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Effects of probiotic supplementation on 12 min run performance, mood management, body composition and gut microbiota in amateur marathon runners: A double-blind controlled trial

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ABSTRACT

Background: Probiotic supplementation has a positive effect on endurance exercise performance and body composition in athletes, but the underlying mechanisms remain unclear. Gut microbiota can provide measurable markers of immune function in athletes, and microbial composition analysis may be sensitive enough to detect stress and metabolic disorders caused by exercise.

Methods: Nineteen healthy active amateur marathon runners (15 male and 4 female) with a mean age of 29.11 years volunteered to participate in this double-blind controlled study. Based on the performance of the Cooper 12-min running test (CRT), the participants were allocated into two groups to receive either a probiotic formulation comprising lactobacillus acidophilus and bifidobacterium longum ($n = 10$) or placebo containing maltodextrin ($n = 9$) for five weeks. Consistency of diet and exercise was ensured throughout the experimental period. Before and after the intervention, all participants were assessed for CRT, emotional stability and gastrointestinal symptoms, gut microbiota composition, body composition and magnetic resonance imaging (MRI) indicators of skeletal muscle microcirculation.

Results: Compared to before the intervention, the probiotics group showed an increase in CRT score (2.88 ± 0.57 vs 3.01 ± 0.60 km, $P < 0.05$), significant improvement in GSRS and GIQLI (9.20 ± 4.64 vs 7.40 ± 3.24, 118.90 ± 12.30 vs 127.50 ± 9.85, $P < 0.05$), while these indicators remained unchanged in the control group, with a significant time-group interaction effect on gastrointestinal symptoms. Additionally, some MRI metabolic cycling indicators of the thigh skeletal muscle also changed in the probiotics group ($P < 0.05$). Regarding microbiota abundance, the probiotics group exhibited a significant increase in the abundance of beneficial bacteria and a significant decrease in the abundance of harmful bacteria post-intervention ($P < 0.05$).

Conclusion: As a sports nutritional supplement, probiotics have the potential to improve athletic performance by optimizing the balance of gut microbiota, alleviating gastrointestinal symptoms.

1. Introduction

A potential bidirectional relationship between exercise and gut microbiota has been demonstrated. Exercise can promote gut health by regulating gut microbiota, but most relevant studies are based on animal models or limited to low-intensity exercise.[1,2] Recent studies have found that endurance exercise, represented by marathons, has adverse effects on intestinal microbiota, such as increased intestinal permeability,

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increased intestinal fatty acid binding protein (I-FABP), and changes in intestinal microbiota.[3,4] These effects can cause gastrointestinal discomfort and emotional symptoms that ultimately lead to decreased athletic performance. In addition, high-intensity and high-duration exercise often cause athletes to suffer from psychological and physical stress,[5] which can increase the risk of symptoms related to intestinal microbiota disorders,[6] including abdominal pain, cramps, and diarrhea,[7] leading to poor performance.[8] In turn, gut microbes can affect the body's inflammatory response, stress adaptation, neurological function, and even psychiatric symptoms, which play a vital role in athletes' performance and post-exercise recovery.[9] Therefore, recent studies have begun to manipulate the microbiome to influence athletic performance and the physical and mental health of athletes, such as by increasing the diversity and abundance of beneficial bacteria in the gut.

Probiotic supplementation is the most direct and effective way to increase the abundance and diversity of gut microbiota, which can significantly improve the endurance exercise performance of athletes [10,11] and reduce fatigue indicators.[12,13] It has been widely used as a nutritional supplement for athletes to ensure excellent physical function.[14] Gut microbiota can provide measurable and valid markers of immune function in athletes, and microbial composition analysis may also be sensitive enough to detect exercise-induced stress and metabolic disorders. The development of high-throughput sequencing (HTS) technology has promoted the understanding of the interaction mechanism between gut microbiota and host health. Previous studies using HTS have found that supplementation of beneficial microbiota can ameliorate exercise-induced changes in gut microbiota abundance.[15] However, previous studies focus on the effects of probiotic supplements on single fecal bacteria, while the overall composition of intestinal flora and the abundance of beneficial or harmful bacteria are not fully understood.

Probiotic supplements can not only effectively support the proliferation of beneficial intestinal flora, but also play a variety of benefits on the body by regulating the gut-muscle axis,[16,17] microbiome-gut-brain axis,[18] and hypothalamic-pituitary-adrenal (HPA) axis,[19] such as improving physiological adaptability,[20] oxidative stress,[21] inflammation,[13] and energy balance.[22] The interactive regulation of the gut-muscle axis reveals the existence of a homeostatic balance between intestinal microbiota and skeletal muscle. Excessive exercise load will break this balance, causing increased intestinal permeability and oxidative stress, accelerating the process of inflammation and lactate metabolism, and negatively affecting the function of skeletal muscle.[23] Preclinical and human studies have shown that the administration of specific probiotic strains can maintain skeletal muscle mass,[24–26] promote recovery from muscle-damaged exercise,[27] and increase exercise endurance.[28,29] In addition, the gut microbiota can contribute to the establishment of bone mass and strength through hormonal and immune system modulation.[30,31] A functioning microbiome can accelerate the healing of exercise-related bone trauma.[32] The regulatory mechanism of the microbiome-gut-brain axis and the HPA axis suggests that there are close links between the gut microbiome and the brain, and between the gut microbiome and the immune system. Gut microbial disturbances or malnutrition are associated with anxiety and depression. Probiotics can cause the age-related normalization of the decline in testosterone levels and decrease in cortisol levels, allowing athletes to have an improved response to physical or mental stress.[27] However, these potential benefits need to be validated in more active population studies. At the same time, how probiotics improve body composition and emotional response in endurance athletes is still unclear, such as its effects on bone mineral density, skeletal muscle mass and metabolic level, and emotional stability.

Therefore, the purpose of this study was to evaluate the effects of probiotic supplements consisting mainly of lactobacillus and bifidobacterium longum on exercise performance, gut microbiota structure, body composition, and emotional management of amateur marathon runners using 16S rRNA HTS, multimodal image quantitative technology and emotional stability scale. We hypothesized that probiotic supplementation would result in improved exercise performance, changes in gut microbiota composition and body composition, and increased emotional stability in athletes.

2. Materials and methods

2.1. Experimental design

Twenty well-trained amateur marathon runners (16 male and 4 female, aged 20–50 years) from the "Hangzhou Sunshine Running Team" were recruited. One participant voluntarily quit due to injury during the experiment. This study used a double-blind controlled design, and the participants were evenly distributed to one of two groups according to the total distance traveled (meters) in 12 min (The 12-min Cooper Running/Walking test): the experimental group (10 participants, male/female: 8/2) and placebo group (9 participants, male/female: 7/2). The experimental process is shown in Fig. 1.

All participants received an intervention with placebo or probiotic supplements for five consecutive weeks, during which the same amount of training was maintained. Before and after the experiment, participants were assessed with a 12-min running/walking distance test and an emotional stability scale respectively. Body composition, thigh muscle, and stool samples were analyzed using dual-energy X-ray absorptiometry, magnetic resonance imaging, and 16S RNA HTS, respectively. During the experiment, all participants worked and rested in a manner formulated by the team and received the assigned diet to ensure consistency of the diet.

2.2. Ethical aspects and randomization

This study was approved by the Research Ethics Committee of the Affiliated Hospital of Hangzhou Normal University, and registered in the Chinese Clinical Trial Center (ChiCTR2200064394). All subjects signed written informed consent before participating in the study. Subjects were randomized into blocks of five and sequentially numbered. To guarantee a balanced 12-min Cooper's test running distance distribution between groups (probiotics versus placebo) we conducted stratification via running distance statistics. The randomization code was performed and kept by a researcher who was not involved in other items of the trial and given to the person responsible for statistical analysis after all data collection had been completed. All participants, data collectors, and data statisticians were blinded throughout the study, and only the person dispensing the probiotics or placebo was aware of the group assignment of all participants.

2.3. Participants

Participants included amateur marathon runners between the ages of 20 and 55 years, excluding those with hypertension, asthma, or skeletal neuromuscular impairment of the upper or lower limbs and a previous history of intestinal surgery. All subjects were required to have no history of tobacco and have no or a limited alcohol use (<100 ml per year). Participants were instructed to cooperate with a uniform intensity of training, to refrain from consuming nutritional supplements, yogurt, prebiotics, other probiotic-related products, or antibiotics during the experimental period, and to abstain from alcohol and tobacco consumption for one week before the experimental period. Throughout the study, each participant was required to fill out a training diary, recording the intensity, duration and mileage of all their weekly aerobic exercises to ensure they maintained the same training intensity as before the intervention. After a detailed explanation of the experimental procedure and content, all participants provided written informed consent before participation. The basic demographics and characteristics of the participants are listed in Table 1.

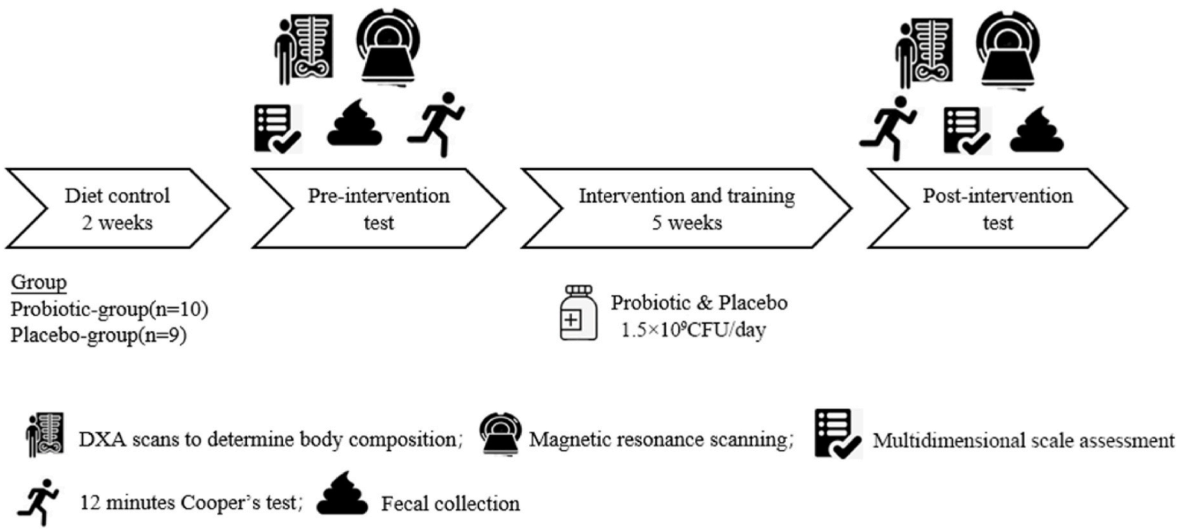

Fig. 1. Experimental design. The study used a randomized, double-blind design. Twenty volunteers were divided into two groups: a placebo group and a probiotic group (1.5×10^9 colony-forming units [CFU]/day). All subjects received placebo or probiotic supplements for five weeks, during which they trained with a uniform amount of exercise. Body composition, emotional stability, and stool samples were analyzed before and after the intervention.

Table 1
Basic information data of the participants.

Variables	Probiotic group	Placebo group	t/χ^2 values	*P* values
Sex (male/female)	8/2	7/2	0.014	0.906[a]
Age (years)	28.50 ± 12.18	29.78 ± 12.39	−0.748	0.497[b]
Education (years)	16.00 ± 0.00	16.44 ± 2.30	−0.528	0.720[b]
BMI (kg/m^2)	20.54 ± 2.24	21.51 ± 1.84	−0.826	0.420[b]
Running experience (years)	5.10 ± 2.02	5.00 ± 2.35	0.100	0.922[b]
Average running distance (km/month)	188 ± 41.31	183 ± 40.93	0.247	0.808[b]

Data are expressed as mean ± standard deviation.
[a] χ^2.
[b] Independent-sample *t*-test, two-tailed.

2.4. Probiotic

The probiotics in the current study was manufactured and supplied by Minsheng Pharm Co., Ltd. (Hangzhou, China). The approval code is G20100366. Each 100g contained 6.8×10^{10} CFU of Lactobacillus acidophilus and 3.3×10^{10} CFU of Bifidobacterium longum. The placebo contained only maltodextrin and was packaged to look indistinguishable from the probiotics. Maltodextrin is made from starch and is so safe that it is often used as a placebo in experiments. The dosage is one bag (1.5g) per day.

2.5. The 12-min Cooper Running/Walking test

The 12-min Cooper Running/Walking test is a simple method to estimate aerobic endurance and physical fitness.[33] A standard sports field of 400 m was marked every 10 m. Time was recorded from the start of the run, and distance was recorded every 3 min (3rd, 6th, 9th, and 12th min). Finally, the total distance traveled in 12 min was counted.

2.6. Body composition

Basic anthropometric measures included weight and height. Dual-energy X-ray absorptiometry (GE Lunar Prodigy) was used to measure total body subtotal fat mass, local (bilateral thighs) fat and muscle content, and bilateral femoral bone mineral density.

2.7. Multidimensional scale assessment

We used the Chinese version of the Eysenck Personality Questionnaire Short Scale to assess emotional stability (ESS).[34] The scale has 12 items, each of which is scored 0 or 1. Individuals who responded "yes" received one point, and those responding "no" received zero points. Higher scores indicate more emotional instability. As a self-report questionnaire for assessing GI symptoms, the Gastrointestinal Symptom Rating Scale (GSRS) includes 15 common GI symptom items, each on a 7-point scale ranging from 1 to 7, with a total score ranging from 15 to 105 points. This questionnaire was shown to be valid and reliable[35] and has been used in large-scale surveys[36,37] and to assess GI symptoms in marathon runners.[38] The Gastrointestinal Quality of Life Index scale (GIQLI) is a 36-item gastrointestinal-specific questionnaire, each question with five response categories and the responses to the questions are summed to give a numerical score, which had been used to evaluate gastrointestinal function in clinical practice and research.[39]

2.8. Running-associated skeletal muscle inflammatory edema and microvascular perfusion

Functional magnetic resonance imaging (fMRI) was used to measure the thigh muscle metabolism of athletes after 30 min of running before and after the intervention (Fig. 2). Specifically, we employed T2 sequences and IVIM sequences to assess inflammatory edema and microvascular perfusion in the biceps femoris long head (BFL), semitendinosus (ST), and semimembranosus (SM), respectively. T2 values reflect the level of inflammation in the muscle and correlate with the severity of muscle injury.[40] MRI-IVIM is sensitive to the diffusion properties of water molecules and can assess microvascular perfusion in skeletal muscle.[41,42] Both techniques have been used to detect exercise-induced changes and recovery of muscle and microvascular perfusion and were used in our previous study.[43] A 1.5-T MRI scanner (Magnetom Avanto, Siemens Healthcare, Germany) with an 18-channel body coil was used for all MRI data collection. Participants were supine with their legs fully extended within the coil. Sponges were added to the gaps in the coils to prevent motion artifacts. The scanning range was from the greater trochanter to the medial condyle of the femur bilaterally. The acquired sequences and parameters were as follows: 1)

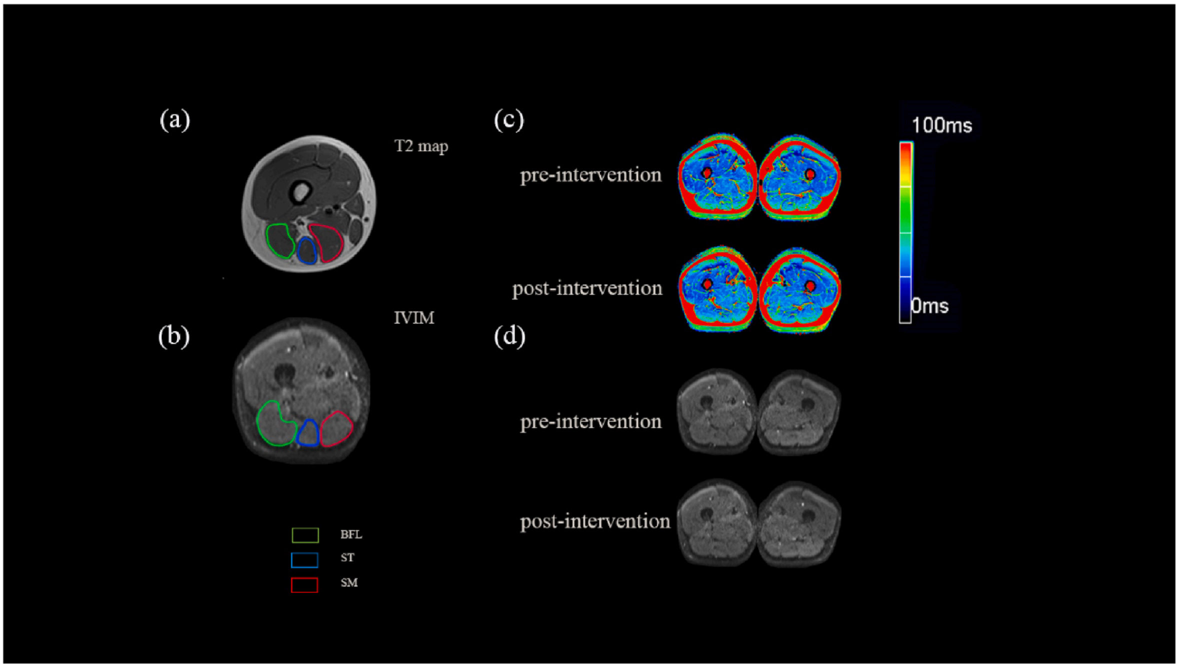

Fig. 2. Example magnetic resonance images for raw T2 (a) and intravoxel incoherent motion (IVIM) b170 images (b), in which regions of interest are outlined for biceps femoris long head (BFL), semitendinosus (ST) and semimembranosus (SM). Panels (c, d) are color-coded maps for T2 mapping and IVIM b170 images of the corresponding plane before and after the intervention, respectively. Light colors on the color-coded maps indicate lower values.

T1-weighted sequences was used to provide an anatomical reference for IVIM images. The specific parameters were as follows: TR = 850 ms, TE = 12 ms, FOV = 400 mm, thickness = 3.5 mm, average = 2, bandwidth = 182 Hz/px. 2) T2 mapping sequence. The parameters were TR = 2300 ms, TE = 13.8 ms, 27.6 ms, 41.1 ms, 55.2 ms and 69.0 ms, FOV = 400 mm, thickness = 5 mm; Bandwidth = 227 Hz/px. 3) IVIM sequence. The parameters were as follows: TR = 5800 ms, TE = 83 ms, FOV = 400 mm, thickness = 5.0 mm, slice number = 26, bandwidth = 1042 Hz/px; b value [mean = 0(1), 10(1), 20(1), 40(1), 80(1), 110(1), 140(1), 170(1), 200(2), 300(2), 400(2), 500(2), 800(3) s/mm^2. The fMRI images were processed by a senior diagnostic radiologist who was unaware of the experimental procedure and participant grouping.

2.9. Bacterial DNA extraction and 16S rRNA sequencing

Stool samples from all subjects were collected in freeze-dried tubes containing freeze-dried solution and frozen at − 80 °C freezer before the start of the trial and before the indicated endpoints. DNA was extracted from the samples, and the target primer 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and reverse primer 806R (5′-GGACTACHVGGGTWTCTAAT-3′) were selected for the V4 region of 16SrRNA gene. PCR products were amplified using AM Pure XP Beads (Beckman Coulter, Indianapolis, IN). Purified PCR products were quantified with the PicoGreen double-stranded DNA Detection kit (Invitrogen, Carlsbad, CA, USA) and then subjected to high-throughput sequencing on an Illumina novaseq 6000 paired-end 2 × 150 bp platform. Through sequence filtering, more accurate and reliable data can be obtained. Low-quality and chimeric sequences were filtered, and sequences at 97 % similarity level were grouped into a cluster operational taxonomic unit (OTU) and subjected to bioinformatics statistical analysis. *P* value of less than 0.05 was considered statistically significant.

2.10. Statistical analysis

Statistical analyses were performed using IBM SPSS software (Version 25, Statistical Package for the Social Sciences, Chicago, IL, USA). One participant had incomplete data and was counted as missing data in the analysis. Final participant numbers for baseline analysis (n = 20) represent pooled groups at pre-intervention. Analysis of the intervention report n = 10 for probiotics and n = 9 for control groups. Normally distributed data were expressed as mean ± standard deviation (mean ± SD). Responses to the intervention were analyzed using repeated measures analysis of variance (ANOVA) and Fisher's least significant difference post hoc test. Paired *t*-test was used for intra-group differences (before and after intervention). $P < 0.05$ was considered statistically significant.

3. Results

Changes in 12-min Cooper test total distance, multidimensional scale score, body composition, thigh bone mineral density, skeletal muscle content, and metabolism before and after the intervention are shown in Table 2. There were no significant differences in the above indicators between the two groups at baseline, and all of them were comparable at baseline.

3.1. Effect of probiotic supplementation on distance in 12-min cooper running/walking test

Before the intervention, the placebo group and the probiotic group achieved distances of 2.68 ± 0.41 km and 2.88 ± 0.57 km, respectively. After five consecutive weeks of probiotic intervention, the probiotics group showed increases in the total distance of the 12-min cooper test ($P < 0.05$), with no change in the control group (Table 2).

3.2. Effects of probiotic supplementation on gastrointestinal symptoms and emotional stability

For GSRS scores, significant interaction effects between time and intervention factors were observed ($F = 5.71$, $P = 0.044$), along with a significant time effect ($F = 6.07$, $P = 0.025$). Regarding GIQLI scores, no interaction effect was found between the two groups before and after treatment. However, the effect dominated by the time factor is significant. Paired *t*-test indicated that both GSRS and GIQLI scores in the

Table 2
Effects of probiotics on each observed index.

Variable	Probiotics		Placebo		Group	Time	Interaction
	Pre	Post	Pre	Post	*P* value	*P* value	*P* value
BMI (kg/m^2)	20.54 ± 2.24	20.35 ± 2.29	21.51 ± 1.84	21.54 ± 2.02	0.278	0.446	0.268
12min-cooper (km)	2.88 ± 0.57	3.01 ± 0.60*	2.68 ± 0.41	2.71 ± 0.44	0.323	0.016	0.122
Multi-dimensional scale scoring							
ESS	4.10 ± 1.66	3.40 ± 1.51	3.11 ± 1.27	3.22 ± 1.72	0.393	0.265	0.131
GSRS	9.20 ± 4.64	7.40 ± 3.24*	6.44 ± 2.74	6.33 ± 2.45	0.226	0.025	0.044
GIQLI	118.90 ± 12.30	127.50 ± 9.85*	129.44 ± 9.11	130.67 ± 8.46	0.118	0.028	0.090
Dual-energy x-ray absorptiometry							
Thigh_MM (g)	16742 ± 2550	17031 ± 2730	16890 ± 3126	16741 ± 3204	0.958	0.050	0.511
Thigh fat (%)	14.33 ± 8.35	13.99 ± 7.97	16.41 ± 7.56	16.03 ± 7.37	0.931	0.082	0.994
Left PF_ BMD	1.08 ± 0.16	1.10 ± 0.16	1.05 ± 0.97	1.06 ± 0.11	0.619	0.171	0.755
Right PF_ BMD	1.08 ± 0.15	1.08 ± 0.15	1.03 ± 0.09	1.03 ± 0.10	0.429	0.610	0.522
Body fat (%)	14.12 ± 8.41	14.43 ± 8.44	17.76 ± 7.60	17.20 ± 7.58	0.248	0.748	0.869
Magnetic resonance imaging/spectroscopy							
BFL_ IVIM	12.06 ± 5.16	13.29 ± 4.72*	11.87 ± 2.50	12.51 ± 2.86	0.819	0.028	0.454
ST_IVIM	11.40 ± 3.65	13.05 ± 3.36*	12.01 ± 1.83	12.36 ± 1.88	0.979	0.015	0.090
SM_ IVIM	13.34 ± 3.91	13.89 ± 3.70	13.30 ± 2.66	13.16 ± 3.32	0.831	0.541	0.305
BFL_T2 value	30.96 ± 1.10	31.69 ± 1.10*	30.04 ± 1.39	30.57 ± 1.24	0.098	0.026	0.700
ST _ T2 value	30.98 ± 1.75	31.54 ± 1.78	30.34 ± 3.48	30.91 ± 2.52	0.618	0.074	0.988
SM _T2 value	32.34 ± 2.23	32.91 ± 1.55	31.13 ± 2.31	31.43 ± 1.93	0.199	0.184	0.666

Table 2: Descriptive statistics for continuous variables were expressed as mean ± standard deviation. Pre- and Post-intervention efficacy was statistically analyzed by paired *t*-test. "*" represents $P < 0.05$; "**" represents $P < 0.01$. BMI, body mass index; 12min-cooper, 12-min Cooper experiment; ESS, Eysenck Personality Questionnaire Short Scale; GSRS, Gastrointestinal Symptom Rating Scale; GIQLI, Gastrointestinal Quality of Life Index scale. Thigh_MM, bilateral thigh muscle content; Thigh fat (%) and Body fat (%), represent whole-body and bilateral thigh fat mass percentages, respectively. Left PF_ BMD and Right PF_ BMD, are left and right lateral femoral bone density, respectively. BFL_IVIM, ST_IVIM, SM_IVIM, are the biceps femoris long head, semitendinosus, and semimembranosus MRI sequence "f" values, respectively; BFL_T2 value, ST_T2 value, and SM_T2 value, are the biceps femoris, semitendinosus, and semimembranosus MRI sequence "T2" values, respectively. T2mapping sequence "T2" value.

probiotic group showed improvement post-treatment compared to pre-treatment ($P = 0.027$, $P = 0.046$), whereas this difference was not statistically significant in the placebo group ($P = 0.729$, $P = 0.351$). No significant time-group interaction effects and time effects were detected for emotional stability scores between the two groups before and after treatment ($P > 0.05$).

3.3. Effect of probiotic supplementation on body composition

There were no significant time-group interaction effects and time effects on body mass index (BMI), total body subtotal fat mass (%), bilateral thigh fat mass (%), bilateral thigh muscle mass (g) and bilateral proximal femur bone mineral density (PF_BMD) between the two groups before and after intervention ($P > 0.05$). These results are described in Table 2.

3.4. Effect of probiotic supplementation on running-associated skeletal muscle inflammatory edema and microvascular perfusion

Four subjects did not undergo MRI scans after completing the running test for personal timing reasons. Therefore, MRI data from 15 participants, 8 in the probiotic group and 7 in the placebo group, were included in the final analysis.

We adopted the perfusion fraction "f" value to indicate the amount of microvascular perfusion in the IVIM sequence. The T2 value represents the level of muscle inflammation. Same as in our previous experiment.[43] The f values of BFL and ST and T2 values of BFL were increased in the probiotics group ($P < 0.05$), while there was no change in the control group. After probiotic supplementation, the f and T2 values of other measured thigh muscles tended to increase but did not reach statistical significance. In particular, no significant differences were found between and within groups in the T2 and f values of thigh muscles measured before 30 min of running for both groups of subjects before and after intervention, so we directly compared the T2 and f values of thigh muscles after 30 min of running.

3.5. Effects of probiotics supplementation on gut microbial composition

We used the 16S rRNA gene to analyze the composition of gut microbiota. At the *Phylum* level (Fig. 3a), there were no significant differences in *Bacteroidetes, Firmicutes, Proteobacteria, or Actinobacteria* between the placebo and probiotic groups before intervention. Interestingly, after five weeks of supplementation, the populations of *Bacteroidetes* were more abundant in the probiotic group than in the control group, while the number of *Firmicutes, Proteobacteria, and Actinobacteria* were less abundant in the control group than in the probiotic group.

At the *Genus* level (Fig. 3b), no significant differences in the dominant flora between placebo and probiotic groups existed before the experimental intervention. After five weeks of supplementation, *Lacticaseibacillus* was significantly richer in the probiotics group than in the control group ($P = 0.001$). In particular, the beneficial bacteria such as *Olsenella, Weissella, and Anaerostipes* were significantly increased after supplementing with probiotics ($P < 0.05$). The abundance of harmful bacteria such as *Cloacibacillus and Alphaproteobacteria_unclassified* were significantly decreased ($P < 0.01$). Unexpectedly, there was no significant increase in *Bifidobacterium* abundance after probiotic administration. As shown in Fig. 3c.

3.6. Safety assessment and blinded post-study evaluation

No adverse event attributable to ingestion of the placebo or probiotic was observed during the study period. Within one month after the end of the experiment, all of the amateur marathon runners were found to have no abnormalities in their blood index or liver and kidney function during routine physical examinations. Six participants (60 %) in the probiotics group and four participants (44.44 %) in the placebo group correctly guessed whether they were taking probiotics or placebo. For the testers, they were only asked to test and evaluate the data, and were completely unaware of the design of the intervention group or the placebo group. Seven patients (77.78 %) in the placebo group are willing to receive probiotics after the trial.

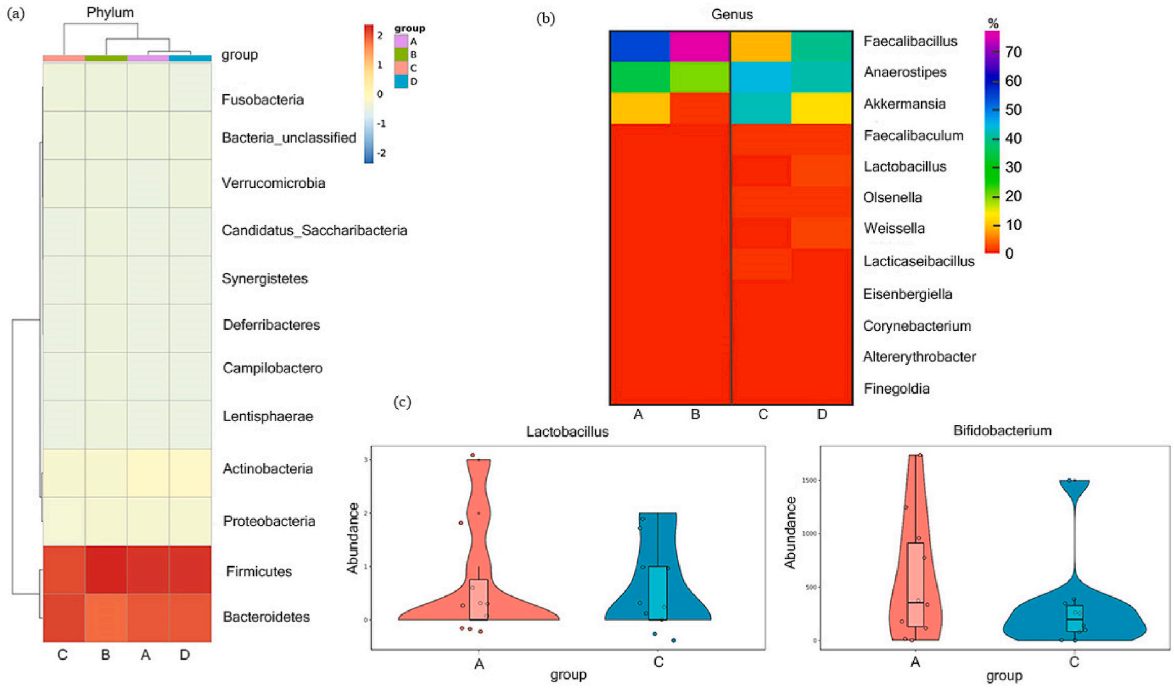

Fig. 3. Heat maps of the top 12 gut microbiota species in abundance for the four groups (before and after the probiotic intervention, before and after placebo intervention) at the phylum level(*a*)and the genus level(b). The abscissa represents the group, and the ordinate represents the species of the flora. (c) Violin plot of target microbiota (Bifidobacterium longum and Lactobacillus acidophilus) in the probiotics group. The abscissa represents the groups and the ordinate represents the abundance of the microbiota. A: probiotic group before intervention; B: placebo before intervention; C: probiotic group after the intervention; D: placebo group after the intervention.

4. Discussion

In this study, we found that after receiving a 5-week intervention of probiotic supplementation, amateur marathon runners had an increase in the abundance of beneficial bacteria and a decrease in the harmful bacteria, and showed positive effects in improving the mood management of athletes and exercise endurance performance. Moreover, we observed that probiotic supplementation increased post-exercise thigh muscle microperfusion and affected the inflammatory response to varying degrees. Finally, it produced neither favorable effects on body composition nor adverse effects on the human body.

After five weeks of supplementation, Lacticaseibacillus was significantly richer in the probiotics group than in the control group, which was consistent with our expectations. Unexpectedly, Bifidobacterium abundance did not increase significantly in the probiotics group. In addition to the changes in the target flora, the probiotic group also experienced an increase in other beneficial flora and a decrease in harmful flora. One study proposed[44] that the observed changes in the abundance of other bacterial clades are likely due to their interactions with other gut microbes. It is also possible that short-term interventions may not be sufficient to colonize “beneficial” bacteria, so further studies with longer interventions are needed. Lactobacillus and Bifidobacteria have been proposed to enhance epithelial barrier function,[45,46] regulate the immune system,[47] and improve pathological inflammation.[48] Our study also confirmed that probiotic supplements with these two bacteria as main components significantly improved intestinal symptoms and intestinal quality of life in athletes.

Many athletes develop stress-related gastrointestinal problems, such as irritable bowel syndrome (IBS), which often leads to withdrawal or poor performance. Previous studies have shown that both pathogenic and nonpathogenic gut bacteria can influence stress-related symptoms and even behavior in animals and humans.[49,50] Supplementing probiotics and other ways to adjust intestinal flora can not only relieve the emotion-related symptoms and behaviors of functional diseases such as chronic fatigue syndrome[51] but also reduce the recovery time after high-intensity training[52] and improve gastrointestinal distress and related psychological problems.[53] This is largely consistent with our findings that probiotic supplementation interventions led to improved gut symptoms and associated quality of life in athletes, but did not result in significant enhancement of emotional stability. The researchers[9] found that Veillonella can induce metabolic transformation of lactic acid to relieve exertional fatigue and improve running performance. As the 12-min Cooper’s test showed significant improvement in the probiotic group in our study, a possible reason for this could be that probiotics promote the enhancement of intestinal symptoms and the recovery of physical performance by modulating the composition of the gut microbiota.[54] Therefore, improving stress-related gastrointestinal problems by influencing the gut-brain axis through the intake of probiotics may be highly effective for endurance athletes to enhance their athletic performance.

Animal experiments and human studies have confirmed that probiotics can increase muscle mass, enhance bone mass density, prevent bone loss[55] and delay the process of osteoporosis[31] in the elderly. Probiotic supplementation caused a decrease in muscle atrophy markers (Atrogin-1, MuRF1, LC3 protein, cathepsin L), while muscle mass and strength increased.[56,57] Buigues et al.[58] found that 13-week probiotic mixture supplementation increased endurance and muscle strength in the elderly. However, we did not observe a statistically significant increase in thigh muscle mass or bilateral femoral bone density in the probiotic group. It is possible that one month was insufficient to produce significant changes in muscle and bone mass. MRI T2 mapping and IVIM sequences are widely used for quantitative monitoring of muscle at the microscopic level, especially to evaluate inflammatory edema and microvascular perfusion after exercise. Studies have shown that the T2 and f values of skeletal muscle increase immediately after running,[59–61] which may be related to the loss of calcium homeostasis, proinflammatory responses, and increased oxidative stress after exercise.[59,62] We found that the significant increase in the f value of BFL and ST after running in the probiotics group indicated an increase in muscle micro blood perfusion, which was conducive to muscle recovery.[63] In addition, an increase in T2 values occurred after probiotic supplementation, which is in contrast to the previous result.[64] Sharafi et al.[65] proposed that the increase in T2 values may be caused by an inflammatory response or may come from an increase in microvascular perfusion after exercise. Lactobacillus acidophilus and Bifidobacterium longum have been found to fermentate non-digestible carbohydrates to produce short-chain fatty acids, which can enhance lipid uptake and oxidation[66,67] and increase GLUT4 expression in skeletal muscle. These effects may contribute in part to improved insulin action and glucose handling and enhanced muscle glycogen storage after exercise training in health and disease.[68] We hypothesized that probiotic supplementation improves exercise performance by affecting the microbial-muscle axis, as indicated by an increase in microvascular perfusion of thigh muscle after running at the microscopic level. Unexpectedly, there were no significant changes in quantitative thigh muscle MRI parameters before running, suggesting that post-exercise measurements of these parameters were more responsive to muscle compensatory function.

This study also has the following limitations. First, the sample size is small and the proportion of male subjects is high, which may have a certain bias to the research results. Second, all tests and interventions were coordinated with the athlete's training program to minimize the impact on daily training and competition, so the duration of the study intervention was short. Future studies are needed to expand the sample size, extend the duration of the intervention, and investigate the effects of these changes during exercise or recovery.

In conclusion, this study confirmed the probiotic supplementation with Lactobacillus acidophilus and Bifidobacterium longum for five weeks can effectively improve gastrointestinal health and increasing Lactobacillus in amateur marathon runners. The proportion of other beneficial bacteria also increased significantly, and the number of certain pathogenic bacteria decreased.

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CRediT authorship contribution statement

Le Wang: conducted statistical analysis and wrote the initial draft. **Fan-Jing Meng:** collected data. **Yi-Han Jin:** collected data. **Li-Qiang Wu:** conducted statistical analysis. **Ruo-Yu Tang:** conducted statistical analysis. **Kuang-Hui Xu:** collected data. **Yun Guo:** collected data. **Jun-Jie Mao:** collected data. **Jian-Ping Ding:** designed the study, were responsible for the project logistics and administration, edited the manuscript. **Jie Li:** designed the study, were responsible for the project logistics and administration, wrote the initial draft, edited the manuscript. All authors approved the final version of the manuscript prior to submission.

Declaration of competing interest

The authors declare no conflict of interest.

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