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The effect of physical exercise and dairy probiotics (*Lactobacillus casei*) on gut microbiome in childhood cancer survivors

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Gut microbial dysbiosis persists months after intensive cancer treatment in children and adolescents. This prospective study compared the intestinal microbiome of children 1-3 years after completion of Berlin-Frankfurt-Münster protocol (BFM)-based pediatric ALL (PALL) treatment and healthy controls. To induce a favorable shift in the bacterial composition of the intestines in PALL with gut microbiome disruptions, 8 weeks of physical activity and probiotic consumption were used. Blood analyses and 16S rRNA sequencing for the gut microbiome were performed on 16 pediatric cases and 16 healthy controls. Significant differences in bacterial diversity were found between pre- and post-intervention, respectively (Shannon index, 3.22±0.45 vs. 3.47±0.24, p=0.04; Simpson index, 0.10±0.05 vs. 0.06±0.02, p=0.02; and Chao1 index, 693.88±238.58 vs. 794.23±116.34, p=0.04). Furthermore, the increase in the relative abundance of *Lactobacillus casei* (5.04E-03±1.62E-02 vs. 2.92E-02±5.03E-02, p=0.04) and the increase in some strains of *Veillonella*, a bacterial genus recently linked to improved physical fitness, were identified. Promisingly, the exercise program combined with dairy probiotics increased bacterial richness and diversity.

Key words: Veillonella; lactate acid bacteria; probiotics; acute lymphoblastic leukemia

Short- and long-term toxicity of treatment considerably affects the quality of life in childhood cancer survivors. The remarkably increased survival of children with acute lymphoblastic leukemia (ALL) has raised awareness of therapy-related toxicity. Children with ALL spend two years undergoing treatment, confronting their diseases and treatments while also dealing with developmental challenges [1]. However, long-term treatment of ALL with multiagent chemotherapy and corticosteroids induces several known complications, including oral mucositis [2] systemic inflammation [3], weakness and fatigue in the skeletal muscles of survivors [4], lower mineral density [5], impaired cardiorespiratory fitness, a high degree of symptom distress [1, 6, 7], muscle strength, and motor function [8, 9].

Alteration in the gut microbiota has been recently linked with childhood leukemia and treatment modalities. The early microbiome shifts at the onset of therapy are thought to be related to chemotherapeutic and corticosteroid treatment [10–12]. However, considerable evidence exists concerning the effects of antibiotic treatment on the intestinal community in children who have survived cancer [13–16]. Loss of bacterial diversity and richness is a well-documented side effect of treatment in children with cancer [17]. Knowledge of the complexity of the gut microbiota and its importance in physiological processes and disease development has been expanding in recent years [18]. Similar to other ecosystems, an individual's microbiome composition is constantly shifting. However, more extensive changes can also be mediated by introducing or destroying particular microbial groups [19].

Data from pediatric and adolescent oncology patients have confirmed the persistence of gut microbial dysbiosis several months after intense cancer treatment [20]. Moreover, children who are cancer survivors may develop metabolic syndrome and dyslipidemia [21]. Therefore, post-treatment care through a healthy diet and physical activities should not be neglected. Exercise training is considered a safe and, at least partly, effective option for the improvement of overall

health as well as for an increase in physical activity levels in childhood cancer survivors [22]. Supportive research on physical exercise in pediatric oncology patients during treatment has shown positive effects on body composition [23], quality of life and fatigue symptoms [24], children's pain [25], and decreasing the risk of infection associated with treatment [26]. Unfortunately, little information is available describing the benefits of exercise training on the gut microbiome in pediatric cancer patients and cancer survivors. Similarly, little data is known on the effects of dairy probiotics on the gut microbiome in pediatric cancer patients in remission.

Therefore, the aim of this pilot prospective study was to compare the structure of the intestinal microbiome between children shortly after treatment for ALL (1–3 years after cancer treatment) and healthy controls. And then to provoke a positive shift of bacterial diversity through physical activity and probiotics in cured pediatric oncology patients with persistent gut microbiome disruptions.

Patients and methods

Study subjects and recruitment. The study subjects' cohort included a) children previously diagnosed and treated for acute lymphoblastic leukemia in remission between 1 and 3 years (PALL), and b) healthy controls. Pediatric cases and healthy controls ranged in age from 6 to 12 years. PALL were from a single institution: Department of Pediatric Hematology National Institute of Children's Diseases Bratislava (NICD). The healthy controls were chosen at random.

The time range of the study was as follows: from May 2022 to June 2022. This research was carried out in accordance with the Helsinki Declaration Principles for Human Experiments. Following the reading of the informed consent form, an explanation of the study steps, and talks with the investigators, all legal representatives signed informed consent, enabling their children to participate in the study (parents). The research was approved by the National Institute of Child Diseases' Ethics Committee EK/1/22. Complete clinical trial registration is deposited at ClinicalTrials.gov (NCT05939791).

Medical treatment. The patients were treated for B-ALL according to the BFM-based strategy: AIEOP-BFM ALL 2009 [27]. Induction treatment consisted of corticosteroids: prednisone for 29 days, 4× anthracyclines, 4× vincristine, and 2× PEG asparaginase; early consolidation with araC, cyclophosphamide, and 6 mercaptopurine; consolidation with 4× high dose methotrexate (5 g/m^2) and 6 mercaptopurine; intensification with corticosteroids: dexamethasone for 22 days; 4× anthracycline, 4× vincristine; 1× PEG asparaginase, and prophylactic treatment with intrathecal methotrexate. Maintenance treatment with once weekly methotrexate and every day 6 mercaptopurine.

Experimental intervention. The individual online (MS Teams) training program for PALL of 8 weeks included 25–45 min of moderate-to-vigorous physical exercise [28], twice a week, under the supervision of a certified sports trainer from the Faculty of Physical Education and Sports Comenius University. The structure of the exercise program was developed to improve endurance and gradually rebuild muscular strength [29]. The exercise program's structure was created to increase endurance and gradually rebuild muscular strength. Large muscle groups were the focus of training sessions, which also placed a strong emphasis on proper technique. A strength exercise squat was given consideration as a movement required to meet necessities (e.g., sitting or standing). Each exercise consisted of between 10 and 15 repetitions in each series and 2 to 3 series overall. According to the degree of difficulty, the exercises generally included three modalities. The exercise was chosen with consideration for the patient's condition and level of ability. When needed, the senior physiotherapist from the NICD's Physiotherapy and Rehabilitation Department was consulted prior to the training session.

The commercial probiotic dairy product (Danone, Belgium) has been provided to PALL along with physical training once a day for 8 weeks. Each serving contained 20 billion CFUs of Lacticaseibacillus paracasei subsp. Paracasei CNCMI-1518 (*Lactobacillus casei* CNCMI-1518). Each patient's legal representative was advised to follow a normal diet when preparing and providing food for children.

Sample collection and microbiome analyses. PALL were asked to collect their own feces one week before the beginning and one week after the end of the intervention. Samples were stored in a DNA/RNA Shield-Fecal Collection Tube in order to preserve the stability of the nucleic acids in the stool samples (ZymoResearch, Irvine, CA, USA). The fecal samples from the controls were collected once. Each stool's DNA was taken out, put on ice, and frozen right away at –80 °C.

The total DNA from fecal samples was extracted with the ZymoBIOMICS DNA/RNA kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's protocol. NGS libraries were prepared with the 16S Microbiome NGS Assay (ViennaLab Diagnostics GmbH, Vienna, Austria). In the first PCR step, the highly variable V3-V4 regions were amplified with locus-specific primers. The first PCR products were evaluated with agarose electrophoresis. The second low-cycle PCR used dual index sequences for the assignment of the reads to individual samples during data demultiplexing. Both PCR products were purified with Agencourt AMPure XP magnetic beads (Beckman Coulter, Brea, CA, USA). DNA libraries were quantified by a Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA), and DNA profiles were verified using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) and a High Sensitivity DNA Kit (Agilent Technologies). DNA libraries were diluted to 4 nM, pooled equimolarly for sequencing, and analyzed using the Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) via 300-bp paired-end reads.

Illumina data processing. Sequencing data were analyzed using the software from The ViennaLab NGS Microbiome

Assay, which was part of the kit. The reads were pre-processed using BBMerge [30], Cutadapt [31], and SeqKit [32]. The read classification pipeline used the CLARK sequence classification system for the species-level classification of reads [33]. The CLARK system was based on discriminative k-mers in a sequence database. The sequence databases used by the classification pipeline were constructed based on sequences from the SILVA [34] and UNITE [35, 36] databases. The species taxonomy used by the read classification was downloaded from NCBI (https://www.ncbi.nlm.nih.gov/taxonomy/). Diversity statistics were calculated from the species-level abundance results using MOTHUR [37].

Biochemical assay. Biochemical variables were measured at the beginning and after 8 weeks of intervention in the Department of Laboratory Medicine (National Institute of Childhood Diseases, Bratislava, Slovakia). C-reactive protein (CRP) levels were measured using an *in vitro* immune kinetic enzymatic assay in human serum on layered reagent media with reflectometry measurements on a Vitros 4600 analyzer (Ortho-Clinical Diagnostics, Inc., Rochester, NY, USA). Lactate dehydrogenase (LDH) was determined in serum using an *in vitro* diagnostic kit based on the UV determination principle (IFCC) on a Cobas Integra 400 Plus analyzer (Roche Diagnostics GmbH, Mannheim, Germany).

Statistical analyses. Statistical analyses were carried out using the SPSS 21.0 program for Windows (SPSS, Inc., Chicago, IL, USA). The Shapiro-Wilk test validated the data's normality. An independent t-test was conducted to achieve a comparison of nonparametric data on the gut microbiome between the cancer survivors and controls. Furthermore, pre- and post-data from cases were subjected to the Welch and Brown-Forsythe versions of the one-way ANOVA. Spearman's rank correlation coefficient was used to assess the relationship between variables (gut microbiome and training variables). The significance level of $p<0.05$ was applied. ClustVis was used to visualize multidimensional data by means of principal component analysis (PCA) [38].

Results

Children, 1–3 years after completion of BFM based ALL treatment (PALL) vs. controls. We assessed 35 cancer children for eligibility, of whom 21 were willing to participate in the experimental and probiotic intervention. Five participants dropped out of the research or reported being less present during exercise training than the required 80% attendance rate. 16 PALL (9 females and 7 males; 8.9±3.3 years) completed the study with the following physical characteristics: height (132.4±18.6 cm), weight (35.4±14.7 kg), and BMI-for-age percentile (68.6±24.8); with the MRD-based classification [39]: standard-risk (n=7), intermediate-risk (n=5), high-risk (n=4), and with the CNS status: CNS1 (n=14), CNS2 (n=1), CNS3 (n=1). 16 subjects (9 females and 7 males; 9.0±3.3 years) were used as healthy controls with the following physical characteristics: height (134.6±18.3 cm), weight (32.1±10.7 kg), and BMI-for-age percentile (55.9±37.4).

The duration of moderate-to-vigorous physical activity per week ranged from 64.3±12.7 minutes in the first month of the intervention to 82.2±9.4 minutes in the second month in PALL. The commercial probiotic drink was administered to children daily.

Fecal microbiota. We identified 20 bacterial phyla in 32 fecal samples from cases (n=16) and controls (n=16). We found significant differences in the most dominant phylum taxa, with a decrease in *Bacteroidetes* (PALL 29.95±10.04; CTRL 43.98±13.75; p=0.005) and an increase in *Firmicutes* (PALL 62.15±10.39; CTRL 43.87±11.46; p=0.001) in cases versus controls. Furthermore, we noticed a significant difference (p=0.009) in the *Firmicutes/Bacteroidetes* ratio between cases and controls (2.4±1.2 vs. 1.2±0.7, p=0.001).

Principal component analysis (PCA) allowed for the visualization of 29 bacterial genera that were significantly different between cases and controls (Figure 1).

At the bacterial species level, we determined 29 taxa significantly different between cases and controls. Selected bacterial species enabled discrimination between both groups within the heat map visualization and PCA (Figures 2, 3). We further reported a significant increase in the SCFA-producing bacterium *Barnesiella intestinihominis* (PALL 0.1860±0.40; CTRL 1.0244±1.14; p=0.014) in controls compared to cases. Interestingly, we detected a significant decrease in pathogenic bacteria, e.g., *Leptospira* (PALL 0.6066±0.30; CTRL 0.1985±0.22; p=0.001) and *Enterocloster* (PALL 0.5160±0.31; CTRL 0.2879±0.28; p=0.018) in controls compared to cases.

Pre- and post-intervention in PALL. Following completion of the 8-week intervention program (physical exercise

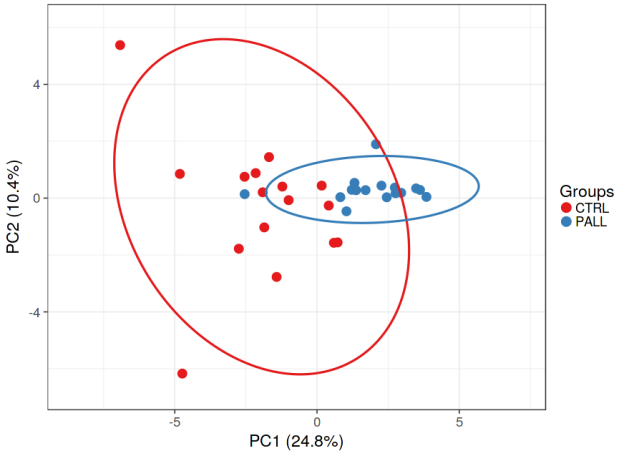

Figure 1. β-diversity of analyzed samples represented by significantly altered (p<0.05) bacterial genus level between PALL (n=16) and controls (n=16) as visualized by PCA. SVD with imputation was used to calculate the principal components. The X and Y axes show principal components 1 and 2, which explain 24.8% and 10.4% of the total variance, respectively. Prediction ellipses are such that, with a probability of 0.95, a new observation from the same group will fall inside the ellipse (n=32 data points).

and probiotic consumption), measurements were taken one week apart. By an α-diversity analysis, we identified a significant increase in Shannon and Chao1 indices and a decrease in the Simpson index in PALL after the intervention (Table 1).

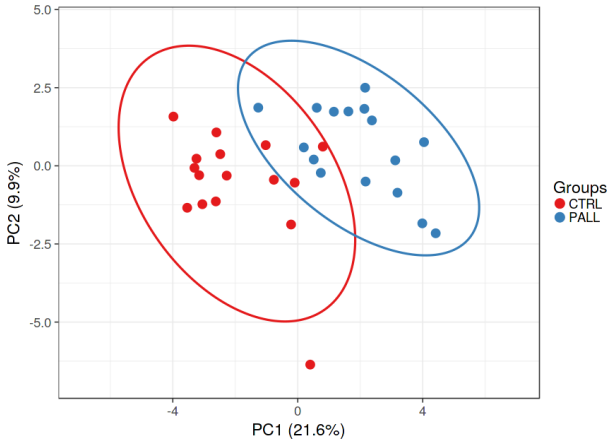

Figure 2. β-diversity of analyzed samples represented by significantly altered (p<0.05) bacterial taxa at species level between PALL (n=16) and controls (n=16) as visualized by PCA. SVD with imputation was used to calculate the principal components. The X and Y axes show principal components 1 and 2, which explain 21.6% and 9.9% of the total variance, respectively. Prediction ellipses are such that, with a probability of 0.95, a new observation from the same group will fall inside the ellipse (n=32 data points).

By conducting an β-diversity analysis in phylum, class, order, and family, we determined 18 significantly different taxa within the PALL groups (Table 1). Moreover, we detected 22 significantly different species in PALL (Table 2).

We identified a significant increase in the relative abundance of *Lactobacillus casei* (p=0.041) after the intervention (Figure 4A).

Moreover, we have found a significant increase in the relative abundance of the bacterial species *Veillonella ratti* (p=0.022) (Figure 4B) and *Veillonella rogosae* (p=0.003) after the intervention in PALL. We also observed an increase in the bacterial genera level of *Veillonella*, although this shift did not reach significance (p=0.30).

We have found associations between lactate-producing bacteria and bacterial taxa belonging to the *Veillonellaceae* family. A significant positive association was observed between the relative abundance of the genera Lactobacillus and *Veillonella* (r=0.566, p=0.001). Moreover, the genus Lactobacillus was positively correlated with the bacterial species *Veillonella ratti* (r=0.461, p=0.008) and *Veillonella magna* (r=0.479, p=0.006). In addition, the bacterial species *Lactobacillus casei*, the main bacterial taxon in the dairy product, was positively correlated with the relative abundance of *Veillonella rogosae* (r=0.522, p=0.002).

Pre- and post-intervention serum lactate dehydrogenase levels were not significantly different in children cancer survivors (3.57±0.62 vs. 3.67±0.71 μkat/l; p=0.62). Pre- and

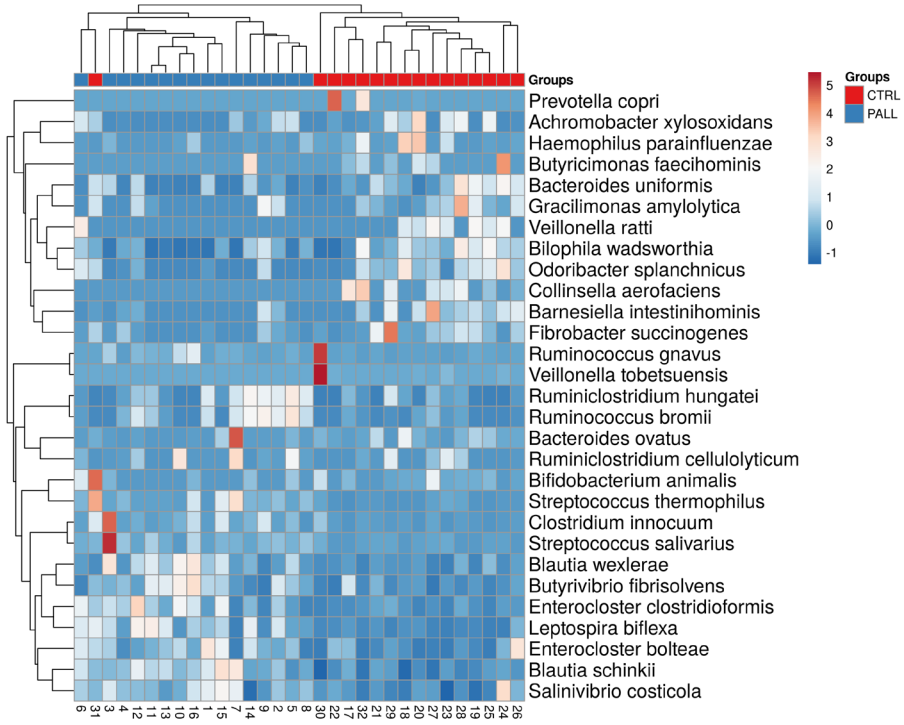

Figure 3. Microbial composition of significantly (p<0.05) distinct bacterial species within the cases (PALL, n=16) and controls (n=16). Selected rows are centered; unit variance scaling is applied to rows. Imputation is used for missing-value estimation. Both rows and columns are clustered using correlation distance and average linkage. Numbers represent samples visualized by the heat map.

Table 1. Bacterial diversity and taxa (phylum, class, order, family, and genus) before and after 8 weeks of exercise training with the consumption of probiotics in cancer survivors (n=16).

		PALL-pre	PALL-post	p-value
Diversity	Shannon index	3.22±0.45	3.47±0.24	0.04
	Simpson index	0.10±0.05	0.06±0.02	0.02
	Chao1 index	693.88±238.58	794.23±116.34	0.04
Phylum (%)	*Balneolaeota*	5.94E-04±1.19E-03	1.59E-03±1.42E-03	0.02
Class (%)	*Balneolia*	5.94E-04±1.19E-03	1.59E-03±1.42E-03	0.02
	Dehalococcoidia	2.15E-03±3.46E-03	3.55E-03±2.47E-03	0.05
Order (%)	*Balneloales*	5.94E-04±1.19E-03	1.59E-03±1.42E-03	0.02
	Halanaerobiales	1.92E-03±3.20E-03	3.52E-04±7.64E-04	0.03
	Legionellales	1.24E-03±2.85E-03	1.87E-03±3.39E-03	0.04
	Marinilabiliales	1.17E-04±4.68E-04	1.15E-03±1.96E-03	0.04
	Oscillatoriales	1.31E-04±5.23E-04	1.00E-03±1.21E-03	0.05
Family (%)	*Balneolaceae*	4.64E-04±8.33E-04	1.59E-03±1.42E-03	0.01
	Dysgonamonadaceae	1.25E-02±4.46E-02	0.00E+00±0.00E+00	0.02
	Halobacteroidaceae	1.57E-03±1.96E-03	3.52E-04±7.64E-04	0.03
	Hungateiclostridiaceae	8.99E-02±1.11E-01	1.51E-01±1.37E-01	0.02
	Leuconostocaceae	3.48E-02±2.80E-02	7.11E-02±3.98E-02	0.01
	Peptostreptococcaceae	8.10E-01±5.64E-01	2.00E+00±2.51E+00	0.01
Genus (%)	*Barnesiella*	2.01E-01±4.19E-01	5.39E-01±7.79E-01	0.04
	Candidatus neoehrlichia	6.83E-04±1.19E-03	0.00E+00±0.00E+00	0.04
	Clostridioides	6.59E-01±4.58E-01	1.56E+00±1.96E+00	0.01
	Ruminococcus	4.03E+00±2.03E+00	4.96E+00±3.36E+00	0.08

Notes: values are presented as mean ± SD; differences were considered significant at $p<0.05$. Abbreviations: PALL-children diagnosed for acute lymphoblastic leukemia

Table 2. Bacterial taxa (species) before and after 8 weeks of exercise training with the consumption of probiotics in cancer survivors (n = 16).

Species (%)	PALL-pre	PALL-post	p-value
Bacteroides fragilis	1.04E+00±1.35E+00	5.72E-01±8.25E-01	0.05
Streptococcus salivarius	2.78E-01±2.21E-01	3.17E-01±7.09E-01	0.05
Paenibacillus pabuli	1.52E-03±2.61E-03	0.00E+00±0.00E+00	0.03
Paraclostridium bifermentans	8.37E-02±8.31E-02	2.87E-01±4.66E-01	0.03
Clostridioides difficile	6.57E-01±4.56E-01	1.56E+00±1.96E+00	0.01
Paeniclostridium sordellii	3.85E-02±2.44E-02	1.15E-01±1.15E-01	0.01
Clostridium carnis	1.25E-03±2.17E-03	7.46E-03±1.08E-02	0.03
Clostridium baratii	1.08E-02±1.35E-02	7.83E-02±1.89E-01	0.05
Lactobacillus casei	5.04E-03±1.62E-02	2.92E-02±5.03E-02	0.04
Mycoplasma feliminutum	1.14E-03±2.25E-03	1.70E-04±6.82E-04	0.04
Clostridium aerotolerans	3.55E-02±3.42E-02	7.67E-02±5.89E-02	0.04
Clostridium quinii	6.90E-03±1.05E-02	2.61E-02±3.23E-02	0.04
Abiotrophia defectiva	9.55E-04±1.56E-03	0.00E+00±0.00E+00	0.03
Clostridium sartagoforme	1.86E-03±1.94E-03	2.22E-02±5.67E-02	0.04
Dorea longicatena	5.07E-04±1.19E-03	1.93E-03±1.98E-03	0.04
Veillonella ratti	2.60E-02±2.62E-02	5.63E-02±6.34E-02	0.02
Prevotella copri	4.92E-04±8.92E-04	1.48E-02±3.30E-02	0.04
Sporotomaculum syntrophicum	2.74E-03±3.46E-03	3.11E-04±8.54E-04	0.02
Bacteroides barnesiae	2.02E-03±5.50E-03	4.95E-03±1.21E-02	0.04
Bacteroides propionicifaciens	1.53E-03±2.56E-03	4.95E-03±5.85E-03	0.04
Veillonella rogosae	1.67E-03±1.88E-03	5.19E-03±3.55E-03	0.00
Alistipes inops	1.90E-02±7.48E-02	1.44E-01±3.91E-01	0.04

Notes: values are presented as mean ± SD; differences were considered significant at $p<0.05$ Abbreviations: PALL-children diagnosed for acute lymphoblastic leukemia

post-intervention serum c-reactive protein levels were not significantly different in children cancer survivors (2.12±6.59 vs. 2.75±7.74 mg/l; p=0.16). We didn't find a significant correlation between LDH, CRP, and the relative abundance of any bacterial taxa within the PALL groups.

Discussion

The reported controlled trial was used to study the effect of an 8-week physical exercise program, along with dairy probiotic consumption, on the gut microbiota composition in children 1–3 years after completion of treatment for ALL. We hypothesized that differences in bacterial richness between healthy controls and pediatric oncology patients ≥12 months after cancer treatment would be apparent. We also hypothesized that the combination of physical exercise and probiotics would have a positive influence on the structure of the gut microbiome in childhood cancer survivors in remission.

In this study, we confirmed differences in the profile of the gut microbiome between childhood cancer survivors and controls. The main findings demonstrate a significant positive effect of exercise and probiotics on a) bacterial diversity (Shannon, Simpson, and Chao1 indices); and b) lactate acid bacteria abundance. Furthermore, we have detected an increase in the relative abundance of the genera *Veillonella* associated in the literature with physical fitness improvements and benefits.

First of all, our results confirmed existing evidence concerning the persistent effects of cancer therapy on the intestinal community in children cancer survivors [13–17]. During and after cancer treatment, the relative abundance of a healthy gut microbiome substantially decreases in children with cancer [40]. Using 16S rRNA gene sequencing, [41] compared the gastrointestinal microbial composition in stool samples from nine survivors of childhood ALL with 10 healthy sibling controls. Years after therapy, the composition of the intestinal microbiome differed between pediatric ALL survivors and healthy sibling controls [41]. Here, we report considerable differences in the relative abundance of commensal and pathogenic bacteria between ALL survivors and controls.

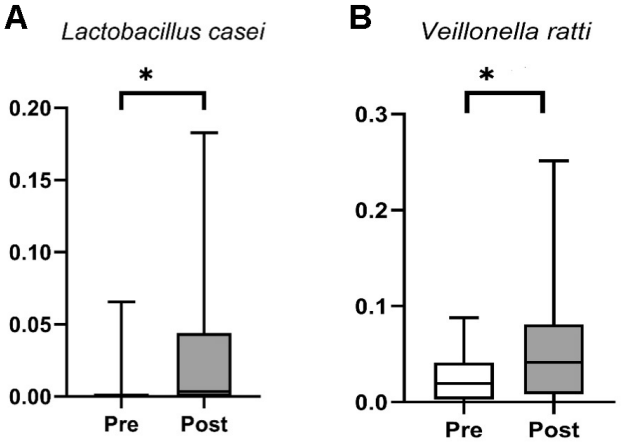

Figure 4. A) The relative abundance (%) of *Lactobacillus casei* and B) the relative abundance (%) of *Veillonella ratti* in PALL (n=16) between pre- and post-intervention, *p<0.05.

Although it is well known that treatment for childhood cancer reduces endurance, strength, and useful mobility, the results of exercise in pediatric cancer patients are less compelling than those in adults [42]. Supportive research on physical exercise in pediatric cancer patients during treatment has shown positive effects on weight, body mass index, body fat, and fat-free mass [23], on quality of life and fatigue symptoms [24], on children's pain [25], and on decreasing the risk of infection associated with treatment [26].

Unfortunately, there is scant information accessible on how exercise training affects a child's gut microbiome when they have cancer. Besides that, we reported a shift in bacterial diversity and changes in the relative abundance of several taxa between pre- and post-treatment in ALL survivors. It is noteworthy that we have found an increased bacterial genus of *Veillonella* (*V. ratti* and *V. rogosae*). This symbiotic bacterium was recently associated with exercise performance [43–45]. This strain may help elucidate two-way traffic on the highway between gut microbiota and muscle [44, 45] demonstrating that serum lactate crosses the epithelial barrier to enter the gut lumen. In addition, they found that *Veillonella atypica* increases athletic performance by metabolically converting exercise-induced lactate into propionate, identifying a natural, enzymatic process that is encoded by the microbiome and improves athletic performance [45].

Therefore, *Veillonellaceae* may also have an adaptive advantage in the guts of athletes by utilizing a distinct metabolic niche: L-lactate metabolism, as this family of microbes has been found to be more prevalent in athletes compared to sedentary controls in numerous independent human studies [46]. The identification and isolation of *Veillonella* in athletes raise the possibility that anaerobic bacteria may affect the Cori cycle, which controls the exchange of information between the gut microbiota and muscle, as well as the Cori cycle itself [46]. A significantly higher abundance of the bacterial genus *Veillonella* was observed after moderate-intensity continuous training compared to baseline in sedentary subjects [47]. Interestingly, Murri et al. [48] found a significant increase in the number of *Veillonella* in children with type 1 diabetes. This strain, which has the aforementioned capacity to ferment lactate to propionate, may play a part in the control of the levels of gut hormones like glucagon-like peptide 1, ghrelin, and glucose-dependent insulinotropic polypeptide [48]. However, it is unclear if *Veillonella* increases specifically in cancer patients after physical exercise. Unfortunately, in this study, we did not detect changes in serum LDH before and after physical exercise intervention. Furthermore, we do not report a significant association between serum LDH and *Veillonella* strains. More research is needed to determine

the relationship between *Veillonella* and physical exercise in cancer patients.

One more goal of our research was to determine whether consuming dairy probiotics can alter the relative abundance of lactate acid bacteria in the patient's collected stools. The use of prebiotics and probiotic foods in children with cancer appears to improve the microenvironment of the gut during chemotherapy treatment rather than at the beginning of treatment [49]. Moreover, both prebiotic and probiotic interventions reduce infection and morbidity risk in pediatric cancer patients [49]. The latest results indicate that the use of a healthy diet and probiotics can be a great alternative for the improvement of gastrointestinal symptoms and the adverse effects associated with cancer treatment [50, 51].Here we report a significantly increased relative abundance of *Lactobacillus casei*. This outcome may have been apparent while L. casei was dominant in the dairy probiotic daily administered to children for 8 weeks. However, interestingly, we have found associations between *Lactobacillus* spp., *L. casei*, and the aforementioned bacterial genus *Veillonella* (*V. ratti*, *V. magna*, and *V. rogosae*). We can therefore speculate that the production of lactate from L. casei found in dairy products may influence the growth of *Veillonella* strains. To gain a better understanding of this relationship, serum and stool metabolomics must be included.

Strengths of our study include the focus on children 1–3 years after completion of BFM-based ALL treatment for cancer survivors with high rates of inactivity and obesity, dairy probiotic use, targeting the early survivorship period, and precise exercise training. Limitations include a lack of physical function and physical fitness measures, serum and fecal metabolomics, and a lack of a control group during the intervention.

In conclusion, our findings suggest that the profile of the gut microbiome negatively differs between healthy children and pediatric patients with acute lymphoblastic leukemia in remission. Promisingly, the exercise program enhanced bacterial richness and diversity when combined with dairy probiotics.

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