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Alimentary Tract

Effects of the supplementation with a multispecies probiotic on clinical and laboratory recovery of children with newly diagnosed celiac disease: A randomized, placebo-controlled trial ☆

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ABSTRACT

Objective: To evaluate the efficacy of a multispecies probiotic on clinical and laboratory recovery of children with celiac disease (CeD) at diagnosis.
Methods: Children with newly diagnosed CeD entered a randomized double-blind placebo-controlled trial. A gluten-free diet (GFD) plus a multispecies probiotic or placebo were administered for 12 weeks. Growth, laboratory, and clinical parameters were recorded at enrollment, after 3 and 6 months of follow-up.
Results: Overall, 96 children completed the study: 49 in group A (placebo) and 47 in group B (probiotic). A significant increase of BMI-Z score was found in both groups after 3 and 6 months of treatment ($p < 0.001$), however the increase of BMI-Z score was significantly higher and faster in Group B than in Group A. Other clinical and laboratory parameters improved in both groups after 3 and 6 months ($p<0.001$), but no difference was found between the groups and a comparable time trend was observed in both groups.
Conclusions: Treatment with a multispecies probiotic induced a higher and faster increase of BMI in children with newly diagnosed CeD. The mechanism of this positive effect remains to be elucidated.

1. Introduction

Celiac disease (CeD) is a permanent T-cell-mediated enteropathy, caused by the ingestion of gluten - the major protein fraction in wheat, rye, and barley - in genetically susceptible individuals. From a clinical perspective, CeD is extremely variable and may present with gastrointestinal symptoms or extra-intestinal manifestations or be discovered by screening in asymptomatic individuals. The only available treatment is a life-long exclusion of gluten-containing cereals from the diet, the so-called gluten-free diet (GFD) [1]. A strict GFD leads to a gradual resolution of symptoms, normalization of serology and intestinal lesion, generally within 12–24 months [2]. The timing of recovery is variable from patient to patient and depends on several factors such as presenting symptoms, age of disease onset, and adherence to the GFD [1,2]. The persistence of a minimal intestinal lesion and/or symptoms is not rare, particularly due to poor adherence to the GFD.

The intestinal microbiota may have a role in the recovery process of CeD. Several studies report imbalances in the intestinal microbiota of patients with CeD [3]. Recent longitudinal studies investigating pre-disease changes in stool microbiome in children at risk have identified several species that increase or decrease prior to disease onset [1]. However, a unique microbiome signature of CeD has not been yet identified [1,4]. Basic and transla-

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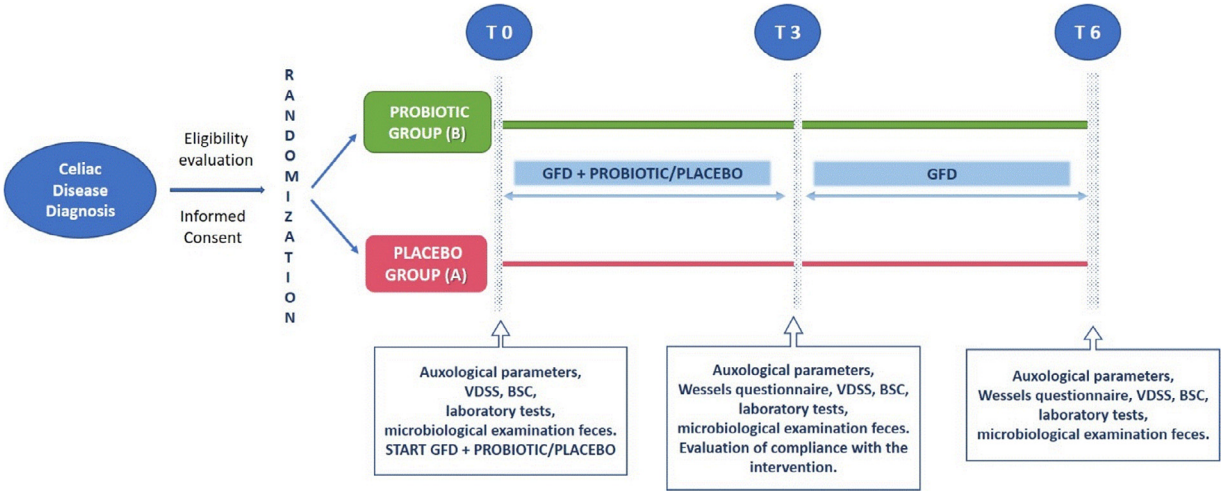

Fig. 1. Study design (GFD: gluten-free diet; VDSS: Validated Disease Specific Symptom Index for Celiac Disease; BSC: Bristol stool chart.).

tional studies have proven a valuable tool to increase mechanistic insight on the role of microbiome in CeD. For instance, bacterial proteases, such as elastase from opportunistic pathogens, increase gluten antigenicity and enhance immune reactivity of gluten specific T cells in CeD. On the other hand, commensal bacteria such as Lactobacillus, aid in the full digestion of gluten peptides, reducing their immunogenicity [1]. Intestinal dysbiosis may persists irrespective of the adherence to the GFD [5]. Modulation of the gut microbiota, by administration of probiotics, is an attractive adjuvant therapy in CeD although their efficacy remains controversial [6]. Some probiotics may attenuate gluten-induced inflammation in patients with CeD [7] while others may digest gluten peptides thereby reducing their toxicity [6]. It has been recently shown that a mixture of five probiotic strains, belonging to the species *Lactobacillus paracasei, Lactobacillus plantarum, Bifidobacterium breve Bbr8 and BL10*, and *Bifidobacterium animalis*, is able to significantly improve the irritable colon-like gastrointestinal symptoms of adult patients with treated CeD [8].

In this double-blind, placebo-controlled randomized trial, we investigated the effect of the previously mentioned five-species probiotic in the clinical and laboratory recovery of children with newly diagnosed CeD. In a sub-group of these patients the stool microbiota was investigated.

2. Methods

2.1. Study design

This was a multicenter, prospective, double-blind, randomized, placebo-controlled study conducted at the Center for Celiac Disease of the Marche Polytechnic University (Ancona, Italy) and the Pediatric Unit of Cava de' Tirreni Hospital (Salerno, Italy).

Between October 2018 and October 2020, all consecutive patients aged between 2 and 16 years, with newly diagnosed CeD were invited to participate, before starting treatment with the GFD. Diagnosis of CeD was performed according to the ESPGHAN 2012 Guidelines [9]. Exclusion criteria were any of the following: (1) presence of autoimmune comorbidity (e.g. type 1 diabetes) or other associated chronic diseases; (2) associated selective IgA selective deficiency; (3) antibiotic or proton-pump inhibitor therapy performed in the previous 7 days.

Fig. 1 shows the study design. At enrollment, patients were randomized to receive a strict GFD plus oral administration of one sachet per day of either placebo (group A) or the study product (group B) for 12 weeks. The active study product consisted of a mixture of 5 strains of lactic acid bacteria and bifidobacteria: *Lactobacillus paracasei* 101/37 LMG P-17504 (5×10^9 CFU/sachet), *Lactobacillus plantarum* 14D CECT 4528 (5×10^9 CFU/sachet), *Bifidobacterium animalis subsp. lactis* Bi1 LMG P-17502 (3.4×10^9 CFU/sachet), *Bifidobacterium breve* Bbr8 LMG P-17501 (3.4×10^9 CFU/sachet), *Bifidobacterium breve* BL10 LMG P-17500 (3.4×10^9 CFU/sachet). The probiotic was given as a sachet once per day. The active study product and the placebo had identical appearance and taste, with the placebo only lacking the viable bacteria. All study products were provided free of charge by Nóos S.r.l. (Rome, Italy), which monitored the stability of the probiotic formulation throughout the study. In both groups, patients were divided into symptomatic and asymptomatic according to the clinical presentation before diagnosis.

The random allocation sequence was generated by two investigators with no clinical involvement in the trial. All investigators, and participants were blinded to the allocation. On the basis of a stratified randomization, children were assigned to one of the two groups.

The study protocol was approved by the institutional review board at each participating center. Written informed consent was obtained from the parents or guardians of the children. ClinicalTrials.gov number: NCT03857360.

2.2. Clinical characteristics

The pattern of clinical presentation at diagnosis was defined as "classical," if the patient presented the classical picture of malabsorption (diarrhea, weight loss and abdominal distension), "non-classical", in cases showing other clinical manifestations including iron deficiency, short stature, aphtous stomatitis, recurrent abdominal pain, etc., or "silent," in individuals apparently asymptomatic, diagnosed as part of a screening program [1]. At baseline, and after 3 and 6 months of follow-up, clinical symptoms were recorded through the Validated Disease Specific Symptom Index for Celiac Disease (VDSS) [10], and stool aspect was evaluated through the Bristol Stool Chart [11].

2.3. Anthropometric measurements

For all children, anthropometric measurements were collected by the same trained operator at baseline, and after 3 and 6 months of follow-up. Body weight was measured using the same mechanical balance (SECA 200); height was measured to the nearest 5 mm using a stadiometer (SECA 220). Body mass Index (BMI) was cal-

culated from weight and height (Kg/m^2). To account for the variability of the BMI according to gender and age, the BMI Z-score (the number of SD units above or below the mean) was calculated using the individual's weight and height and the World Health Organization (WHO) reference values [12].

2.4. Laboratory parameters

In all children the following laboratory parameters were assessed at baseline, and after 3 and 6 months of follow-up: hemoglobin (g/dL), ferritin (ng/mL), AST (U/L), ALT (U/L), albumin (g/dL), vitamin D (ng/mL), fecal calprotectin (mcg/g), and IgA anti-tissue transglutaminase (anti-tTG) (U/mL). IgA anti-tTG were determined by an ELISA test (Eu-tTG IgA; Eurospital SpA, Trieste, Italy).

2.5. DNA extraction from fecal samples

Ninety-nine fecal samples were collected in a sub-group of enrolled children (at baseline: $n = 18$ in group A and 21 in group B; after 3 months: $n = 13$ in group A and 15 in group B; after 6 months: $n = 13$ in group A and 19 in group B). Stool samples were shipped under frozen conditions to the laboratory of microbiology of the Department of Agriculture, Food and Environment, University of Catania (Italy) and immediately processed. In detail, total genomic DNA (gDNA) was isolated following the previously described protocol [13]. The concentration of the purified gDNA was estimated by using the fluorimeter Qubit 4.0 (Invitrogen, Carlsbad, CA, USA).

2.6. Libraries preparation for Illumina MiSeq sequencing

The V3-V4 region of the 16S rRNA gene was amplified by PCR as previously described [14,15]. PCR products were purified using the AMPure XP beads (Beckman Coulter s.r.l., Cassina De' Pecchi, Milan, Italy), to remove free primers and primer dimer species. Dual indices and Illumina sequencing adapters were added using the Nextera XT V2 Index Kit (Illumina, San Diego, CA, USA). A further clin up step of the final library, using the AMPure XP beads (Beckman Coulter s.r.l., Cassina De' Pecchi, Milan, Italy), was performed before quantification. The resulting libraries were validated using the Agilent Bioanalyzer 2100 (DNA 1000 chip) to check size distribution. Indexed DNA libraries were normalized to 4 nM and then pooled in equal volume with all the other samples. The pool was loaded onto an Illumina Flowcell v2 with 25% of Phix control. The samples were sequenced using the Illumina MiSeq platform at the Synbiotec (Camerino, Italy) facilities using 2 × 250 bp paired end run. 16S rRNA raw data were deposited at NCBI Sequence Read Archive (SRA) under the accession code PRJNA923292.

2.7. Outcome measures

Primary outcome measures were the differences T3-T0 and T6-T0 between the two treatment groups related to all study parameters and in particular: (1) VDSS score; (2) BMI Z-score; (3) IgA anti-tTG level; (4) hemoglobin and ferritin level; (5) vitamin D level; (6) fecal calprotectin.

2.8. Statistical analysis

The sample size was estimated using VDSS score as the primary response variable and calculating 95% CI for the difference of the primary response in the 2 groups. One hundred thirteen patients were required to estimate a 95% CI considering a clinical difference between the 2 groups of 20% as maximum, a standard deviation of 4.5, a level of significance of 0.5, and a power of 90%, considering a drop-out rate of 20%.

A descriptive analysis of the baseline demographic and clinical characteristics of patients was performed. According to the Shapiro-Wilk test results median and interquartile range (IQR) were used to summarize quantitative variables while absolute and percentage frequencies were used to summarize qualitative variables. Wilcoxon rank-sum test and Chi-square test were used for comparison between the two groups.

Median of the differences in VDSS score between baseline and 3 months values were estimated in both groups with 95% confidence interval and the distribution of the differences in two groups of treatment were compared with the Wilcoxon rank-sum test.

A nonparametric analysis of longitudinal data in factorial experiments was used to test differences between the two treatment groups (A and B), the differences between time points (baseline, 3 months, 6 months) and the interaction between groups and time for each of the following dependent variables: VDSS and Bristol score, IgA anti-tTG, Hemoglobin, Ferritin and Vitamin D value. Results were graphically reported with box plots of the distribution of the dependent variable at each time point for both groups and the plot of the estimated marginal relative effects. If the effect of time was found to be statistically significant, multiple comparisons were performed between the distributions of the dependent variable at each time point.

2.9. Bioinformatic analysis of next generation sequencing data

The produced reads were initially quality checked through the FastQC [16] application. Then, the main metagenomics analysis was performed by using QIIME2v2021.11 suite [17]. Reads were trimmed (first 20 bases), filtered (by read average quality), end-joined and depleted by chimeric constructs, by using the QIIME2 integrated DADA2 [18] module. The QIIME-integrated Mafft [19] software was implemented for read alignment, while rooted and unrooted 16S phylogenetic trees were constructed through the FastTree tool [20]. Taxonomic classification was obtained by using the Naïve Bayes fitted classifier, pre-trained on the most recent Greengenes Reference Sequence Database (ver. 13.8) [21]. Effect of sequencing coverage depth on sample microbial diversity was evaluated by the examination of the rarefaction curves (at 10,000 and 20,000 read sampling cutoffs), through the "diversity" QIIME module. Shannon's entropy [22], Number of Observed Features, Faith Phylogenetic Distance [23] and Pielou's evenness [24] alpha diversity measures were computed and group-specific distributions were compared by Kruskal-Wallis statistical tests. Dissimilarity among samples was also visually evaluated through Principal Coordinate Analysis (PCoA) plots using Emperor [25] web application at https://view.qiime2.org. Differences for beta diversity indexes (Jaccard, Bray-Curtis, Unweighted and weighted Unifrac dissimilarities [26–28]) across sample groups were evaluated by Permutational Multivariate Analysis of Variance (PERMANOVA) tests. Differences in abundance profiles of the detected taxa were examined by using three "compositionally-aware" methodologies. At first, from the QIIME2-produced feature table, non-bacterial contaminant sequences were removed. Coda-lasso, Clr-lasso, and Selbal methods [29–31] were applied on the filtered feature table (collapsed at genus level), in order to evidence taxa significantly over or down-represented in pairwise group comparison [32–33]. R packages Microbiome 1.20.0 and Phyloseq 1.42.0 [34–35] were additionally used for metagenomics data parsing and graphics.

3. Results

The flow diagram of the study is shown in Fig. 2. One-hundred thirteen children were recruited, 64 of them in Ancona and 49 in Cava dei Tirreni, and 17 withdrew from the study. The number of children withdrawing from the study was not significantly

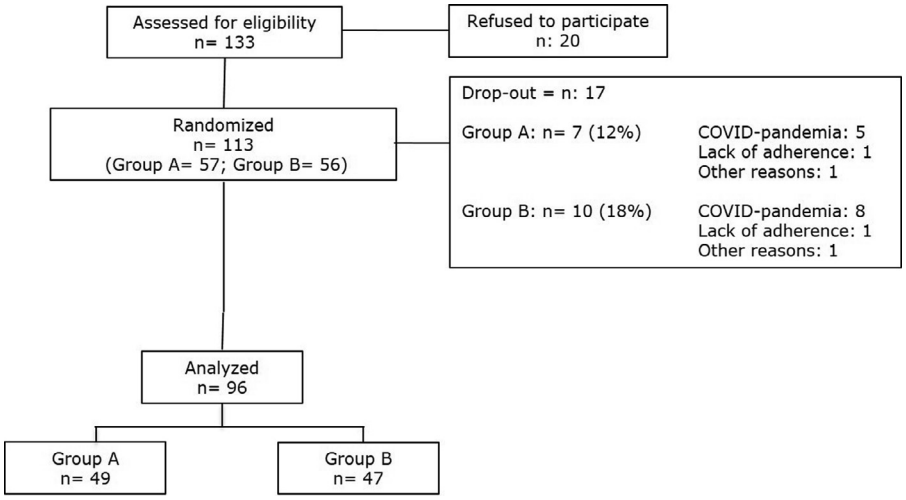

Fig. 2. Study flow-diagram.

Table 1
Baseline demographic and clinical characteristics of the two study groups. Values are summarized as median and interquartile range.

	Group A (n = 49)	Group B (n = 47)	p
Age, years [median (IQR)]	7.50 (3.88; 11.71)	8.09 (5.22; 10.75)	0.773[a]
Sex, female [n (%)]	28 (57.1)	25 (53.2)	0.858[b]
BMI, kg/m^2 [median (IQR)]	16.46 (15.21; 20.30)	16.59 (14.20; 18.43)	0.246[a]
BMI Z score [median (IQR)]	0.35 (−0.60; 1.01)	0 (−1.04; 0.67)	0.190[a]
Symptoms, yes [n (%)]	33 (67.3)	27 (57.4)	0.532[b]
Anti tTG-IgA, U/ml [median (IQR)]	200 (71.5; 300)	178 (106; 300)	0.900[a]
Hb, g/dl [median (IQR)]	13.15 (12.5; 13.6)	12.8 (11.78; 13.93)	0.500[a]
Ferritin, ng/ml [median (IQR)]	14 (9.75; 22)	17.1 (10.5; 38.2)	0.750[a]
AST, U/l [median, (IQR)]	28 (23; 34)	36 (26; 41)	0.017[a]
ALT, U/l [median, (IQR)]	21 (18; 25)	25 (18; 35.5)	0.065[a]
Albumin, g/dl [median, (IQR)]	4.15 (4; 4.3)	4 (3.9; 4.5)	0.130[a]
Vitamin D, ng/ml [median, (IQR)]	22 (17.3; 27.7)	21.4 (16.77; 26.8)	0.924[a]
Calprotectin, mcg/g [median, (IQR)]	15 (15; 38.5)	17 (15; 40.5)	0.633[a]
VDSS score [median, (IQR)]	32 (27; 39.5)	37 (26; 40.5)	0.554[a]
Bristol chart score [median, (IQR)]	3 (2; 4)	3 (2; 3.5)	0.421[a]

BMI: Body Mass Index; tTG: tissue transglutaminase; Hb: hemoglobin; AST: aspartate aminotransferase; ALT: alanine aminotransferase; VDSS: Validated Disease Specific Symptom Index for celiac disease. *p refers to p-value of:*.
[a] Wilcoxon sum-rank test.
[b] Chi-square test.

different between the two groups of patients; in both groups the most common reasons for giving up were inability to ingest the assigned sachet or to attend the follow-up visits due to COVID-pandemic. Overall, 96 children completed the study: 49 in group A (placebo) [28 females (57%); median age 7 years], and 47 in group B (probiotic) [25 females (53%); median age 8 years). The demographic and clinical characteristics of the 96 enrolled patients are reported in Table 1. No significant differences were found between the two treatment groups. No side/adverse clinical effect of probiotic/placebo supplementation was reported.

Figs. 3a-f show box plots (left panels) and 95% confidence interval (right panels) for the relative treatment effects for the two study groups at each time point for VDSS score, IgA anti-tTG, hemoglobin, ferritin and vitamin D value, respectively. Significant median differences of the VDSS score from baseline to 3 months of 7 point (95%CI: 5; 11) in Group A and of 6 points (95%CI: 5; 12) in Group B were observed; the distribution of the differences between the two groups were not significantly different (p = 0.809). A significant decreasing trend was found in both group (Fig. 3a) over the three time points: the VDSS was significantly lower after 3 and 6 months compared to baseline (p<0.001); significant lower values were also reported after 6 months respect to 3 months (p < 0.001); no difference was found between groups A and B (p = 0.327) and a comparable time trend was observed (p = 0.183) in the two groups. No significant differences were observed in Bristol stool chart score between groups and time points.

A significant increase of BMI Z-score at 3 (p = 0.009) and 6 months (p<0.001) compared to baseline values was found in both groups. The interaction between time and group was statistically significant (p = 0.032), indicating that the increase of BMI-Z score was significantly higher and faster in Group B than in Group A (Fig. 3b).

For IgA anti-tTG a significant decreasing trend was found in both groups (Fig. 3c, p < 0.001); the time trend was similar between the groups (p = 0.134) and no difference was detected between groups (p = 0.242).

No significant differences were observed between values of hemoglobin at baseline and at 3 months, while significant higher values were found at 6 months compared to baseline and 3 months (Fig. 3d, p < 0.001, p = 0.004 respectively), but no significant difference was found between groups. A significant increasing trend was found for ferritin (p <0.001), with higher values after 6 months compared to baseline and 3 months (p = 0.002 for both). The time trend was similar in both groups (p = 0.936) and no significant difference was found between groups (p = 0.200) (Fig. 3e). Significant higher values of vitamin D were found after

3 months compared to baseline ($p = 0.039$) while no difference was found between 3 and 6 months ($p = 0.999$). The pattern over time was similar in both groups and no significant difference was found between groups (Fig. 3f). No significant differences were observed between values of AST, ALT, albumin, and fecal calprotectin at baseline and values at 3 and 6 months in both groups.

3.1. Fecal microbiota

Overall, an average number of 63,000 amplicon reads were obtained and 60–80% of them resulted of good quality and usable for taxonomic assignment and diversity analyses.

Rarefaction curves for alpha diversity indices were produced for evaluating the coverage depth effect on such metrics. Given the good sequencing yield, no remarkable effect was evidenced, then no sampling depth cutoff was imposed for diversity tests. Samples were clustered according to treatment ("A"/"B") and sampling time (0, 3, 6 months). Based on Kruskal-Wallis pairwise test, no significant differences were found between group-specific alpha metrics distributions (Fig. 4 panels a-d) for all groups. No clear clusterization among samples was obtained as shown in the PCoA plot (Fig. 5). Indeed, no significant differences were detected across groups through PERMANOVA tests on four Beta diversity dissimilarities matrices.

The global composition of fecal samples showed the presence of 5 bacterial phyla, 41 families, and 97 genera, occurring at different abundances across samples. Differential abundance analyses were performed at genus level. Variation of taxa abundance

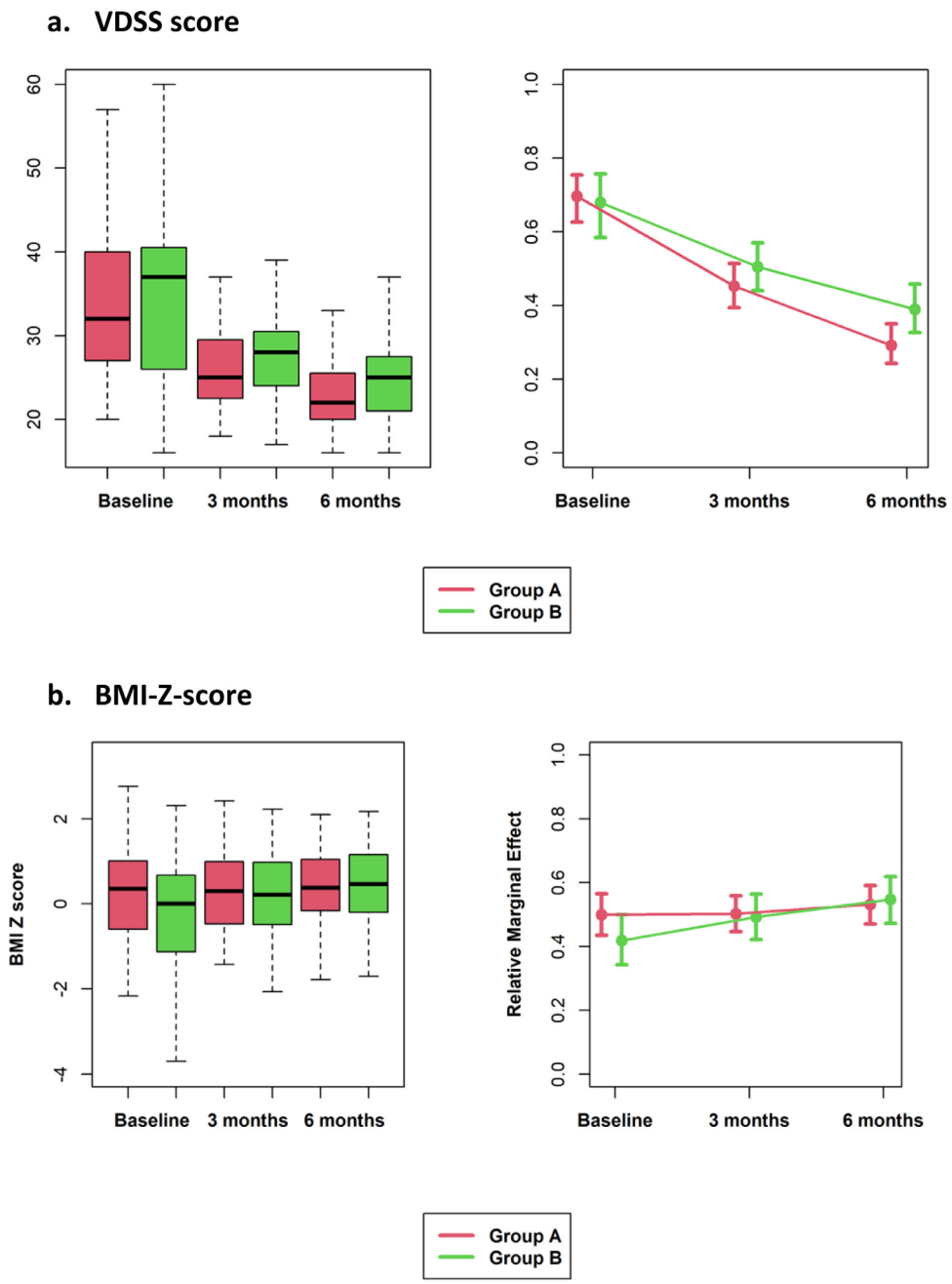

Fig. 3. a-f. Changes [box plots (left panels) and 95% confidence interval (right panels)] of Validated Disease Specific Symptom Index for Celiac Disease (VDSS) score (a), Body mass index (BMI) Z-score (b), IgA anti-tissue transglutaminase antibodies (IgA anti-tTG) (c), Hemoglobin (d), Ferritin (e), Vitamin D (f) over time according to the study group.

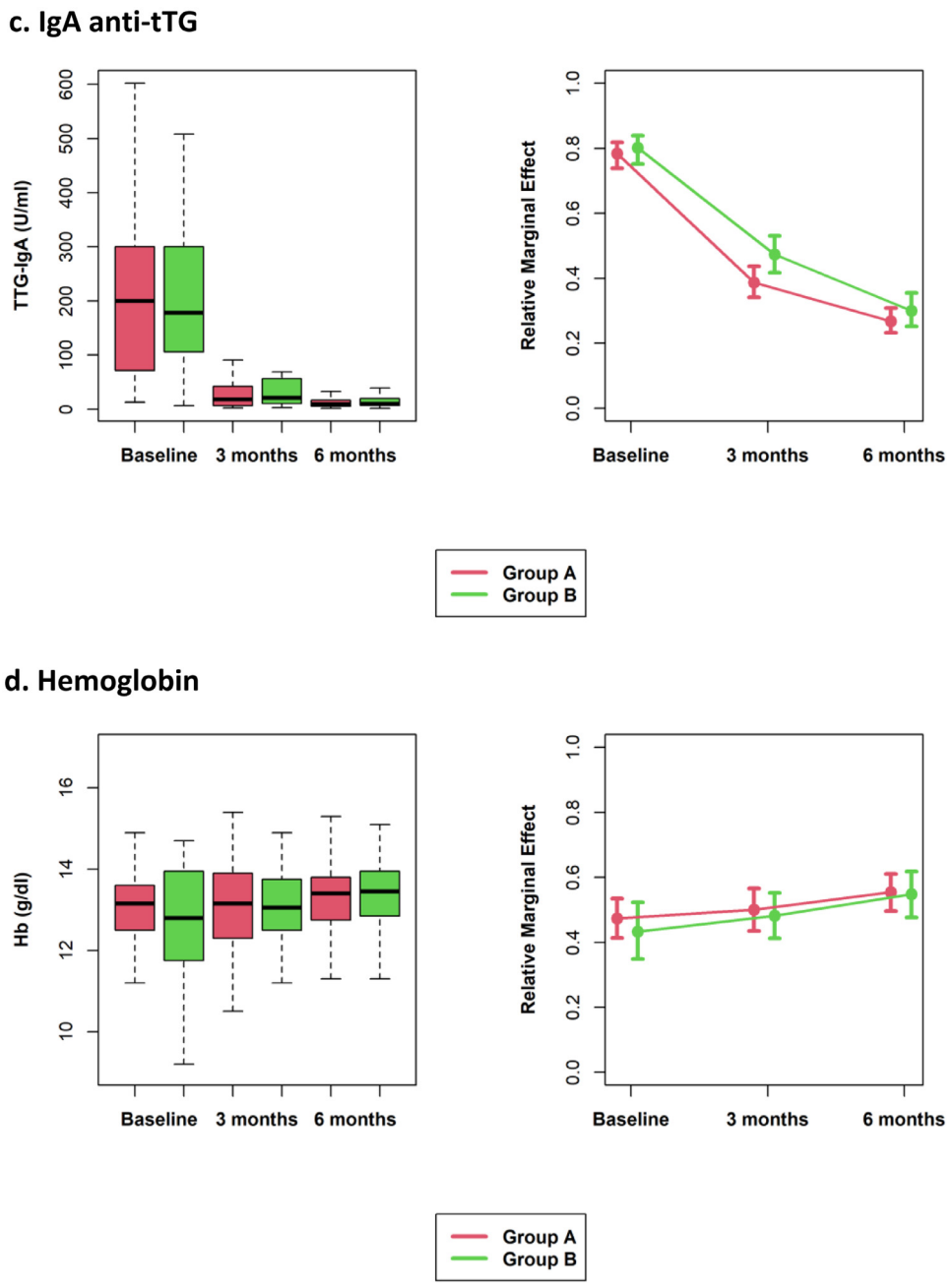

Fig. 3. Continued

profiles along time was estimated by using Selbal, Coda_lasso and Clr_lasso methodologies and features consistently over-represented or down-represented by at least two methods, in a specific pairwise comparison, are reported in Supplemental Table 1.

4. Discussion

This double-blind, placebo-controlled randomized trial showed that supplementation with a multispecies probiotic formulation induces a significantly higher and faster improvement of BMI Z-score in children with CeD at diagnosis, as compared to the standard treatment with GFD alone. No other significant effect on gastrointestinal symptoms and laboratory parameters was found, probably due to the covering beneficial effect of the GFD in both probiotic- and placebo-treated patients.

Growth retardation may be found in children at CeD diagnosis, both in classical and non-classical presentations. Adherence to the GFD is associated with a positive effect on anthropometric parameters with an increase in lean body mass, normalization of BMI, and acceleration of linear growth [36–39]. The maximum catch-up growth is expected within the first six months of GFD but can continue for 2–3 years, at which time the child is predicted to reach the expected height [2]. The acceleration of BMI recovery in our patients treated with probiotic supplementation was essentially related to a significant improvement of weight. Although increased, the mean BMI Z-score, did not reach the range of overweight or obesity for age and sex.

There are a few studies on the effect of probiotics in patients with CeD at diagnosis. In a double-blind, randomized, placebo-controlled study, 33 newly diagnosed pediatric patients with CeD were given *Bifidobacterium longum* CECT 7347 for three months,

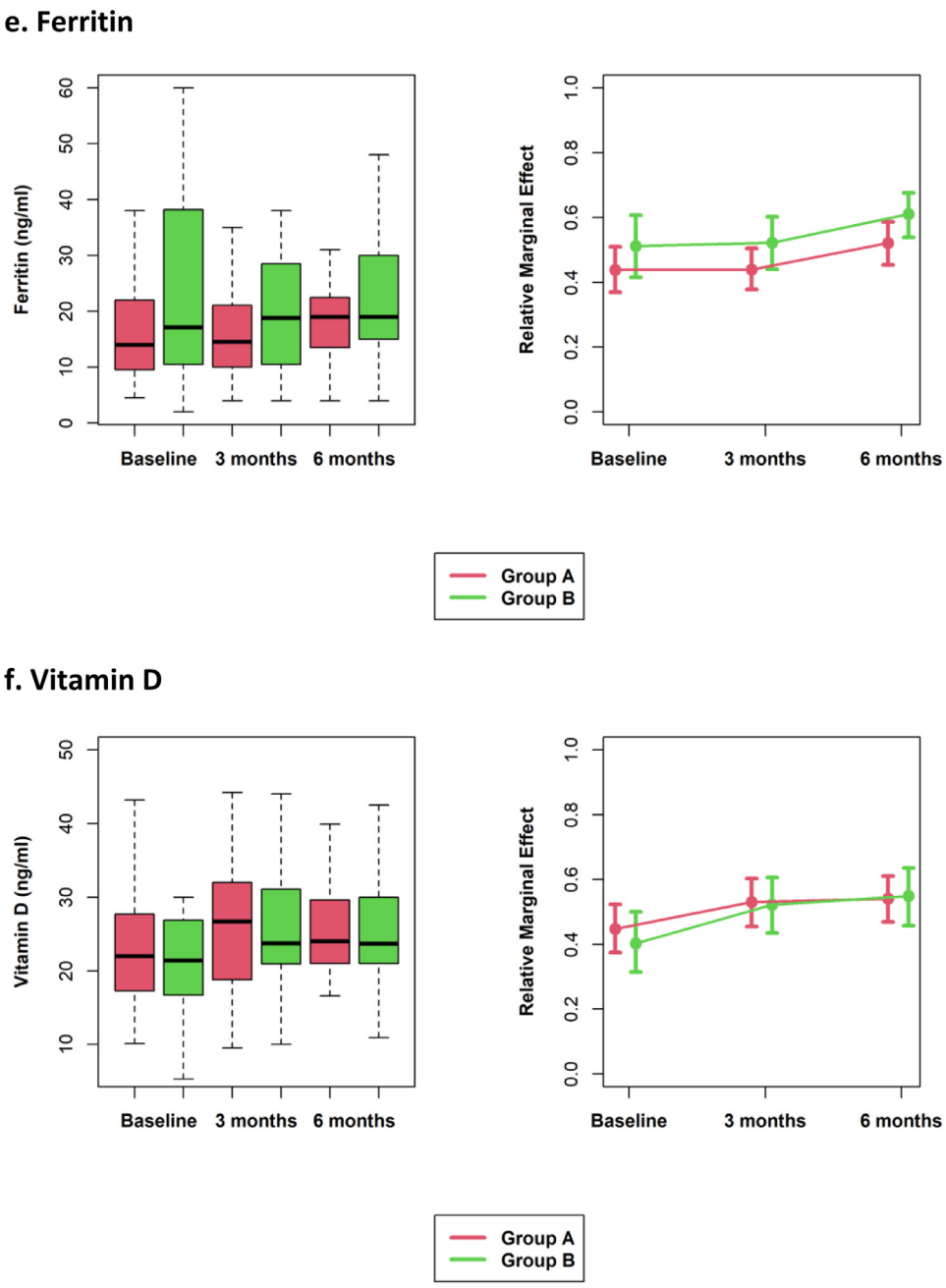

Fig. 3. Continued

together with the GFD. Children receiving the probiotic supplement showed an increased height velocity that paralleled a reduction in the intestinal inflammation, as measured by peripheral CED3+ T and TNF levels [40]. In a another double-blind randomized placebo-controlled study on untreated CeD patients at the start of the GFD ($n = 12$), *Bifidobacterium infantis* (Natren Life Start, NLS) treatment for three weeks determined a significant improvement in Gastrointestinal Symptom Rating Scale and reduction of IgA anti-tTG antibodies but had no effects on intestinal permeability [41]. The effect of *Bifidobacterium breve* strains B632 and BRO3 was evaluated in a double-blind placebo-controlled trial on 49 children showing a reduction of TNF and IL-10, and a restoration of the physiological *Firmicutes*/*Bacteriodetes* ratio in the probiotic-treated group [42,43]. Besides a positive effect on intestinal inflammation, probiotics may accelerate CeD clinical remission by eliminating traces of gluten that may accidentally contaminate the GFD. In a clinical study where GFD-treated CeD patients underwent a 60-day challenge with Lactobacilli-predigested gluten, there was no worsening of symptoms, intestinal permeability or serological markers, suggesting that Lactobacilli-derived endopeptidase may be capable of completely degrading gluten thereby reducing its toxicity for CeD patients [1]. Studies of the gut microbiota (fecal and duodenal biopsies) revealed that Lactobacilli and Bifidobacteria may reduce symptoms in CeD patients on a GFD, by mitigating the effect of accidental/contaminant gluten exposure or other beneficial effects [44,45].

The specific mix of bacterial strains tested in our study had previously been investigated in both *in-vitro* and clinical works. A study performed on human Caco-2 cells showed that this probiotic mix of *Lactobacilli* and *Bifidobacteria* can hydrolyze gliadin

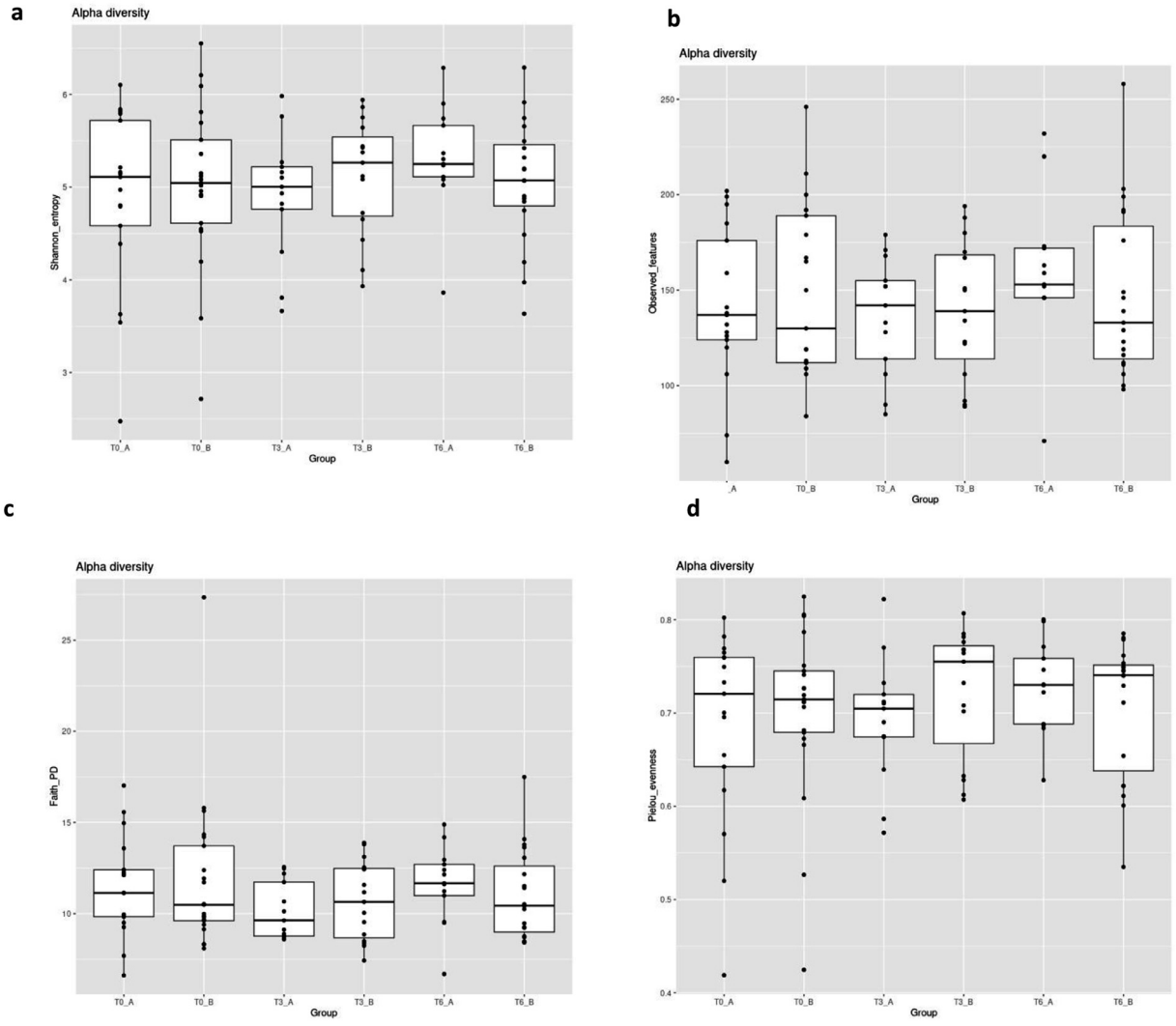

Fig. 4. Group-specific alpha metrics distributions.

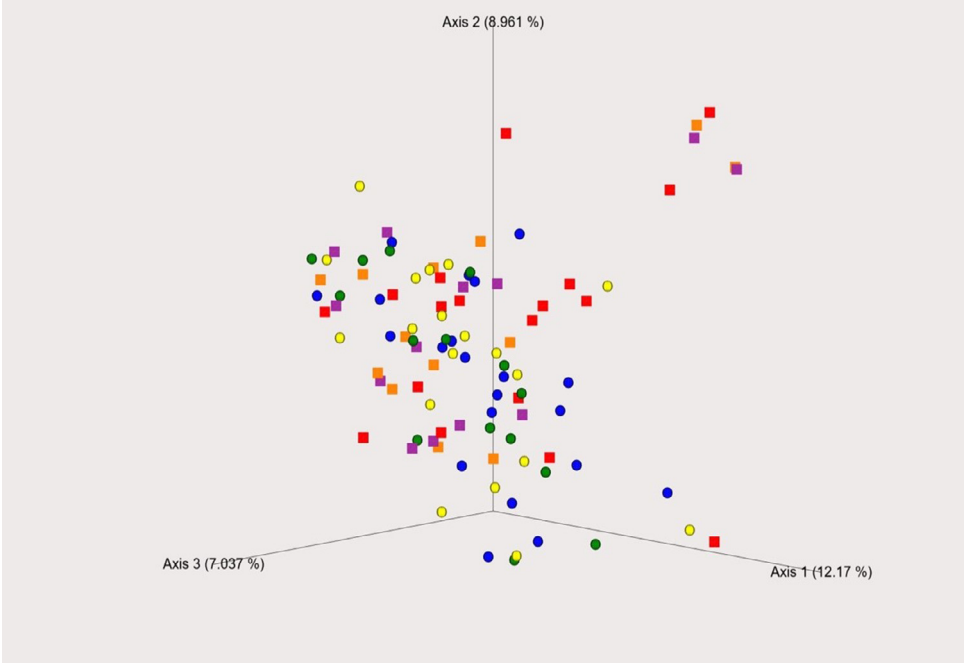

Fig. 5. PCoA plot of the analysed samples.

fragments into smaller fragments and that the digestion of gliadin fragments with probiotic strains modulated the inflammatory status and damage of tight junctions induced by gliadin in intestinal epithelial cells [46]. Francavilla et al. recently showed that this probiotic mix improved persisting IBS-symptoms in CeD patients treated with the GFD [8]. By contrast, we found no favorable effect of probiotic supplementation on gastro-intestinal symptoms. These variable results could depend on several differences between the two studies, such as age (children vs adults) and characteristics (at diagnosis or in follow-up) of patients, and diagnostic tools used to measure clinical manifestations (VDSS vs GSRS score). The greater positive effect of the GFD at the beginning of treatment might have overwhelmed a small addictive benefit of probiotic supplementation. Likewise, we found no effect of probiotic supplementation on the levels of CeD autoantibodies and other laboratory parameters. Full normalization of anti-tTG levels may take more than two years, particularly in those with severe small bowel lesions and high anti-tTG levels at diagnosis [2]. Therefore, treatment for a longer time and/or longer observation period than our study-design may be required to find a significant effect of probiotic supplementation on the course of CeD serology normalization.

Using the same probiotic mix, the Francavilla's study also showed an increase in lactic acid producing bacteria, *Staphylococci*, and *Bifidobacteria* [8]. By contrast, no significant changes in the microbiota composition were showed in our study, probably due to the small number of analyzed samples. Nonetheless, an overexpression of both *Anaerostipes* and *Lactobacillus* genera was revealed after probiotic administration. It is well known that members of the genus *Anaerostipes*, besides being butyrate-producing commensals, play a key role in the maintenance of the gut barrier function [47,48], whereas lactobacilli are considered an essential component of a healthy microbiota [49].

The strength of our study was an appropriate study-design that was powered in advance to evaluate the clinical effect of tested probiotics. The main limitations were the high number of dropouts, particularly due to the concurrent COVID pandemia, and the restricted number of cases performing the analysis of microbiota.

In conclusion, this study suggests that supplementation with a multispecies probiotic, coupled with the GFD, is safe and may accelerate weight recovery in children with growth retardation at diagnosis of CeD. The mechanism of this favourable effect, likely related to changes in the gut microbiota, should be clarified by further studies.

Author contributions

Carlo Catassi is the guarantor of article. C.C. and E.L. were responsible for: conceptualization, funding acquisition, project administration, supervision, validation, writing original draft, review and editing. E.L., V.D., M.I., E.C., I.A., S.G., C.M., A.P., C.L.R., and G.C. contributed to data curation, investigation, methodology. A.F., R.G., A.P., C.L.R analyzed the data. All authors approved the final version of the article, including the authorship list.

Funding and Conflict of Interest

Carlo Catassi served as consultant for dr Schaer. The other authors have no conflict of interest to declare.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.dld.2023.04.021.

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