed [28]. In the present study, no significant difference between the PCFG and SCFG with positive or negative bacteriology was found on lung function measured by FEV_1. Likewise in a study involving 61 adolescents and adults with CF (median age: 17.5 years; interquartile range: 6 to 29 years) given a supplement with the probiotic *L. reuteri* (10^{10} CFU) for 6 months. Although, the authors found a significant reduction in the number of lung exacerbations and upper airway respiratory infections among patients with mild to moderate lung disease [29]. Even as in a study involving 10 adults with CF (mean age: 26.2 ± 12.9 years) given a supplement of probiotics (*L. acidophilus*, *L. bulgaricus*, *B. bifidum*, *Streptococcus thermophilus*) for 6 months, the authors also found a significant reduction in the number lung exacerbations (1.3 ± 1.0 to 0.6 ± 0.7, $p = 0.002$) [11]. In contrast, in a study involving 38 adolescents with CF (mean age: 13.2 ± 4.2 years) given a supplement with *L.* GG for 6 months, a significant increase in FEV_1 was found ($3.6 \pm 5.2\%$ vs. $0.9 \pm 5\%$, $p = 0.020$). The authors hypothesizes this improvement in FEV_1 as the consequence of the increase in weight and reduction in the number of lung exacerbations found during the study [10].

The synbiotic and/or duration of supplementation were not sufficient to affect the nutritional status of the participants in the present study independently of positive or negative bacteriology. A similar finding is reported in a previous study analyzing the effect of supplementation with probiotics (*L. acidophilus*, *L. bulgaricus*, *B. bifidum*, and *S. thermophilus*) for 6 months on BMI [11]. However, in another study evaluating supplementation with the probiotic *L.* GG for 6 months, a significant increase in weight was found in patients with CF in comparison to those who only received an oral hydration solution (1.5 ± 1.8 kg and 0.7 ± 1.8 kg, respectively, $p = 0.020$) and the authors suggest that the increase in weight may have been associated with the reduction in the number of lung exacerbations [10].

In the present study, there was no improvement in nutritional status and lung function, which may be related to shorter supplementation time, considering that some of the strains used, as well as the amount of them, resemble previous studies [10, 11] that found a significant improvement.

Regarding the inflammatory markers IL-12 and MPO in the SCFG, the significant interaction found that when assessed individually was not maintained, may be due to biological changes not related to supplementation that occurred during the study with the participants, because at the baseline moment no significant differences were found between the placebo and synbiotic groups.

The synbiotic evaluated in this study led to a significant reduction in serum levels of NOx ($p < 0.030$, $d = 0{,}79$) in the SCFG. As well the synbiotic had a medium effect on NOx in the SCFG negative and positive bacteriology, that it was "likely to be perceived" according to Cohen's statistics [23]. Measure NO is difficult due to short half-life, however, measure the serum levels of NOx is accepted, because it has a high correlation with the NO endogenous production [30, 31]. As seen previously NOx levels are high in the circulation system of individuals with CF [12], which may be a consequence of an increase in NO production through induced nitric oxide synthase (iNOS) [32]. Its production is triggered by activated macrophages, TNF-α and IL-1β and, in excess, can cause tissue damage [32].

After supplementation with the synbiotic in the present study, the cytokine IL-1β underwent a reduction, which, although non-significant, may be related to the reduction in NOx levels and consequent attenuation of the inflammatory process.

The reduction, albeit non-significant, in concentrations of the inflammatory markers IL-6 and IL-8 in the SCFG may also be an indication of the effectiveness of the synbiotic at attenuating the pro-inflammatory response. This effect was evident when the PCFG and SCFG were stratified based on bacteriology (negative and positive), as individuals in the SCFG with positive bacteriology sowed a significant reduction in these markers (IL-6: $p = 0.033$, $d = 0.72$; IL-8: $p = 0.009$, $d = 0.58$ for). The authors of a study evaluating the effect of supplementation with the probiotic *L.* GG for 6 months found no significant difference in the level of IL-8 in the sputum of patients with CF [11]. Likewise, the authors of a study evaluating the effect of supplementation with *L. reuteri* found no difference in levels of IL-6 or IL-8 in the feces of patients [33].

The comparison of studies is hampered by the use of different methods for the evaluation of the IL-6 and IL-8. Investigations report an increase in these pro-inflammatory markers in patients with CF, especially in groups with positive bacteriology as well as those with positive results for *P. aeruginosa* in the blood and bronchial epithelial cells [12, 34]. Like TNF-α and IL-1β, the cytokines IL-6 and IL-8, are mediators of the acute phase. In an inflammatory process, IL-1β and TNF-α can induce the synthesis of these cytokines by macrophages and epithelial cells [35]. The synthesis of IL-8 by epithelial cells of the airways in an endogenous manner or in response to pathogens through the

activation of nuclear factor kappa B plays a central role in the physiopathology of inflammation in CF [1].

The effects of the synbiotic on the biomarkers assessed in this study may have been limited due to the time of supplementation as well as due to the evaluation methods used. The serum evaluation of inflammatory markers, unlike some studies evaluated in the gastrointestinal tract, were performed with the objective of evaluating synbiotic effects in a systemic way. Although the alterations found are small, the long-term effect that the synbiotic can have should be considered. Another limitation of this study, we did not evaluate the inflammatory markers in the gastrointestinal tract. As strengths we have the study design, sample size considering the difficulty of adherence to supplementation in this population and the precision of the methods used.

In conclusion, strategies are needed to attenuate inflammatory process in order to diminish the consequent lung damage and negative influence on the nutritional status of patients. Supplementation with the synbiotic tested in the present investigation shown promise at diminishing the pro-inflammatory markers IL-6, IL-8, and NOx in children and adolescents with cystic fibrosis. However, further studies with a longer evaluation period are needed to gain a better understanding of the effects of synbiotic dietary supplements in this population.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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