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Lifestyle related changes with partially hydrolyzed guar gum dietary fiber in healthy athlete individuals – A randomized, double-blind, crossover, placebo-controlled gut microbiome clinical study

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ABSTRACT

The gut microbiomes association towards a favorable health profile is a rapidly emerging active area of research. This randomized, double-blind, crossover, placebo-controlled study elucidates the impact of prebiotic partially hydrolyzed guar gum (PHGG) dietary fiber on the relative abundance of the gut microbiomes (16S rRNA gene sequencing) and their significant correlation with fecal defecation characteristics among healthy male athletes. Subjects followed a daily six grams dietary intake of either PHGG or placebo for four weeks, following a three weeks washout period, subjects followed another four weeks of crossover alternative administration. Fecal defecation characteristics were assessed using a questionnaire along with quality of life (QOL) questions. The results demonstrated that genus level alteration in gastrointestinal microbiomes relative abundance was correlated to representative changes in fecal defecation characteristics, especially in reduction of diarrhea ($P = 0.035$) and fecal excretory feeling ($P = 0.038$). The PHGG intake led to a significant increase in phylum *Actinobacterium* ($P = 0.04$), along with a significant decrease in *Bacteroidetes* ($P = 0.036$). Also, a trending decrease in *Firmicutes* ($P = 0.09$) was noticed. At genus level, a significant alteration in the relative abundance of *Bifidobacterium* ($P = 0.047$) and *Clostridium* subcluster XI ($P = 0.044$) were observed with PHGG intake compared with placebo. Reduction in diarrhea was associated with the relative abundance of phylum *Firmicutes/Bacteroidetes* ratio and genera *Bacteroides/Prevotella* ratio for the PHGG intervention compared to placebo. In summary, the finding reveals that even at relatively low dosage, intake of prebiotic PHGG dietary fiber modulates gastrointestinal microbiomes, and thus improves gut health.

1. Introduction

The widespread impact of the gut microbiota in human health has emerged in recent years. The gut microbiota composition promotes digestion and food absorption for host energy production (Hsu et al., 2015). Numerous factors can affect the modulation of the gut microbiomes; however, diet and stress are crucial and plays a significant role to maintain health and prevent disease. Changes in diet and exercise can alter the gut microbiomes of humans; however, a very few studies to date have assessed the microbiomes of highly fit athletes and shown the impact of moderate exercise on the gut microbiomes (Mach & Fuster-Botella, 2017). An estimated 20–60% of athletes suffer from the stress caused by excessive exercise and inadequate recovery (Purvis, Gonsalves, & Deuster, 2010). Depending on the type of exercise and intensity factor, between 30 and 60% of athletes suffer gastrointestinal symptoms (Lamprecht et al., 2012). Also, endurance exercise could have a profound impact on intestinal permeability, systematic inflammation and immune responses (Davies, Packer, and Brooks (1981) in an attempt to regain homeostasis and recovery (Morgan, Corrigan, & Baune, 2015). Nevertheless, recognition of the effect of exercise on gut microbiomes composition and structure are still in the early stages and remains inconclusive. Very few studies have shown the impact of moderate exercise on the gut microbiomes (Clark & Mach, 2016). Contrary to American Dietetic Association (ADA) guidelines for low dietary fiber in meals and shakes for athletes to facilitate gastric emptying and minimize gastrointestinal distress (Rodriguez, Di, & Langley,

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2009), insufficient intake of dietary fiber may promote a loss of microbiota diversity and function in the gastrointestinal tract (David et al., 2014; Kuo, 2018). Therefore, a favorable intestinal microbiome profile is vital for the critical nutritional, metabolic, and immunological processes (Ramakrishna, 2013; Belkaid & Hand, 2014) of the healthy athletes.

Plant-based foods are the primary source of bioactive compounds and dietary fiber for active athletes, which are metabolized by microbes to produce different metabolites. Dietary fiber's complex structure includes a variety of compounds that vary significantly in physicochemical properties such as viscosity, solubility, and fermentability that contribute an important role in diversifying the gut's microbiomes (Lattimer & Haub, 2010). In addition, dietary fiber is a nutrient of a public health concern due to under consumption and a lack of fermentable dietary fiber may negatively impact the natural intestinal barrier system and have had an adverse impact on the gut microbiota (Simpson & Campbell, 2015). Metabolites of dietary fiber have, justifiably, received significant attention in the search for possible explanations for the health benefits of dietary fiber. Dietary fiber also has other benefits including increased fecal bulking and laxation, to help maintain intestinal health by increasing both the cell proliferation and cellular specialization functions. Moreover, dietary fiber metabolites help with immune functions and increase absorption of minerals in the gut to support a healthy metabolism, endocrine response, and signaling between the kidneys, liver, and gut.

Partially hydrolyzed guar gum (PHGG), a prebiotic soluble dietary fiber remains undigested in the upper gastrointestinal tract and is readily fermented and metabolized by gut colonic bacteria into short-chain fatty acids (SCFA), mainly acetate, propionate, and butyrate in the colon. After absorption by the colonocytes, these SCFAs either are used locally as fuel for colonic mucosal epithelial cells or enter the portal bloodstream (systemic circulation) and inhibit the expression of specific pro-inflammatory cytokines (Ríos-Covián, Ruas-Madiedo, Margolles, Gueimonde, De Los Reyes-gavilán, & Salazar, 2016; Nastasi et al., 2015). The quantity of SCFAs in the colon is essential for host immune-regulation. It has recently been reported that PHGG increases butyrate-producing bacteria in the intestine, which is an immune-enhancing compound and serving as a significant energy source for gut epithelia (Ohashi, Sumitani, Tokunaga, Ishihara, Okubo, & Fujisawa, 2015) PHGG is also promising for its ability to absorb water during digestion and exerts satiety via slow down the digestion functions (Rao et al., 2015), support adiposity reduction, lower hyperglycemia/ hyperlipidemia, improve glycemic control and metabolic syndrome-related functions such as aberrant lipid and glucose metabolism (Dall'Alba et al., 2013; Kapoor, Ishihara, & Okubo, 2016). A recent systematic review and meta-analysis revealed the significant role of PHGG in the prevention of constipation in healthy subjects, elderly/ children and subjects on laxative aids (Kapoor, Sugita, Fukuzawa, & Okubo, 2017). PHGG has been reported to improve the symptoms associated with both constipation- and diarrhea-predominant forms of irritable bowel syndrome (IBS) (Giannini, Mansi, Dulbecco, & Savarino, 2006). The beneficial effects of dietary PHGG have also been demonstrated in pediatric functional gastrointestinal pain and constipation (Romano, Comito, Famiani, Calamarà, & Loddo, 2013; Ustundag, Kuloglu, Kirbas, & Kansu, 2010). PHGG does not form gel even at high doses, had been facilitated its use as dietary fiber in many kinds of food and widely used in beverage applications (Kapoor & Juneja, 2009; Yoon, Chu, & Juneja, 2006). PHGG safety is well known with established clinical uses. A number of *in vitro*, animal and human clinical studies have confirmed its safety and many physiological health benefits (Rao et al., 2015; Kapoor et al., 2016; Anderson, Fisher, & Talbot, 1993; Finley et al., 2013; Slavin & Greenberg, 2003) and PHGG was generally recognized as safe (GRAS) in the United States of America (Heimbach, 2006).

The present study was designed to examine the influence of prebiotic PHGG dietary fiber intake on gut microbiota responsiveness by assessing the microbiomes relative abundance and their association with fecal defecation characteristics among the healthy athletes using a randomized, double-blind, placebo-controlled design. Among around thousands of bacterial species colonized in healthy human guts, the dominant classified phylum includes *Bacteroidetes, Firmicutes*, and *Actinobacterium* (Lay, Sutren, Rochet, Saunier, Doré, & Rigottier-Gois, 2005; Icaza-Chávez, 2013; Milani et al., 2013). Also, the *Firmicutes* to *Bacteroidetes* ratio is regarded to be of significant relevance in human gut microbiomes composition (Ley, Turnbaugh, Klein, & Gordon, 2006; Thursby & Juge, 2017; Mariat et al., 2009). We hypothesize, a measurable association between gut microbiomes composition and the fecal defecation characteristics in healthy male athletes could be observed from consumption of prebiotic PHGG dietary fiber. Herein, we evaluated the influence of PHGG on concurrent relative changes in several associated variables such as fecal defecation frequency, fecal excretory feelings (relief from stomach discomforts), loose defecation, hard defecation, and feeling of incomplete defecation. Finally, the results are summarized as a framework for common constituents of the gut community in athlete individuals who follow an exercise-rich lifestyle.

2. Materials and procedural methods

2.1. Participants and preliminary selection

The ethical committee of Otsuma Women's University approved the study protocols and agreement was registered at the university information network. The study was conducted according to the guidelines laid out in the declaration of Helsinki, and based on the Ethics Guidelines for Epidemiological Study (Noticed from the Ministry of Education, Culture, Sports, Science and Technology, and the Ministry of Health, Labor, and Welfare of Japan). Participants gave informed written consent and were well informed about the purpose of the study. The study was designed as a randomized, double-blind, placebo-controlled, crossover trial of 157 healthy Japanese male athletes mainly the soccer players. Participants were recruited from the Sports Faculty of University of Tsukuba, Japan (Age, 19–22 years; BMI, 19.8–24.6 Kg/m^2). A questionnaire was distributed regarding quality of life (QOL), anthropometric, diet, lifestyle, sleep, bowel habits, supplement uses, medical conditions, etc. to 154 individuals who expressed interest in the study (Fig. 1A). Quality of life (QOL) covariates determined from the questionnaire responses from the study participants are listed in Table S1. Inclusion criteria of the study included male athlete only, aged 18 years or older, of Japanese ethnic background. Healthy, male athletic subjects had to be willing and able to provide blood (in case required), stool specimens and provide signed informed consent. While exclusion criteria of the study were; Body mass index (BMI) higher than 30 kg/m^2, blood pressure greater than 160/100, an oral temperature higher than 100°F, and pulse rate higher than 100. Subjects were excluded if they use any drugs that influence the gut microbiome including systemic antibiotics, antifungals, antivirals, and anti-parasitic (oral, intravenous, intramuscular, nasal or inhaled) within the last six months.

Further, the subjects were excluded they consumed commercial probiotics. Also, the subjects were excluded for any acute disease or chronic pulmonary, cardiovascular, gastrointestinal, hepatic or renal functional abnormality. Also subjects with major surgery of the gastrointestinal tract in the past five years; any primary bowel resection at any time; history of active uncontrolled gastrointestinal disorders or diseases such as inflammatory bowel disease (IBD), Crohn's disease; irritable bowel syndrome (IBS); *Clostridium difficile* infection or *Helicobacter pylori* infection; and chronic constipation were excluded from the study. Lastly, subjects with the unstable dietary history as defined by significant changes in diet during the previous month were excluded. Based on the screening as mentioned above, procedures and QOL covariates statistical correlation, fifty-three (n = 53) subjects were preliminarily accepted for further study (see Fig. 1A).

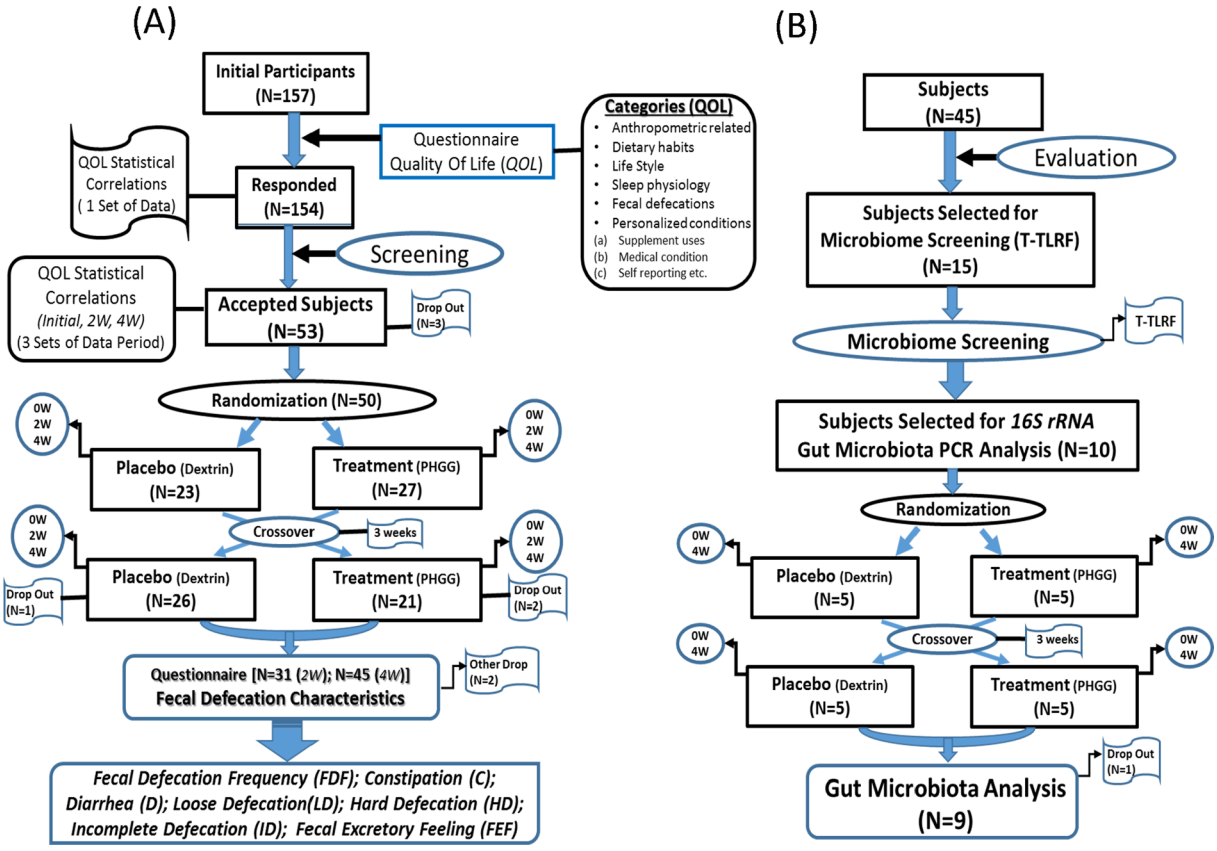

Fig. 1. (A) Schematic flow diagram of study protocol and procedures. The interventions are blinded to the lead researcher, analysts, and participants. (B) Illustration of trial procedural details to evaluate the effect of prebiotic PHGG dietary fiber intervention on the relative abundance of fecal microbiome determined using real-time PCR amplification of the 16S rRNA gene.

2.2. Material and characteristics

Partially hydrolyzed guar gum (PHGG) derived from guar gum, commercially available under a brand name Sunfiber® (or Guar fiber), is mostly studied as unique water-soluble, almost tasteless, colorless, and odorless dietary fiber with relatively low viscosity. PHGG manufactured by controlled enzymatic hydrolysis of guar gum seeds (*Cyamopsis tetragonolobus L.*; β-1,4-linked mannose with α-1,6-linked galactose units) is relatively low viscous (< 12 cps@5% of PHGG), and lower molecular weight (~20,000 Da; approximately a 10-fold reduction in the degree of polymerization found in intact guar gum) dietary fiber consists of nearly 80% soluble dietary. In dry form it absorbs less than 7.0% moisture, contains less than 1.0% proteins and less than 2.0% ash (Kapoor & Juneja, 2009).

2.3. Supplementation and dosages

Subjects consumed a daily dietary supplementation of six gram (6 g) PHGG { > 80% total dietary fiber, 5 g soluble fiber as measured by Association of Official Agricultural Chemists (AOAC) method 2009.01; Taiyo Kagaku, Co. Ltd., Japan} or placebo (dextrin; Sanwa Starch Co., Ltd., Japan) during the meal every day with either water or tea for entire duration of the trial without modifying the diet regimen, lifestyle and/or daily exercise habits. Identical PHGG or placebo containing sachets were supplied, and compliance was assessed at every visit by counting the number of remaining sachets that subjects were asked to return. Also, subjects were asked to describe any unusual symptoms or signs in order to evaluate the possible adverse effects related to PHGG supplementation. Subjects were instructed not to abruptly change their food intake pattern two weeks before the study and during the trial period. Also, to ensure that all subjects were roughly in energy balance, they were asked to continue their routine and structured physical activity as well as physical training as an athlete. A six gram PHGG containing nearly five gram soluble fiber was chosen because it was an appropriate level known to be effective in glycemic responses, health-related circulated metabolites (Dall'Alba et al., 2013; Kapoor et al., 2016) as well as reported for objective evidence of efficacy in functional gastrointestinal disorders (Kapoor et al., 2017).

2.4. Study design and enforcement parameters

The study design is a randomized, double-blind, crossover, and placebo-controlled study. Before randomization and start of the study, the three (n = 3) subjects were eliminated from the study after reexamination of the inclusion/exclusion criteria. Therefore, fifty (n = 50) subjects were randomized assigned to begin with PHGG (active) and placebo (dextrin) according to a computer-generated randomization plan by an independent researcher. A detailed schematic protocol of the present study is illustrated in Fig. 1A. No significant differences were observed in anthropometric between the PHGG and placebo groups at baseline. Placebo group (n = 23) and PHGG group (n = 27) received blinded sachets to consume every day with the meal for four weeks according to the study protocol. After the four weeks of respective intakes, a three weeks washout period was implemented before crossover the study. Two (n = 2) subjects who received placebo and one (n = 1) subject of PHGG group dropped out of the study after the first treatment period (see Fig. 1). Subjects completed a quality of life (QOL) questionnaire at baseline and every two weeks during supplementation. Subjects were instructed to complete a questions related to fecal defecation characteristics including fecal defecation frequency (FDF), constipation (C), Diarrhea (D), loose defecation (LD), hard defecation (HD), incomplete defecation (ID), and fecal excretory feeling (FEF). Subjects

provided fecal samples for the subsequent study. Finally, at the end of the study trial, the thirty-one (n = 31) subjects who completed the QOL questionnaire at baseline, after two weeks and four weeks during their both respective intakes of prebiotic PHGG and placebo, had their data statistically analyzed. Whereas, the forty-five (n = 45) subjects who at least completed the prescribed QOL questionnaire at baseline and after four weeks of their respective intakes during the study period are also analyzed and reported for the significance levels. Two (n = 2) subjects were excluded from analysis due to noncompliance to the study protocol.

2.5. Laboratory evaluations

We explored the capability of 16S-ribosomal-RNA (16S rRNA) gene-based sequence profiling assigned to operational taxonomic units (OTUs) using terminal restriction fragment length polymorphism (T-RFLP) analysis and real time polymerase chain reaction (PCR) analysis. Particular PCR primers for amplification of the targeted region of the 16S rRNA gene were employed, pivotal for facilitating the intestinal microbiomes abundance among healthy human athlete subjects (Nagashima, Hisada, Sato, & Mochizuki, 2003; Li, Hullar, & Lampe, 2007). Isolation of fecal bacterial DNA, the amplification of the fecal 16S ribosomal DNA (rDNA), restriction enzyme digestion, size-fractionation of terminal restriction fragments (T-RFs) and the T-RFLP analysis were carried out as described previously (Nagashima et al., 2003; Nagashima, Mochizuki, Hisada, & Shimomura, 2006).

2.5.1. DNA extraction, PCR conditions and quantitative PCR amplification

Fecal bacterial DNA from healthy athletes was stored at 4 °C until analysis. DNA was extracted after enzymatic digestion treatment of the washed fecal samples (Liu, Marsh, Cheng, & Forney, 1997) followed by bead-beating steps employing bead treated suspension by using benzyl chloride as described by Zhu, Qu, and Zhu (1993), and QIAamp Stool DNA mini Kit (Qiagen, Tokyo, Japan). The procedural details on the purification of PCR products and restriction enzyme digestion are already described in Nagashima et al. (2003). In brief, PCR was performed using the extracted bacterial fecal DNA and the barcoded fusion primers of 5_ FAM-labeled 516f (5¢-TGC CAG CAG CCG CGG TA-3¢; Escherichia coli positions 516 to 532) and 1510r (5¢-GGT TAC CTT GTT ACG ACT T-3¢; *E. coli* positions 1510–1492) for PCR amplification of the targeted region of the 16S rRNA gene. In the case of amplicons digestion with BslI (5¢–CC NNNNN|NNGG-3¢) or BseLI, which is an isoschizomer of BslI, a reaction mixture (10 μl) containing 2 U of BslI (New England BioLabs, Ipswich, MA, U.S.A.) or BseLI (MBI Fermentas, Amherst, N.Y.), 1x NEB buffer 3 (New England BioLabs, Ipswich, MA, U.S.A.), 2 μl of the PCR product from the fecal DNA, and 0.5 μl of either of two kinds of the internal control DNAs was incubated at 55 °C for 1 h.

2.5.2. Terminal restriction fragment length polymorphism (T-RFLP) analysis

Gut microbiome abundance was determined in the Techno Suruga Laboratory Co., Ltd. Shizuoka, Japan. Terminal restriction fragment length polymorphism (T-RFLP) analysis with suitable combinations of primers that provide more accessible prediction/ validation of the kind of intestinal bacterial communities corresponded to each operational taxonomic units (OTU) based on the terminal restriction fragment length compared to the conventional T-RFLP was adopted. The fluorescently labeled T-RFs were analyzed by electrophoresis on an ABI PRISM 3130xl Genetic Analyzer automated sequence analyzer (DNA Sequencer: Applied Biosystems, Carlsbad, CA, U.S.A) in GeneScan mode using GeneMapper (Applied Biosystems) according to a modified procedure as reported previously (Nagashima et al., 2003). Two microliters of the restriction enzyme digestion mixture were mixed with 0.5 μl of four fold-diluted GeneScan-2500 size standard (Applied Biosystems) and 12 μl of deionized formamide, followed by denaturation at 96 °C for 2 min and immediate chilling on ice. The injection time was 40 s for that of T-RFs from the digestion with BslI or BseLI. The run time was 40 min.

The lengths and peak areas of T-RFs were determined with the GeneScan software. A standard curve was drawn by using DNA fragments. To compare the T-RFLP patterns among samples, the dissimilarity (D) index was used. The major T-RFs were identified by computer simulation using a T-RFLP analysis MiCA3 program (Shyu, Soule, Bent, Foster, & Forney, 2007). MiCA3, is a phylogenetic assignment database for T-RFLP analysis of human colonic microbiota (PAD-HCM) and Microbiota Profiler (InfoCom Co., Tokyo, Japan; [http://mica.ibest.uidaho.edu/]). Cluster analysis was performed using analysis software GeneMaths (Applied Maths, Belgium) based on OTUs distributed from the T-RFs by the method reported in Nagashima et al. (2006). The Euclidean distances between correlation and coefficients were calculated (unweighted pair-group methods with arithmetic mean algorithm; UPGMA) to determine any similarity among the samples.

2.6. Gut microbiome study protocol and assessments

To further explore the changes in gut microbiota diversity and relative abundance during the respective intakes of PHGG or placebo, the subjects were further evaluated based on the overall scores concerning to the fecal defecation characteristics determined using the QOL questionnaire during the study periods. A schematic illustration of the gut microbiome study protocol is presented in Fig. 1B. The fifteen (n = 15) subjects were selected for the microbiomes screening test using terminal restriction fragment length polymorphism (T-RFLP) analysis. Initially, the ten (n = 10) subjects who scored 10 points for combined fecal defecation characteristics parameters were selected. Besides, four (n = 4) subjects who scored 3 points for a tendency to have diarrhea (D) were considered, while one (n = 1) subject who scored 3 points for fecal excretory feeling (FEF) as of secondary selection criteria were included for the microbiomes analysis. The subjects who scored at least 9 points for combined fecal defecation characteristics parameters through secondary selection were included for the gut microbiota analysis. The fecal samples collected from the subject were examined for the screening study. After the gut microbiomes screening by T-RFLP analysis, the results were evaluated at genera level to monitor the relative abundance of the bacterial population in the gut. Notably, the abundance of phyla A*ctinobacterium*, *Bacteroidetes*, and *Firmicutes* were evaluated for all selected subjects. Also, the significant genera were identified, including the *Bifidobacterium, Bacteroides, Prevotella, Lactobacillales, Clostridium* clusters, and *Roseburia* & *Eubacterium rectale*, etc. The relative abundance of unclassified genera was decided to compare under other categories.

Based on the aforementioned screening, finally the ten subjects (n = 10) were chosen to evaluate the study to determine the effect of prebiotic PHGG dietary fiber on the gut microbiome diversity and /or relative abundance using T-RFLP analysis of 16S rRNA and PCR gene amplicons technology wherein gene was amplified from fecal DNA from selected subjects employing the real-time PCR analysis. The final ten subjects (n = 10) were chosen on the essential criteria of the total abundance of *Bifidobacterium* and *Clostridium* subcluster XIVa genera. Of the fifteen subjects (n = 15) screened using T-RFLP analysis, the five subjects (n = 5) were excluded due to high total of *Bifidobacterium* and *Clostridium* subcluster XIV. Further, using specific primers, the relative change in the abundance of phyla *Actinobacterium*, *Bacteroidates*, and *Firmicutes* were evaluated for selected subjects with real-time PCR analysis. In a computer-generated randomization plan by an independent researcher, the subjects (n = 10) were assigned to order of either prebiotic PHGG dietary fiber or placebo as of two groups of the equal block size (n = 5). The study was conducted as a randomized, double-blind crossover, and placebo-controlled manner (see Fig. 1B). Subjects were instructed to submit their fecal samples at baseline, after four weeks of the either prebiotic PHGG or placebo intakes, after the washout period, and again after four weeks of the other treatment.

Subjects also maintained the same diet throughout the study, and answered the QOL questionnaire with particular emphasis on fecal defecation characteristics as described previously. During the crossover duration of the study, one (n = 1) subject was removed from the study due to noncompliance to the study protocol. Fecal samples were collected and stored at 4 °C until microbiomes analysis.

2.7. Statistical analysis and data processing

Apart from the T-RFLP analysis including quantification of T-RFs and cluster analysis to pattern the OTUs, the statistical analyses were performed using the one-way analysis of variance (ANOVA), analysis of cofactor variance (ANCOVA) with study duration as a repeated measure followed by Tukey's post hoc test, or pre/post ANCOVA model. The P value ≤ 0.05 was considered statistically significant and at ≤ 0.10 was regarded as trending significance. Spearman's correlation coefficients (r) and P values were used to determine the correlation between covariates evaluated using QOL questionnaire. The related variables of the categorized questionnaire on QOL were evaluated used a post-hoc analysis of baseline versus values after both PHGG and placebo supplementation. All values are expressed as means ± SEM unless otherwise stated. An independent two-tailed Student's *t*-test was performed to verify the statistical significances further where necessary.

At the beginning of the gut microbiome study, the sample size was tentatively calculated to assure physiological relevant changes in studied variables with a power of 90%, assuming a significance level of $P \leq 0.05$ and drop out of $< 10\%$. Also, post hoc power calculations with sample size used in the gut microbiomes analysis were performed using online StatWeb system version 1.1.1 (Department of Statistics, University of British Columbia, Canada), to assess the power and validation of study parameters. Taking a two-sided 5% significance level, finally, the retrospective power analysis was performed to confirm the achieved effect size (i.e., power > 80%), which is theoretically necessary to detect a difference (changes from baseline) in study variables between the both PHGG and placebo groups. Usually of continuous coordinates related to microbiomes abundance was assessed; however, data were Log transferred to adjust the perspective comparisons to control the false revealing rate. To explain the identified microbiome features (phyla, genera or taxa), the ANCOVA algorithm was used to verify the significant differential abundance concerning the class of interest, and ANOVA test to assess biological consistency according to the established protocol.

3. Results

3.1. Subjects and baseline characteristic

The primary baseline characteristics of the athlete Japanese participants were evaluated at different stages of the study and are presented in Table 1. No statistical differences were noted at the baseline confirming a homogeneous representation of the study participants. Of the fifty-three accepted subjects (n = 53) who provided informed consent, three subjects (n = 3) did not start in the study for personal reasons. Further, of the fifty (n = 50) eligible subjects randomized to placebo and PHGG intake groups, three (placebo = 2; PHGG = 1) did not complete the study as they could not wholly compliance with the study protocols. Forty-seven subjects completed the study; however, two subjects were excluded from the PHGG intervention because data were not collected. The data collected from forty-five subjects (n = 45) at baseline and after four weeks, as well as from thirty-one subjects (n = 31) who completed the PHGG and placebo periods at baseline, after 2 weeks, and 4 weeks were included in the data analysis (see Fig. 1A).

3.2. Quality of life (QOL): Features of prebiotic PHGG dietary fiber rich diet

The initial QOL variables were subjected to a Principal Component Analysis (PCA) to evaluate the related datasets to explain a maximal amount of variance. Standardization was performed by subtracting the mean and dividing by the standard deviation for each value of each variable, so all the variables were transformed and simplified to the same scale. Screen plot evaluates the variation that each principal component captures from the datasets, and in each case, the first two principal components (PC1 & PC2) were found sufficient to describe most of the dataset. The unweighted PCA biplot displays both principal component scores (dots) and loadings of variables (vectors). Typically, a small angle implies a positive correlation, a large angle (> 90°) suggests a negative correlation between variables. Also, the further away the vectors are from a principal component origin, the more influence they have on the respective principal component. Principal component analysis of initial 154 subjects responded the QOL questionnaire demonstrate that there was sizeable inter-individual variability in datasets responses regarding QOL while displaying a positive correlation between significant fundamental covariates concerning to fecal defecation characteristics, body condition, total body health, gastrointestinal physique and mental physique (Fig. S1a). The detail on sub-parameters of each covariate along with cluster analysis (Fig. S1b) is also listed in the supplementary material.

Furthermore, the Fig. S2 shows the well-separated clusters for different covariates in a principal component analysis (score and loading plots) from selected forty seven (n = 47) subjects of this study responsive to PHGG dietary fiber and/or placebo intervention at the baseline (Fig. S2a) and after completion of the study (Fig. S2b). The covariates such as fecal defecation characteristics, gastrointestinal physique, and meal intake were positively correlated to food and exercise after placebo and PHGG fiber intake compared to the baseline. The Fig. S3 displays a heat map and hierarchical clustering analysis representing an overview of the distribution of QOL covariates of the

Table 1
Baseline characteristic profiles comparison of healthy male athletes at different stages of the study indicate a homogeneous representation of subjects during the trial periods (values are presented as Mean ± SEM).

Characteristics	Healthy Male Athlete Subjects*			P- Value†
	Responded n = 154 (Analysis: 1 set)	Participated n = 47 (Analysis: 3 sets)	16S rRNA (PCR) n = 10 (Analysis: 3 sets)	*ANOVA*
Height (Cm)	172.7 ± 0.43	172.3 ± 0.73	172.5 ± 1.19	0.98
Body Weight (Kg)	66.5 ± 0.45	66.4 ± 0.80	66.3 ± 1.73	0.94
BMI (Kg/m^2)	22.3 ± 0.10	22.1 ± 0.27	22.3 ± 0.40	0.85
Total Body Fat (%)	10.9 ± 0.15	10.9 ± 0.31	11.5 ± 0.91	0.54
Lean Body Mass (Kg)	59.5 ± 0.38	58.5 ± 0.88	58.6 ± 1.27	0.62
Age (Years)	20.06 ± 0.29	20.49 ± 0.16	20.9 ± 0.28	0.93

† One Way ANOVA.
* Soccer athletes.

Table 2
Changes in QOL covariate fecal defecation characteristics during the placebo and PHGG dietary fiber intervention in healthy athletes (n = 31) who completed the study according to the protocol. (Mean ± SEM values).

	Placebo group (Dextrin); n = 31‡			Treatment group (PHGG); n = 31‡			ANCOVA (Between Groups)	
							P-Value; F-Value	
	0 W	*2 W*	*4 W*	*0 W*	*2 W*	*4 W*	*2 W*	*4 W*
Fecal Defecation Frequency							P = 0.73; F = 0.12	P = 0.25; F = 1.36
Mean ± SEM	0.90 ± 0.14	0.81 ± 0.13	0.87 ± 0.13	0.87 ± 0.13	0.81 ± 0.12	0.81 ± 0.13		
ANOVA: P-Value; F-Value		P = 0.18; F = 1.85	P = 0.67; F = 0.19		P = 0.33; F = 1.0	P = 0.34; F = 0.99		
Constipation							P = 0.33; F = 0.97	P = 0.73; F = 0.12
Mean ± SEM	0.81 ± 0.12	0.90 ± 0.14	0.87 ± 0.13	0.94 ± 0.14	0.84 ± 0.12	0.84 ± 0.13		
ANOVA: P-Value; F-Value		P = 0.26; F = 1.3	P = 0.42; F = 0.66		P = 0.18; F = 1.85	P = 0.18; F = 1.85		
Diarrhea							P = 0.93; F = 0.01	**P = 0.045*; F = 4.2**
Mean ± SEM	1.26 ± 0.18	1.39 ± 0.19	1.40 ± 0.21	1.39 ± 0.19	1.29 ± 0.19	1.19 ± 0.18		
ANOVA: P-Value; F-Value		P = 0.16; F = 2.07	P = 0.21; F = 1.63		P = 0.41; F = 0.69	**P = 0.05*; F = 3.94**		
Loose Defecation							P = 0.71; F = 0.14	P = 0.65; F = 0.21
Mean ± SEM	1.26 ± 0.18	1.26 ± 0.9	1.19 ± 0.18	1.29 ± 0.19	1.19 ± 0.18	1.23 ± 0.18		
ANOVA: P-Value; F-Value		P = 1.0; F = 0	P = 0.54; F = 0.34		P = 0.18; F = 1.85	P = 0.49; F = 0.49		
Hard Defecation							P = 0.86; F = 0.03	**P = 0.09#; F = 2.8**
Mean ± SEM	0.90 ± 0.13	0.90 ± 0.13	0.97 ± 0.14	0.94 ± 0.14	0.94 ± 0.15	0.77 ± 0.12		
ANOVA: P-Value; F-Value		P = 1.0; F = 0	P = 0.42; F = 0.66		P = 1.0; F = 0	P = 0.13; F = 2.37		
Incomplete Defecation							P = 0.41; F = 0.69	P = 0.42; F = 0.67
Mean ± SEM†	1.17 ± 0.17	1.24 ± 0.18	1.25 ± 0.19	1.21 ± 0.19	1.14 ± 0.19	1.18 ± 0.19		
ANOVA: P-Value; F-Value		P = 0.42; F = 0.66	P = 0.18; F = 1.85		P = 0.42; F = 0.66	P = 0.57; F = 0.33		
Fecal Excretory Feelings							P = 0.12; F = 2.51	**P = 0.089#; F = 2.99**
Mean ± SEM	1.23 ± 0.18	1.32 ± 0.19	1.32 ± 0.20	1.19 ± 0.18	1.10 ± 0.18	1.13 ± 0.17		
ANOVA: P-Value; F-Value		P = 0.26; F = 1.3	P = 0.26; F = 1.3		P = 0.26; F = 1.3	P = 0.16; F = 2.07		

Trending Significance (P ≤ 0.10).
* Significant (P ≤ 0.05).
‡ n = 31; ANOVA (P ≤ 0.05): 0 W, 2 W and 4 W.
† n = 28.

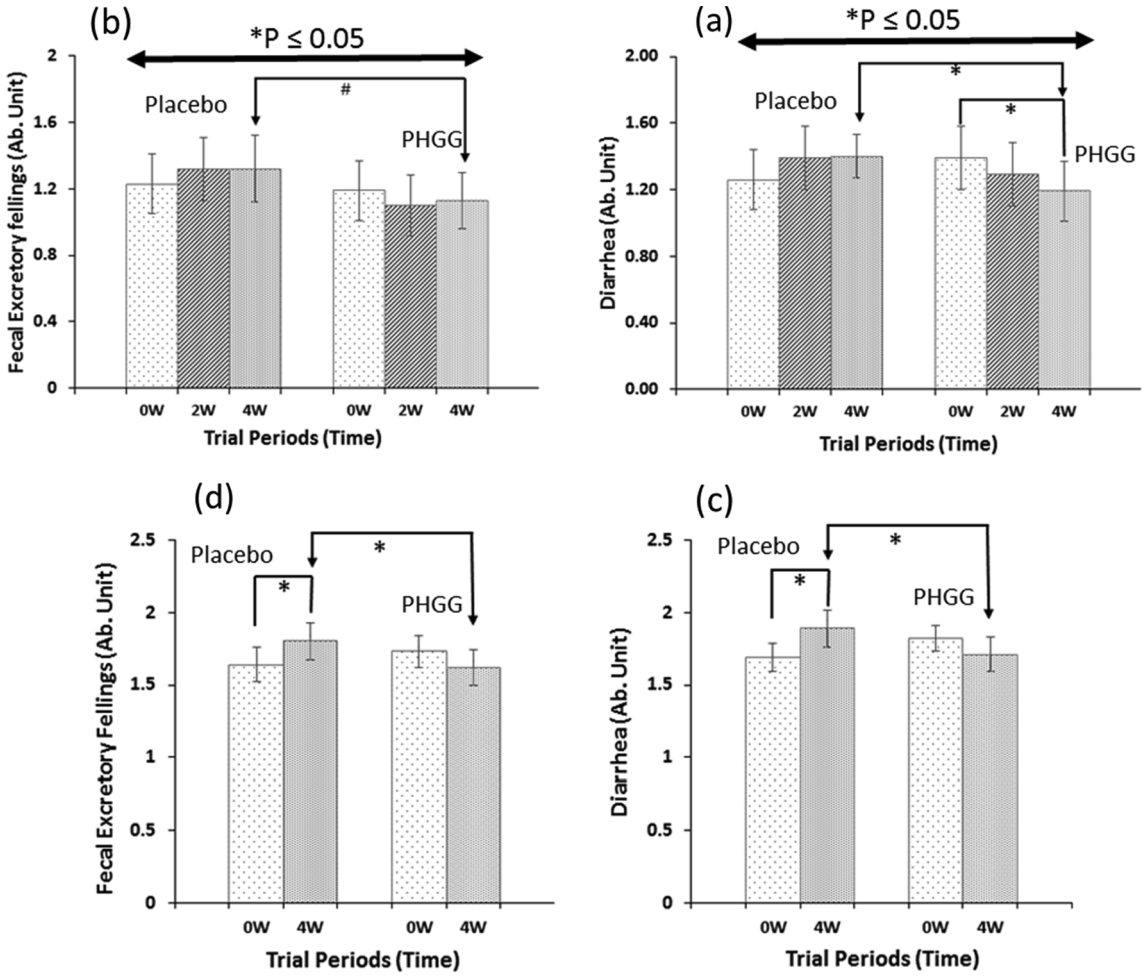

Fig. 2. Time-dependent effect of PHGG dietary fiber and placebo interventions on QOL covariate fecal defection characteristics showed (a) reduction of diarrhea, and (b) improved fecal excretory feelings in thirty-one subjects (n = 31). And, revealed further significance differences in (c) diarrhea reduction and (d) fecal excretory feelings estimated in forty-five subjects (n = 45). One-way ANOVA (intergroup); repeated measure ANCOVA (between the PHGG and placebo groups @ time interaction; n = 31 subjects only; two-sided arrow dark line); ANCOVA (between the PHGG and placebo groups). Significance: *P < 0.05; Trending Significance: #P < 0.10.

individual participant at baseline, and after placebo and PHGG fiber intakes of the study.

3.3. Fecal defecation characteristics

Thirty-one subjects (n = 31) completed the QOL questionnaire at baseline, two weeks and four weeks. The results related to the fecal defecation characteristics section (Table 2) indicates the steady reduction of diarrhea symptoms in the PHGG intake group. A statistically significant (P = 0.05; F = 3.94) reduction in diarrhea episodes was observed after four weeks of PHGG supplementation, while diarrhea symptoms were aggravated in the placebo period. Whereas, ANCOVA analysis confirmed the significant difference between the PHGG and placebo groups after four weeks (P = 0.045; F = 4.2) (Fig. 2a). Corresponding relief in fecal defecation frequency, incomplete defecation, and loose/hard type defecation was observed in PHGG group. Further, when comparing the PHGG and placebo periods, a significant trend toward improvement in both interrelated fecal excretory feelings (P = 0.089; F = 2.99) symptoms was observed (Fig. 2b). Whereas, results of ANCOVA analysis on fecal defecation characteristics of the participants (n = 45) responded to the QOL questionnaire at baseline and after four weeks (Table 3) also suggests an identical trend confirming the reduction of diarrhea episodes (P = 0.035; F = 4.61) and significance improvement (P = 0.038; F = 4.42) in fecal excretory feelings (relief from stomach discomforts) between the PHGG and placebo periods (Fig. 2c & 2d). Also noteworthy that the significant differences noticed for reduction in diarrhea, fecal excretory feelings and trend for incomplete defecation worsened during the placebo period (see Table 3).

3.4. Gut microbiota analyses

The results describing a standard profile of relative abundance of OTUs of the selected 15 healthy athlete subjects obtained from terminal restriction fragment length polymorphism (T-RFLP) analysis are presented in Fig. S4A. The selective criteria are already described earlier in Section 2.6. The OTU's profile presents the relative abundance of phyla *Actinobacterium*, gram-negative *Bacteroidetes* phylum, and gram-positive *Firmicutes* as a dominant phylum in healthy athlete subjects of this study. At the genus level, phyla *Actinobacterium* comprised the *Bifidobacterium* as a dominant genus, while phyla *Bacteroidates* consists of genera *Bacteroides* and *Prevotella.* The most abundant phylum in the gut microbiomes was the phyla members *Firmicutes*, mainly the genera *Lactobacillales*, *Roseburia-Eubacterium rectale*, and members *Clostridium* clusters. Wherein, the subdominant groups of the *Clostridium* genus includes the *Clostridium* Clusters IV, XI, and XIVa. Using specific primers, relative changes in the abundance of phyla *Actinobacterium*, *Bacteroidates* and *Firmicutes* were evaluated for ten (n = 10) selected

Table 3
Changes in QOL fecal defecation characteristics during the placebo and prebiotic PHGG dietary fiber intervention in the whole cohort of healthy athletes (n = 45) at baseline and after completion of the study. (Mean ± SEM values).

	Placebo period (Dextrin); n = 45		Treatment period (PHGG); n = 45		ANCOVA *P-Value; F-Value*
	0 W	*4 W*	*0 W*	*4 W*	*Between Groups*
Fecal Defecation Frequency					P = 0.45; F = 0.57
Mean ± SEM	1.53 ± 0.11	1.58 ± 0.16	1.49 ± 0.11	1.62 ± 0.16	
ANOVA: P-Value; F-Value	P = 0.70; F = 0.15		P = 0.18; F = 1.83		
Constipation					P = 0.88; F = 0.02
Mean ± SEM	1.18 ± 0.08	1.27 ± 0.10	1.27 ± 0.08	1.33 ± 0.11	
ANOVA: P-Value; F-Value	P = 0.25; F = 1.34		P = 0.41; F = 0.69		
Diarrhea					**P = 0.035*; F = 4.61**
Mean ± SEM	1.69 ± 0.10	1.89 ± 0.13	1.82 ± 0.09	1.71 ± 0.12	
ANOVA: P-Value; F-Value	**P = 0.027*; F = 5.21**		P = 0.28; F = 1.2		
Loose Defecation					P = 0.55; F = 0.36
Mean ± SEM	1.78 ± 0.10	1.73 ± 0.12	1.69 ± 0.10	1.73 ± 0.12	
ANOVA: P-Value; F-Value	P = 0.62; F = 0.25		P = 0.20; F = 0.66		
Hard Defecation					P = 0.45; F = 0.57
Mean ± SEM	1.38 ± 0.09	1.42 ± 0.09	1.36 ± 0.10	1.36 ± 0.10	
ANOVA: P-Value; F-Value	P = 0.62; F = 0.25		P = 1.0; F = 0		
Incomplete Defecation					P = 0.37; F = 0.81
Mean ± SEM†	1.38 ± 0.13	1.50 ± 0.15	1.43 ± 0.14	1.65 ± 0.11	
ANOVA: P-Value; F-Value	**P = 0.095#; F = 2.91**		P = 0.22; F = 1.55		
Fecal Excretory Feelings					**P = 0.038*; F = 4.42**
Mean ± SEM	1.64 ± 0.12	1.80 ± 0.13	1.73 ± 0.11	1.62 ± 0.12	
ANOVA: P-Value; F-Value	**P = 0.05*; F = 4.02**		P = 0.23; F = 1.49		

† n = 40.
# Trending Significance (P ≤ 0.10).
* Significant (P ≤ 0.05).

subjects with real-time quantitative PCR analysis to determine whether there was a correlation between baseline phylum relative abundance and after prebiotic PHGG fiber intervention. Almost the same pattern and profile with T-RFLP analysis was observed. There were some prebiotic PHGG fiber or placebo driven changes in gut microbiome relative abundance among the subjects (see Fig. S4B). *Firmicutes* was the most abundant phyla in the microbiome, consisting of approximately 58% of all sequence reads, while equally proportioned (nearly 21–22%) *Bacteroidetes* and *Actinobacterium* were the second most abundant phyla identified (Table 4). At the phyla level, *Actinobacterium* relative abundance significantly increased (P = 0.04; F = 5.39), and the gram-negative *Bacteroidetes* (P = 0.036; F = 6.31) relative abundance significantly decreased, while there was also a trending non-significant reduction in gram-positive *Firmicutes* (P = 0.09; F = 3.48) relative abundance during the prebiotic PHGG period. Although, a similar trend was noted during placebo period; no statistically significant changes were detected from baseline (see Table 4). Comparison the prebiotic PHGG and placebo periods, showed a significant difference for *Actinobacterium* (P = 0.001; F = 17.62); whereas no significant differences were found for both *Bacteroidetes* and *Firmicutes* abundance between PHGG and placebo periods. The relative abundance of *Firmicutes* and *Bacteroidetes* decreased during both placebo and PHGG intake periods. The *Firmicutes/ Bacteroidetes* ratio (F/B) was significantly influenced (P = 0.031, F = 6.84) during the PHGG period.

At a genus level, PHGG increased in the relative abundance of *Bifidobacterium* and a significant difference (Fig. 3a) was observed between the PHGG and placebo groups (P = 0.047; F = 4.66). Furthermore, the relative abundance of both *Prevotella* (P = 0.04; F = 6.16), and *Bacteroides* (P = 0.05; F = 5.47) were significantly decreased compared to baseline during PHGG period, but not during the placebo. Notably, no significant differences were found for both *Bacteroides* and *Prevotella* abundance between prebiotic PHGG and placebo groups (Fig. 3b & 3c). The relative abundance of the *Lactobacillales* had a non-significant increase compared with baseline during both placebo and PHGG periods (Fig. 3d). In contrast, the relative abundance of *Roseburia-Eubacterium rectale* were significantly decreased in placebo (P = 0.04; F = 6.32) group, but exhibited greater significance during the PHGG (P = 0.02; F = 10.45) period (Fig. 3e). The most abundant *Clostridium* cluster genus of phyla *Firmicutes* family showed a decrease in relative abundance compared to baseline during both PHGG and placebo periods, but a trending significance was achieved in PHGG group (P = 0.10; F = 3.33). Moreover, while comparing between the PHGG and placebo groups, virtually no significant difference were found between any members of the *Firmicutes* phylum. Subdomain bacterial groups of *Clostridium* cluster showed a decrease in their relative abundance compared to baseline in the PHGG group. In contrary, the *Clostridium* subcluster IV relative abundance compared to baseline was nearly significant (P = 0.06; F = 5.01) in placebo intake group, while no significance was noticed in PHGG group (Fig. 3f). Similarly, the *Clostridium* subcluster XIV was found significant (P = 0.02; F = 9.35) in placebo group, but only trending (P = 0.07; F = 4.20) in PHGG group compared to baseline (Fig. 3g). ANCOVA analysis revealed a clear statistical difference (P = 0.04, F = 4.83) in the relative abundance of the *Clostridium* subcluster XI between groups (Fig. 3h; see Table 4).

3.5. Correlation between relative abundance of gut microbiomes & quality of lifestyle covariates

To determine whether intake of prebiotic PHGG dietary fiber influenced quality of life via the abundance of gut microbiomes, a correlation analysis was performed. (Fig. 4; also see supplementary material). The best fit to a multivariate model explaining the well-separated clusters of correlated (Spearman's) covariates derived from the final ten subjects participated in fecal microbiomes study are presented (Fig. 4a). As expected, diet impacts microbiome relative abundance among healthy athletes, with food and exercise listed among top covariates. The clustered heat maps presented revealed a highly positive correlation between *Bacteroidetes* and *Firmicutes* (including their genera) with food and exercise covariate (P < 0.01), whereas *Firmicutes* and showed a significant positive correlation (P < 0.01) with body fat, exhausted feelings, mood swings and total body health Also,

Table 4
The relative abundance of gut microbiome at phyla and genus levels before and after the placebo and PHGG intervention in the subgroup of subjects (n = 9) who participated in the 16S rRNA gene sequencing study. (Mean ± SEM values).

(*n = 9*)	Placebo group (Dextrin)		Treatment group (PHGG)		ANCOVA (Between Groups)
	0 W	*4 W*	*0 W*	*4 W*	*P-Value; F-Value*
Actinobacterium (~21%)					**P = 0.001*; F = 17.62**
Mean ± SEM	16.63 ± 0.42	16.48 ± 0.21	17.15 ± 0.09	17.49 ± 0.11	
ANOVA: P-Value; F-Value	P = 0.63; F = 0.25		**P = 0.04*; F = 5.39**		
Bifidobacterium					**P = 0.047*; F = 4.66**
Mean ± SEM	10.22 ± 0.38	10.30 ± 0.18	10.70 ± 0.10	10.77 ± 0.09	
ANOVA: P-Value; F-Value	P = 0.80; F = 0.07		P = 0.18; F = 2.19		
Bacteroidetes (~21%)					P = 0.77; F = 0.09
Mean ± SEM	16.38 ± 0.80	15.27 ± 0.42	16.62 ± 0.76	15.18 ± 0.59	
ANOVA: P-Value; F-Value	P = 0.15; F = 2.53		**P = 0.036*; F = 6.31**		
Bacteroides					P = 0.35; F = 0.95
Mean ± SEM	8.87 ± 0.41	8.48 ± 0.22	8.97 ± 0.37	8.14 ± 0.34	
ANOVA: P-Value; F-Value	P = 0.16; F = 2.07		**P = 0.05*; F = 5.47**		
Prevotella					P = 0.53; F = 0.41
Mean ± SEM	7.51 ± 0.39	6.79 ± 0.25	7.65 ± 0.41	7.04 ± 0.30	
ANOVA: P-Value; F-Value	**P = 0.06#; F = 5.21**		**P = 0.04*; F = 6.16**		
Firmicutes (~58%)					P = 0.49; F = 0.50
Mean ± SEM	45.69 ± 1.35	45.16 ± 0.84	46.94 ± 1.38	45.22 ± 1.01	
ANOVA: P-Value; F-Value	P = 0.53; F = 0.43		**P = 0.09#; F = 3.48**		
Lactobacillales					P = 0.89; F = 0.02
Mean ± SEM	7.64 ± 0.16	8.25 ± 0.40	8.30 ± 0.29	8.54 ± 0.35	
ANOVA: P-Value; F-Value	P = 0.18; F = 2.12		P = 0.54; F = 0.41		
Roseburia & Eubacterium rectale					P = 1.0; F = 0
Mean ± SEM	8.57 ± 0.62	7.90 ± 0.50	8.67 ± 0.58	7.97 ± 0.45	
ANOVA: P-Value; F-Value	**P = 0.04*; F = 6.32**		**P = 0.02*; F = 10.45**		
Clostridium clusters Total					P = 0.39; F = 0.80
Mean ± SEM	29.47 ± 0.77	29.02 ± 0.68	29.98 ± 0.71	28.71 ± 0.65	
ANOVA: P-Value; F-Value	P = 0.24; F = 1.64		**P = 0.10#; F = 3.33**		
Clostridium cluster IV					P = 0.73; F = 0.12
Mean ± SEM	9.53 ± 0.51	9.16 ± 0.44	9.79 ± 0.45	9.43 ± 0.26	
ANOVA: P-Value; F-Value	**P = 0.06#; F = 5.01**		P = 0.41; F = 0.75		
Clostridium cluster XI					**P = 0.044*; F = 4.83**
Mean ± SEM	8.85 ± 0.18	9.26 ± 0.24	9.07 ± 0.39	8.51 ± 0.39	
ANOVA: P-Value; F-Value	P = 0.14; F = 2.7		P = 0.14; F = 2.7		
Clostridium cluster XIV					P = 0.52; F = 0.44
Mean ± SEM	11.09 ± 0.16	10.60 ± 0.22	11.12 ± 0.12	10.77 ± 0.47	
ANOVA: P-Value; F-Value	**P = 0.02*; F = 9.35**		**P = 0.07#; F = 4.20**		
Firmicutes/ Bacteroidetes (Ratio)					P = 0.92; F = 0.01
Mean ± SEM	2.82 ± 0.10	2.97 ± 0.08	2.85 ± 0.09	3.00 ± 0.10	
ANOVA: P-Value; F-Value	P = 0.11; F = 3.32		**P = 0.031*; F = 6.84**		
Bifidobacterium + Lactobacillales					P = 0.21; F = 1.73
Mean ± SEM	17.87 ± 0.38	18.54 ± 0.39	19.0 ± 0.24	19.31 ± 0.33	
ANOVA: P-Value; F-Value	P = 0.30; F = 1.22		P = 0.41; F = 0.74		

* Significant (P ≤ 0.05).
\# Significant (P ≤ 0.05).

the *Firmicutes/Bacteroidetes* ratio revealed a significant positive correlation (P < 0.05) with most of the covariates studied (Fig. 4b). Further, a significant correlation with microbiome diversity were found with gastrointestinal physique and fecal defecation characteristics. Further, we investigated the correlation between featured fecal defecation characteristics and relative abundance of the gut microbiome and presented in the heat map of 16S rRNA gene sequencing analysis of fecal content at the genus level (Fig. 5). Somewhat surprising finding was the identification of diarrhea reduction and fecal defecation frequency correlation with a relative abundance of microbiomes among healthy athletes of this study. Wherein, phyla *Bacteroidetes* and its genera *Bacteroides* and *Prevotella* along with phyla *Firmicutes*, especially *Clostridium* IV genus, showed a positive association with fecal defecation frequency (Fig. 5a). Also, a negative correlation of all studied phyla and their genera support the reduction of diarrhea and further confirmed by the significant correlation with some of the microbiome relative abundance (P < 0.05; Fig. 5b).

Fig. 6 reveals the well-resolved clusters for different QOL covariates and relative microbiomes abundance in the principal component analysis plots derived from the ten subjects in the fecal microbiome study. Wherein, most of the covariates were accumulated around the loading plot of principal component 2 (PC-2). At baseline or before intake of placebo (Fig. 6a) or prebiotic PHGG dietary fiber (Fig. 6b), the subjects of both groups showed a somewhat similar positive correlation between food and exercise, to most of the microbiome. Also, a weak correlation between the *Firmicutes/Bacteroidetes* ratio to the gastrointestinal physique and fecal defecation characteristics was observed. After either placebo (Fig. 6c) or PHGG dietary fiber (Fig. 6d) supplementation, most of the covariates were found to shift toward the loading plot of principal component 1 (PC-1). Although a positive correlation between food and exercise to most of microbiome remained the same as before supplementation of either placebo or PHGG, the positive correlation between *Firmicutes/Bacteroidetes* ratio to the gastrointestinal physique and fecal defecation characteristics was noticed. Interestingly, these three covariates found to be more closely facing the score plot of the principal component 2 (PC-2) with PHGG intake compared to placebo (Fig. 6c & 6d). Their heat maps along with hierarchical clustering analysis, representing an overview of the relative correlations, are provided in the supplementary material (Figs. S5 & S6).

Further, the Spearman's rank correlations at baseline and after four weeks of respective intakes of placebo and PHGG are presented in Table S2. *Bifidobacterium* revealed a significant trending correlation (P = 0.088) with placebo intake, while a significant correlation (P = 0.042) was found with PHGG supplementation. Some notable

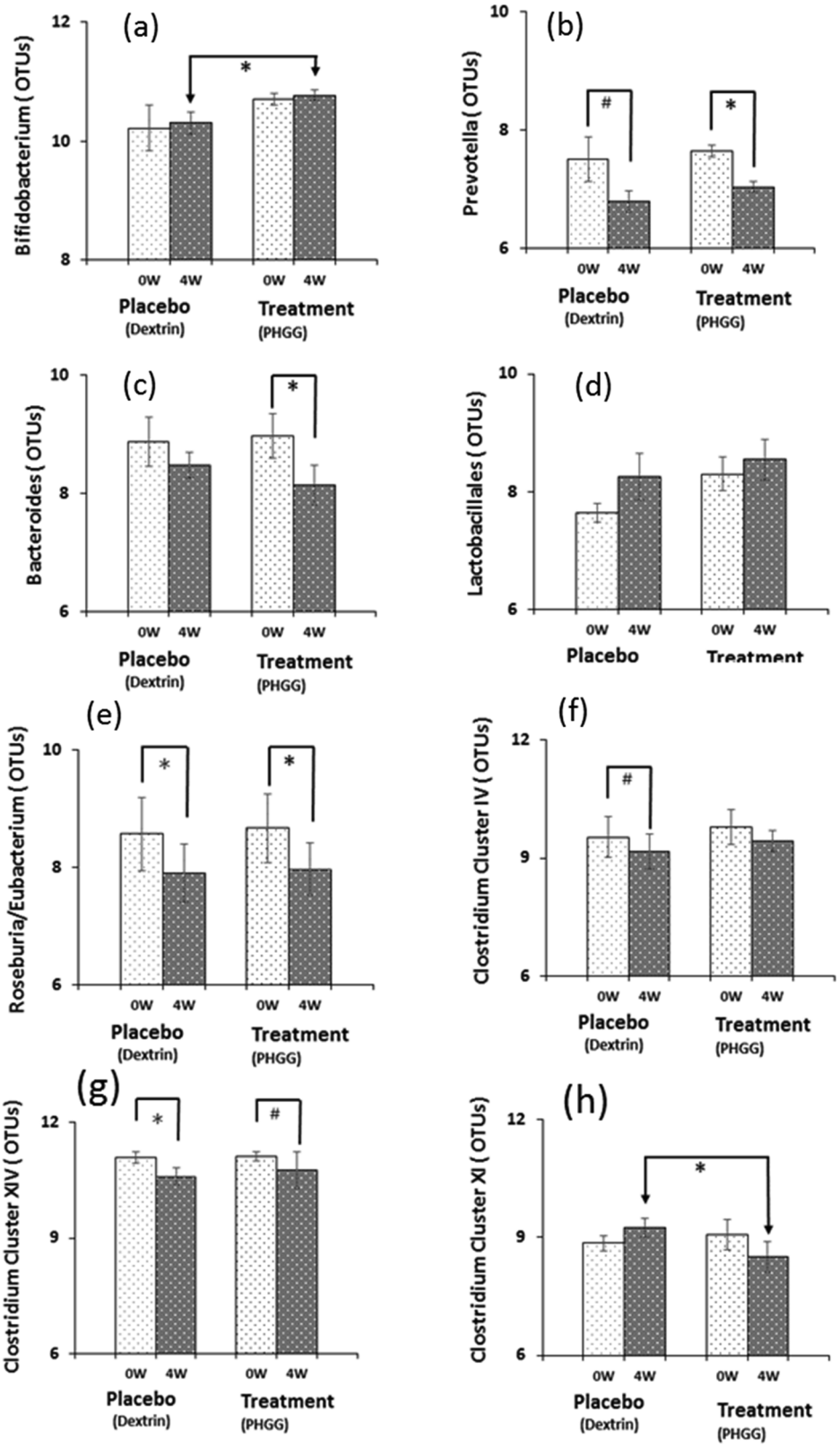

Fig. 3. Time-dependent effect of PHGG dietary fiber and placebo interventions on relative abundance of fecal microbiome at genera level estimated for nine subjects (n = 9) revealed (a) a significant increase in genera *Bifidobacterium* compared to placebo, a decrease in genera (b) *Prevotella* and (c) *Bacteroides* along with (d) a non-significant increase in *Lactobacillales*. (e) *Roseburia-Eubacterium* abundance was significantly decreased in both placebo and PHGG groups. A decrease in the relative abundance of *Clostridium* cluster members (f, g, and h) with significant difference in (h) *Clostridium* cluster XI between placebo and PHGG dietary fiber group. One-way ANOVA (intergroup); ANCOVA (between the PHGG and placebo groups). Significance: *P < 0.05; Trending Significance: #P < 0.10.

alteration in the correlations were monitored for the abundance of *Prevotella, Roseburia-Eubacterium rectale*, and *Clostridium* cluster attributable to microbiome amelioration. The relative abundance of gut microbiome was correlated with fecal defecation characteristics that were found to be ameliorated during the PHGG intake period (Table S3). The most relevant findings were the significant correlation of the abundance of *Bacteroides* (P = 0.016) and *Prevotella* (P = 0.034) with fecal defecation frequency during the placebo intake that might negatively impact on the gut health, found to be altered into an effective non-significant (average) correlation during prebiotic PHGG fiber intake duration. Similarly, a negatively correlated relative abundance of *Bacteroides* and *Prevotella* with fecal excretory feelings during placebo

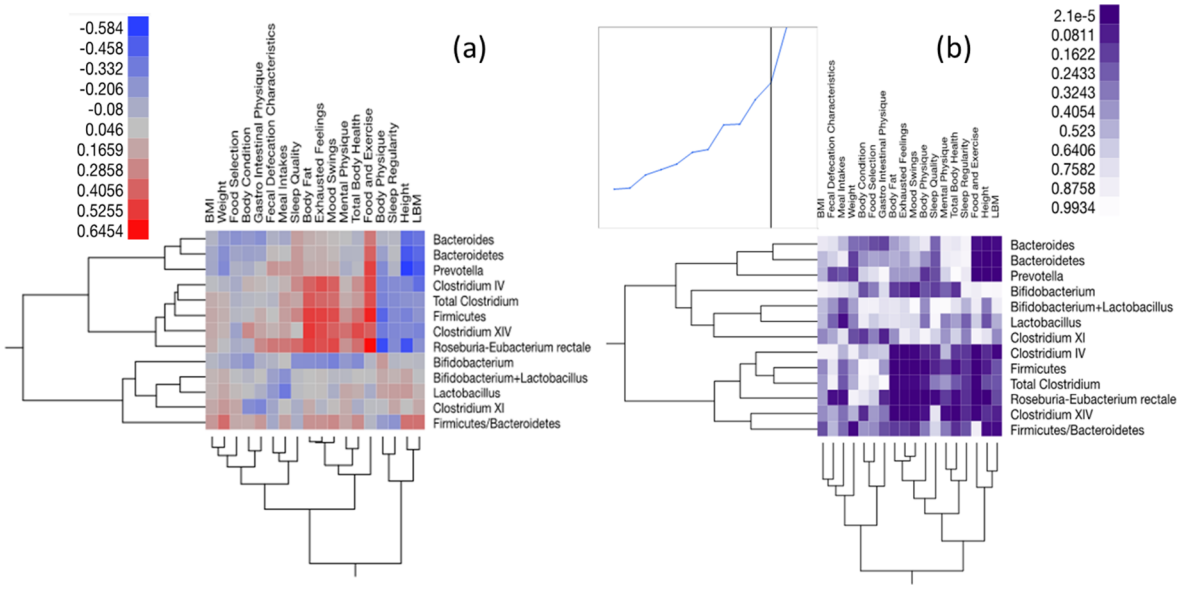

Fig. 4. Multivariate analysis of QOL covariates presented as heat map and hierarchical clustering analysis representing the correlation of different QOL multivariate with individual fecal microbiomes abundance determined using real-time PCR amplification of 16S rRNA gene (at phyla and genera levels). (a) Spearman's rank correlation coefficients and (b) corresponding P value were calculated in JMP (SAS software) to determine the confounding QOL covariates upregulated with a change in the relative abundance of gut microbiome upon PHGG intervention. Dendrogram of QOL covariates is also correlated with dendrogram of structural relative abundance of fecal microbiomes.

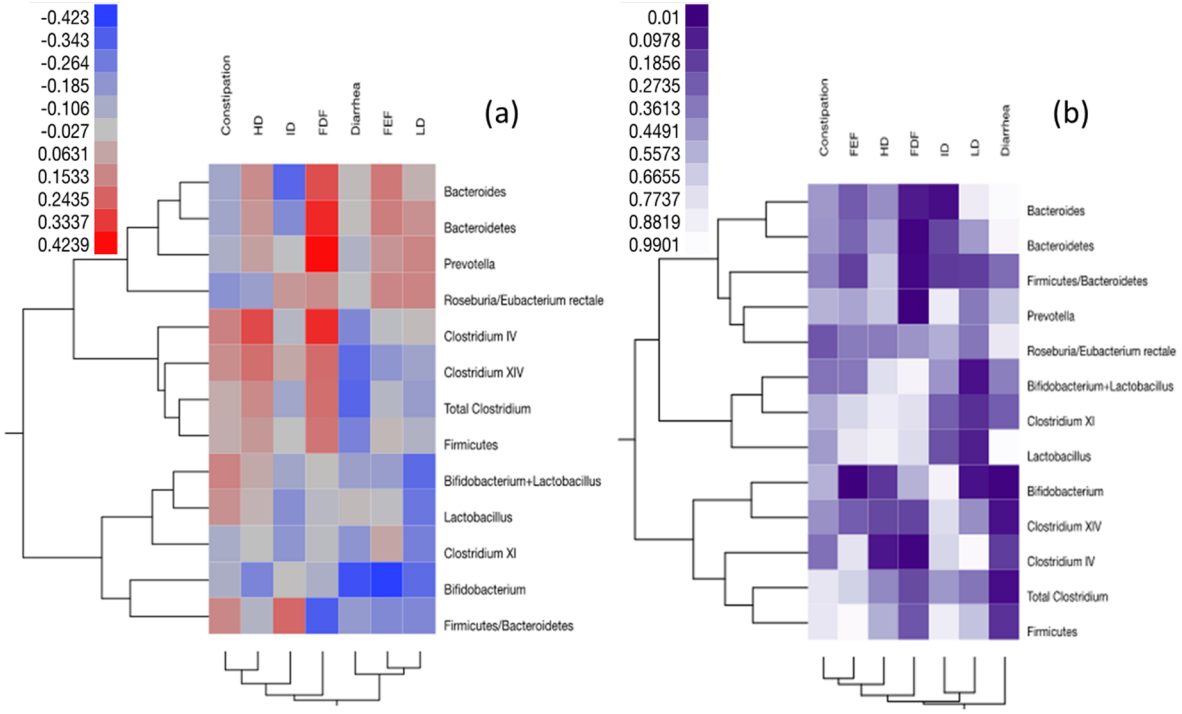

Fig. 5. Extended multifactor analysis of QOL covariate fecal defecation characteristics covariate is presented as heat map and hierarchical clustering analysis. The representative correlation of different fecal defecation characteristics variables with individual fecal microbiomes determined using real-time PCR amplification of 16S rRNA gene (at phyla and genera levels). (a) Spearman's rank correlation coefficients and (b) corresponding P value were calculated in JMP (SAS software) to determine what confounding fecal defecation characteristics variables are upregulated with an alteration in the relative abundance of gut microbes with PHGG intervention. Dendrogram of fecal defecation characteristics is also correlated with dendrogram of structural relative abundance of fecal microbiomes. The acronyms refer to HD, hard defecation; ID, incomplete defecation; FDF, fecal defecation frequency; FEF, fecal excretory feelings; and LD, loose defecation.

period changed to a significant positive correlated covariate during PHGG intake period (*Bacteroides*, P = 0.07; *Prevotella*, P = 0.046, respectively). A negative correlated significant relative abundance of genera *Clostridium* subcluster XIV (P = 0.009) with QOL covariate diarrhea symptom reduction also revealed somewhat identical trending, which can be further supported by a significant positive correlation of the relative abundance of genera *Clostridium* subcluster XIV (P = 0.026) with ameliorating QOL covariate hard type defecation after the PHGG intake.

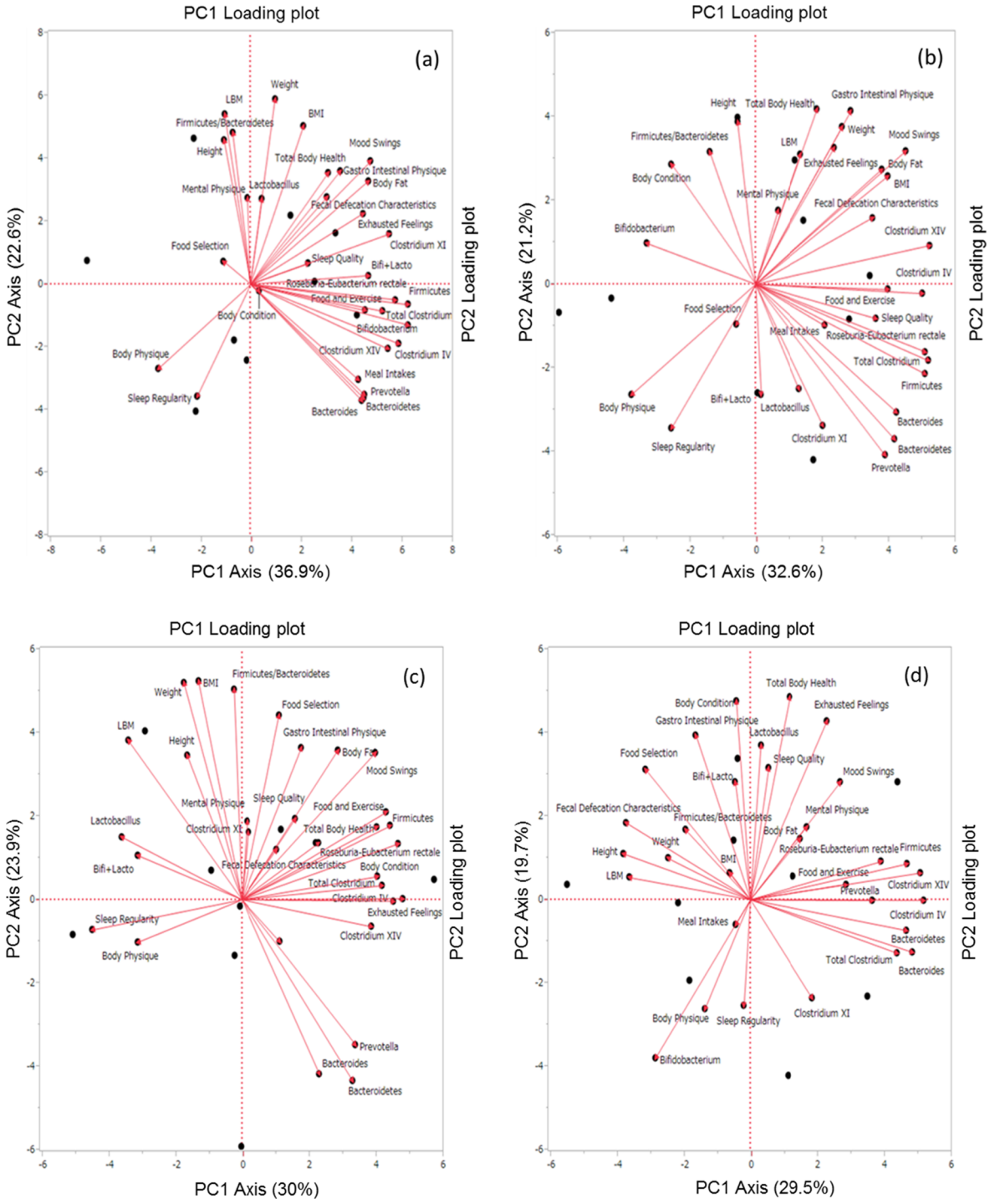

Fig. 6. Quality of life covariates correlated to the relative abundance of fecal microbiomes in healthy male athlete subjects at baseline (a) Placebo, or (b) PHGG, showing no baseline difference between the placebo and PHGG dietary fiber intervention groups, and after either (c) placebo, or (d) PHGG dietary fiber intervention. Standardized principal component analysis (PCA) biplot depiction illustrates the between sample difference, wherein arrow representing the effect size and direction of the major microbiomes and QOL covariates according to their best explaining mutual correlations. The position of arrows indicate the correlation differences between the placebo and PHGG dietary fiber groups.

4. Discussion

Athletes suffer from exercise-related stress, gastrointestinal symptoms, and inadequate recovery. Clinical and animal studies have confirmed the importance of a high fiber diet. Proper intestinal barrier function is crucial for maintaining immune and gastrointestinal health. Low fiber intake results in a low diversity of the beneficial microbiota among athletes, which may be associated with an increase in choric diseases (Makki, Deehan, Walter, & Bäckhed, 2018; Thorburn et al., 2015). There are suspected pathways through which the dietary fiber intake impact the gut microbiomes composition improve athlete health. For example, dietary fibers are capable of modulating undesirable gut microbes that are associated with relative energy deficiency in sport and regulates local inflammatory changes leading to gut wall permeability that may allow increased systematic migration of bacterial components. Prebiotic dietary fibers resist digestion in the upper

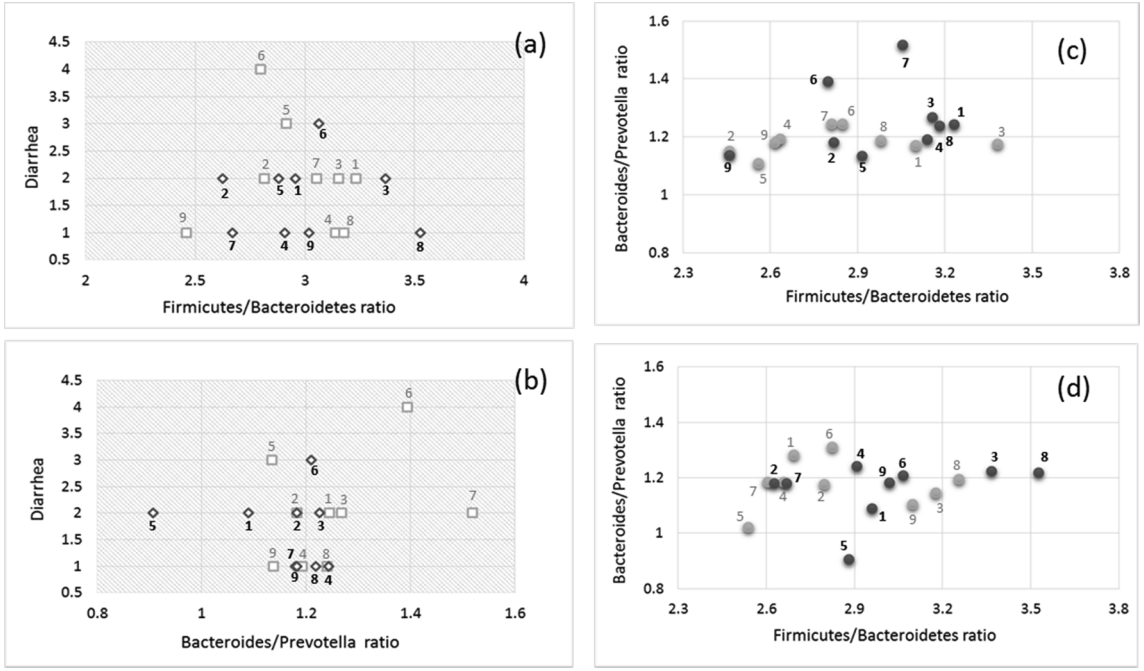

Fig. 7. Reduction in diarrhea is associated with the relative abundance of (a) *Firmicutes/Bacteroidetes* ratio as well as (b) *Bacteroides/Prevotella* ratio confirmed the reduction of diarrhea at both phyla and genus levels (grey squares: placebo (dextrin); black diamonds: PHGG dietary fiber). (Lower *Firmicutes/Bacteroidetes*, as well as *Bacteroides/Prevotella* ratio reveals the reduction in diarrhea symptoms among the subjects). Association of relative abundance of *Firmicutes/Bacteroidetes* ratio to *Bacteroides/Prevotella* ratio with either (c) placebo (dextrin) or (d) prebiotic PHGG dietary fiber intervention (gray and black circles represent; pre and post intake, respectively).

gastrointestinal tract and stimulate growth of beneficial microbes in the distal intestine and colon (Carlson, Esparza, Swan, Taussig, Combs, & Slavin, 2016). In the present randomized, double-blind, placebo-controlled, crossover study, we demonstrated that PHGG significantly influenced the relative abundance of the microbiome in healthy athletes. PHGG undergo fermentation in the large intestine, which can modulate of the intestinal microbiome. The relative abundance of *Bacteroidetes* and *Firmicutes* were decreased upon PHGG supplementation. The relative proportion of protective commensal gut anaerobic bacteria, i.e., *Firmicutes to Bacteroidetes* ratio (F/B) has been already shown to be of significant relevance in humans (Ley et al., 2006). The reduction in diarrhea symptoms with relative abundance of *Firmicutes/Bacteroidetes* ratio and *Bacteroides/ Prevotella* ratio confirmed the reduction of diarrhea at both phyla and genus levels (Fig. 7). As evident from the results, the relative abundance of *Firmicutes* and *Bacteroidetes* decreased during both placebo and PHGG periods, but was only significant in the PHGG group. Thus, the lower phylum *Firmicutes/Bacteroidetes*, as well as genera *Bacteroides/Prevotella* ratio with PHGG intervention reveals the reduction in diarrhea symptoms among the subjects (Fig. 7a & b). This also support the meaningful association of relative abundance of phylum *Firmicutes/Bacteroidetes* ratio to genera *Bacteroides/ Prevotella* ratio with PHGG intervention compared to placebo (Fig. 7c & d). Our results illustrated a statistically significant increase in the *Firmicutes to Bacteroidetes* ratio in PHGG group that appeared to be associated with reduction in diarrhea. Similar to a recent observational study that reported the fecal bacterial profiles of elite male rugby players (Clarke et al., 2014), the athletes in this study also had lower levels of *Bacteroidetes* and higher levels of *Firmicutes.* A plausible explanation is that *Firmicutes* are usually better at extracting energy from gut contents than *Bacteroidetes,* thus help promote more efficient absorption of calories in athletes, which could support stamina enhancement and performances. Therefore, with a relative decrease in *Firmicutes* and the corresponding reduction in *Bacteroidetes* can be associated with an increase in energy recovery among the healthy athletes. Some published review articles also suggest how dietary fiber interventions regulate the gut microbiome to exert beneficial effects in elite athletes (Mach & Fuster-Botella, 2017; Jumpertz et al., 2011). Thus, prebiotic PHGG may offer feasible ways to impact *Firmicutes/ Bacteroidetes* ratio in healthy athletes, although the precise function of many gastrointestinal microbes remain unclear.

Consistent with previous prospective studies, we also found an increase in *Bifidobacterium* and *Lactobacillus* after PHGG supplementation. The present finding show subjects with low baseline *Bifidobacterium* showed a somewhat higher increase in *Bifidobacterium* levels after prebiotic PHGG fiber intakes compared to those with relatively high baseline *Bifidobacterium* abundance. *Bifidobacterium* is a leading producer of folate, a vitamin involved in vital host metabolic process, including DNA synthesis and repair (Pompei, Cordisco, Amaretti, Zanoni, Matteuzzi, & Rossi, 2007). An increased prevalence of *Bifidobacterium* and *Lactobacillus* may prevent the colonization by *Proteobacteria.* The ability of PHGG to regulate the relative abundance of the gut microbiome could be hypothesized as the mechanism leading to improved fecal consistency characteristics and reduction in diarrhea and normalization of constipation episodes. Low fiber diets intakes are known to strongly associate with lower abundances of *Prevotella* (Wu et al., 2011), and it is also evident that *Prevotella* and *Bacteroides* is more abundant in loose defecation and leads to diarrhea (Vandeputte et al., 2016). The result of the present study shows that preferential decrease in the abundance of *Bacteroides* along with *Prevotella*, and *Roseburia-Eubacterium rectale* in PHGG intake group, and thus resulted in a sustained satisfactory fecal excretory feelings and reduction of diarrhea among athletes. Although PHGG ingestion regulates the relative abundance of certain taxa, overall beta-diversity and alpha-diversity were not significantly different between PHGG and placebo periods as there were no differences in the number of microbiome species. Nevertheless, the data presented here provide valuable insights into the gut microbiome communities of healthy athletes. A significant positive correlation between exercise load and the abundance of *Prevotella* (Petersen et al., 2017) has been previously reported. We found a lower abundance of *Bacteroides* and *Prevotella* in our athletes after PHGG

intake, which reveals a significant correlation between habitual exercise and prebiotic fiber. This would be a direct benefit for athletes because increased fermentation of PHGG in the distal colon increases fermentation metabolites such as short-chain fatty acids (SCFAs) that could be absorbed and utilized for energy. It is also interesting to account how *Prevotella* responded to the exercise load regime of athletes, and in turn, may positively influence health and athletic performance. Furthermore, we quantified the *Prevotella* as the resident microbiota, *Bifidobacterium* as the beneficial bacteria, the *Lactobacillales* as the lactate-producing bacteria. Mainly, *Clostridium* subcluster XIV, the *Clostridium* subcluster IV, and *Bacteroides* are identified as the SCFAs-producing indigenous bacteria, while the *Clostridium* subcluster XI as the harmful bacteria including other *Clostridium* members, concerning the fecal bacteria profiles of athletic subjects.

The bacterial metabolites such as SCFAs have often considered as growth regulators of the intestinal epithelial cells (Fukuda et al., 2011; Sanna et al., 2019), and significant association is reported between SCFAs levels and the gut microbiota composition (Scott, Duncan, & Flint, 2008; Duncan, Louis, Thomson, & Flint, 2009). Gastrointestinal disorders during physical exercise increase the body temperature of athletes and blood pools away from the gastrointestinal tract to periphery muscles and organs, resulting in a reduced intestinal permeability by the alteration of gut microbiota composition and activity (the so-called dysbiosis). Conversely, the microbiota's production of SCFAs derived from PHGG fermentation (Ohashi et al., 2015; Carlson, Esparza, Swan, Taussig, Combs, & Slavin, 2016) can increase transepithelial resistance, by acting as energy source for the intestinal epithelium, which improves intestinal barrier function and decreases inflammation (Flint et al., 2008; Macfarlane & Macfarlane, 2003; Hamer, Jonkers, Venema, Vanhoutvin, Troost, & Brummer, 2008; Puddu, Sanguineti, Montecucco, & Viviani, 2014). In the human gut, propionate is mainly produced by *Bacteroidetes,* whereas the production of butyrate is dominated by *Firmicutes* (Macfarlane & Macfarlane, 2003; Louis & Flint, 2017; Morrison & Preston, 2016). PHGG as a butyrate producing substrate (Ohashi et al., 2015) that can play an essential role in gut homeostasis possibly by increasing mucin production, which could result in enhanced tight junction integrity (Peng, Li, Green, Holzman, & Lin, 2009). PHGG helps increase fecal bulking and may bind to bile acids in the gut, which can reduced bile acid reabsorption and elimrinate higher levels of bile acids in feces (Martínez et al., 2013). In the bile acid metabolism, microbial enzyme bile salt hydrolases that causes deconjugation and form secondary bile acids, are mainly present in gram-positive bacteria phyla *Firmicutes* (especially in genera *Lactobacillus*) and also certain gram-negative bacteria phyla *Bacteroidetes* (Long, Gahan, & Joyce, 2017; Jones, Begley, Hill, Gahan, & Marchesi, 2008; Mullish et al., 2018). Also, some critical secondary enzymatic transformation of bile acids may occur by genera *Clostridium* cluster members (Mullish et al., 2018). Although bile acids are critical for digestion and absorption of fats and fat-soluble vitamins in the small intestine, however less bile acids are beneficial as elevated concentration of bile acids in serum causes many structural liver diseases. Elimination of bile acids can also reduce serum cholesterol levels. While, conjugation is beneficial as it detoxifying bile salts.

Furthermore, the prebiotic PHGG dietary fiber intake can be attributed to a higher abundance of major lactate producing bacteria *Bifidobacterium* and *Eubacterium ractale*. *In-vitro* studies have shown the possibility *Bifidobacterium* metabolites can be further metabolized by *Eubacterium ractale* genus to produce lactate (Ríos-Covián et al., 2016; Belenguer, Duncan, Calder, Holtrop, Louis, Lobley, & Flint, 2006). Thus, it can be postulated that *Eubacterium* may attenuate the detrimental effects of lactate production in the gastrointestinal tract. Lactate possibly metabolized by lactate-utilizing bacteria (LUB) and converted largely into butyrate by the gut microbiome. Butyrate are known as a potential source to salvage energy for the fit athletes. Further, a keystone species genera *Roseburia* and *Eubacterium ractale* within the gut microbiomes associated with *Clostridium* clusters XIV, IV, and XI were significantly more abundant in our athletes in addition to the genera *Bacteroides* after PHGG supplementation. Conversely, the gut microbiome exhibited non-significant changes in the abundance of members of *Clostridium* cluster IV and XIV, but a significant change of *Clostridium* cluster XI, after PHGG consumption compared to placebo. The results support the observation that PHGG intake does not cause gas distension issues as its selective fermentation includes a relatively lower abundance of gas forming bacteria in the *Clostridium* clusters. The findings demonstrate that athletic subjects with low baseline *Bifidobacterium* and high *Eubacterium ractale* and *Clostridium* cluster are more responsive to PHGG supplementation due to microbiome modulation.

Variation in fecal defecation characteristics revealed an association with a shift in the fecal microbiota abundance during the PHGG intervention, and it correlates differently with abundance gradients of the individual genera. Some of these associations remain significant when applied to taxonomic assignments. The fact *Prevotella* is capable of binding collagen and degrading mucin oligo-derivatives, and that could reflect in increased fecal water binding capacity attributable to PHGG consumption, may control and diminish the selective force imposed by accelerated transit. Thus, may hamper the robustness of specific motility in loose stools and resulting in reduction of diarrhea and normalization of constipation episodes as well as improving other fecal defecation characteristics. While a decreased *Prevotella* abundance could reflect the fitness of the genus to dwindle in conditions of fecal characteristics amelioration. Further, while comparing the studied lifestyle covariates, the impact of only well-correlated covariates on microbiota abundance was found considerable. Judging from the distribution of correlation coefficients in cluster analysis, the relative abundance of phyla *Firmicutes* and *Bacteroidetes* showed a positive correlation with food and exercise, and attributable to an improved fecal defecation frequency.

The present study has strengths and limitations. The major strength of the present study is a strong randomized, double-blind, placebo-controlled crossover design, that minimizes confounding variables on the study outcomes. More precise results may have been observed if more participants had their fecal microbiomes analyzed, which is a limitation of this study. Another limitation is the lack of the data on SCFAs and fecal bile acids, necessary to establish a relationship between microbiome composition and fecal consistency characteristics, with detailed mechanistic understanding at the molecular level including their overall impact on the human health and quality of life. Further, gaining a better understanding of the factors implicated in the correlation of QOL covariates in gut microbiomes responsiveness could help improve gastrointestinal health of athletes. Overall, more research in this area would be of value to positively impact performance and athletes gut health.

5. Conclusion

We reported relative gut microbiome abundance, fecal defecation characteristics and a hypothesized mechanistic pathway of how prebiotic PHGG supplementation shapes the gut microbiome and lifestyle quality. The comparison demonstrated that several gut bacterial genera responded distinctively between the placebo and PHGG treatments. Although PHGG intervention had little or no effect on the diversity of the gut bacteria, it did alter the relative abundance of specific gut microbiomes and improved fecal defecation characteristics, particularly the reduction in diarrhea symptoms. A significant correlation was present between baseline abundance of the microbiome, and prebiotic PHGG has driven relative changes in microbiomes abundance. Overall, quality of life significantly improved with PHGG consumption compared to baseline. The consistency of the results to the majority of literature supports the conclusion that fecal composition provides reasonably good representation for the response of sizeable intestinal microbiomes (at the genus level) to prebiotic PHGG dietary fiber. The results from the present study also provide proof PHGG acts as a

substrate for the selective growth of beneficial bacteria such as *Bifidobacterium* thus confirming its prebiotic nature. PHGG was well tolerated among the healthy athletes and led to an increase in the relative abundance of *Actinobacterium* and *Bifidobacterium*. PHGG dietary fiber is enzymatically degraded into lower molecular weight galactomannan, consisting of a mannose backbone with galactose side group, which is the primary carbon source for intestinal bacteria (Balascio, Palmer, & Salyers, 1981; Doublier & Launay, 1981; Carlson, Gould, & Slavin, 2016). In conclusion, the consumption of prebiotic PHGG dietary fiber among healthy athletes had a positive effect on the gut microbiome and quality of life and thus is an effective and safe dietary ingredient for the reduction of diarrhea episodes among active healthy individuals. Partially hydrolyzed guar gum is generally recognized as safe (GRAS) in the United States of America and approved worldwide by regulatory agencies.

6. Ethics statements

The ethical committee approved the study protocols and the study was conducted according to the guidelines laid out in the declaration of Helsinki. Participants gave informed written consent and were well informed about the purpose of the study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

All authors equally contributed in the study design and conception, data collection, analysis, and interpretation provided their expertise, as well as writing the manuscript, revision and approval the final version of submission of as an article for the publication.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2020.104067.

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