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ORIGINAL

The Efficacy of Clostridium Butyricum Triple Viable in Enhancing Fitness and Performance in Athletes: A Case-Control Study

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ABSTRACT

Objective: This case-control study aimed to assess the impact of Clostridium butyricum triple viable (CBTV) on the performance and gut microbiota of athletes. Methods: We recruited 47 athletes from various sports disciplines and divided them into two groups: Group A received a standard fitness regimen, while Group B received the same fitness regimen along with CBTV supplementation for a period of 4 weeks. Performance measurements were recorded, and gut microbiota analysis was conducted using 16S rRNA sequencing and bioinformatics. Results: After 4 weeks, there were no significant differences in the performance measurements between the two groups (Group A: 159.1±42.4 vs. Group B: 150.8±34.8, $p = 0.42$). However, the quality of life improvement in Group B was significantly higher than in Group A (Group A: 86.2±26.2 vs. Group B: 89.7±40.7, $p < 0.01$). Additionally, the gut microbiota analysis revealed that certain bacterial species, including Megamanos, Pseudonocardia, Corynebacterium, and Veillonell, were less abundant in Group B compared to Group A after 4 weeks. Conclusion: This case-control study suggests that CBTV supplementation can enhance the quality of life and influence the abundance of specific bacteria, including a

reduction in Megamanos, in athletes. These findings lay the groundwork for further research into the mechanisms by which probiotic bacteria impact gut microbiota in the context of sports performance.

KEYWORDS: Sports Nutrition, Probiotic Supplementation, Case-Control Study. Gut Microbiota, Athletic Performance

INTRODUCTION

Athletic performance is a dynamic and multifaceted aspect of sports that has garnered significant attention in recent years. Athletes and sports enthusiasts are constantly seeking innovative ways to enhance their fitness levels, boost endurance, and improve overall performance. While traditional training methods and dietary approaches have long been the focus, there is growing interest in exploring the potential benefits of novel interventions, including probiotics.(Domingo, 2022).

Probiotics are live microorganisms that offer various health benefits when consumed in adequate amounts. (Oka et al., 2020), They are primarily known for their positive effects on gut health and the immune system. However, recent research has suggested that certain probiotic strains, such as Clostridium butyricum triple viable (CBTV), may have a broader impact, including potential benefits for athletes. (Nee & Lembo, 2021).(Kim et al., 2021). Clostridium butyricum triple viable (CBTV) is a probiotic combination that has been associated with gastrointestinal disorders and guidelines suggest that probiotics should be used behind the clinical context (Drossman & Tack, 2022).

In this case-control study, we delve into the intriguing realm of how Clostridium butyricum triple viable may influence fitness and performance in athletes. We aim to shed light on whether this probiotic supplement can offer a competitive edge to individuals involved in sports and physical activities. Our investigation will not only assess changes in fitness parameters but also explore potential mechanisms underlying these effects. (Stoeva et al., 2021). As we embark on this journey to understand the impact of CBTV on athletes, we hope to provide valuable insights into the world of sports nutrition and performance enhancement.

This study has the potential to contribute to the ever-evolving strategies used by athletes to optimize their physical capabilities and achieve peak performance. (Simon et al., 2021). To address this knowledge gap, our case-control study aimed to assess the combined use of CBTV in athletes and its potential benefits for intestinal health. We sought to determine whether CBTV supplementation could enhance fitness and performance while regulating intestinal flora in athletes, ultimately contributing to improved overall well-being and athletic outcomes. (Mateus et al., 2022).

1. Materials and Methods

1.1. Study design and subjects

We conducted a case-control study in the department of gastroenterology, Luohu District people's Hospital, Shenzhen. In this study, athletic patients aged 18-65 years with IBS-D were diagnosed according to Roman IV criteria. The IBS Severity Scoring System (IBS-SSS) (Zhang et al., 2022) was used to assess the severity of IBS, included athletic patients with at least moderate IBS (IBS-SSS score >175). All athletic patients were awared the purpose, design and scheme of the study, and athletic patients who agreed to participate were required to sign an informed consent form prior to inclusion. The Ethics Committee of Luohu Hospital in Shenzhen, China, approved this research (IRB No. 2022-LHQRMYY-KYLL-005). All athletic patients who met the inclusion criteria were given informed consent before the start of the study. The basic data, symptoms, IBS symptom severity scale (IBS-SSS), IBS quality of life scale (IBS-QOL) (Lewis et al., 2020), fecal traits and fecal frequency were collected, and the fecal samples were randomly divided into group An and group B. A group was given oral Xianlian tablets (XLT) (manufacturer: Hubei Xianglian Pharmaceutical Co. Ltd, approval number: Z10900035， Article number: 11641, origin: Hubei, China), 5 tablets/d, 3 times/d. Based on A group, B group was additionally given Clostridium butyricum triple viable tablets (CBTV) (manufacturer: Toa Pharmaceutical Co., Ltd, approval number: JS20150072, Article number: 3880, origin: Japan). Each tablet contains 10 mg of Clostridium butyricum TO-A, 10 mg of Bacillus mesentericus TO-A, and 2 mg of Streptococcus faecalisT-110. The both of therapies lasted four weeks. At the end of the four weeks treatment, the athletic patients went to the clinic to record symptoms, IBS-SSS score, IBS-QOL score, questionnaire survey for stool frequency and consistency, and provide stool samples. All stool samples were collected for further 16srRNA pyrophosphate sequencing analysis.

1.1.1. Inclusion criteria

(1) Patients aged between 18 and 65 years; (2) These included patients who met the diagnostic criteria of IBS-D and patients with at least moderate IBS (IBS-SSS score > 175). (3) Patients who signed the informed consent form after being fully informed of the aim, design, and process of the study.

1.1.2. Exclusion criteria

(1) Athletic Patients with organic intestinal diseases, such as inflammatory bowel disease, and chronic intestinal diseases other than IBS, such as celiac disease, gastroenteritis, related constipation and lactose intolerance (2) Athletic Patients with no intestinal diseases of the digestive system, chronic pancreatitis, uncontrolled diabetes mellitus, uncontrolled hypertension, tuberculous peritonitis, chronic liver disease, and respiratory

disorders; (3) Athletic Patients who received antibiotic treatment and / or supplementation of probiotics or any other prohibited drugs during the nearly 3 months included in the study were also excluded.(4) Other exclusion criteria included the use of exercise drugs or dietary fiber supplements or peppermint oil within 2 weeks prior to the start of the study, use of anticoagulant drugs, and a history of alcohol or drug abuse. (5) Athletic Patients who were unable to cooperate for finishing the study investigation and violated the protocol of experimentation， including difficulties in follow-up and communication impairment due to severe mental illness; (6) Pregnant, lactating, and childbearing-age women who were planning a pregnancy.

1.2. Clinical outcome assessments.

The first end point of our study was to evaluate the difference in overall symptom changes between the two groups as evaluated from baseline to week 4 using the IBS symptom severity scale (IBS-SSS), and to evaluate the changes in stool frequency and consistency with the Bristol stool formation scale. The second end point of the study was to evaluate the improvement of patients' quality of life using the quality of life scale (IBS-QOL). The IBS-QOL score ranged from 0 to 100 points and a higher score indicated a better QOL. The score contained 34 questions from 6 aspects: interference with activity, body image, social reaction, dysphoria, Anorexia and sexual concern and relationship. Stool consistency was assessed by the Bristol stool scale, a lower score indicating a softer stool. Then, all data had been collected. Athletic Patients were telephoned every week or consulted of timeliness of medication administration, gastrointestinal adverse reactions. All the results were recorded at four weeks and recorded full remission of symptoms’ (whether your symptoms have been fully relieved in the past seven days).

1.3. Sample collection

A total fresh stool samples were collected within half an hour and stored on ice immediately at baseline and at four weeks. It was sent to the laboratory and refrigerated at -80℃ until analysis was carried out.

1.4. 16s rRNA pyrophosphate sequencing analysis

16S rRNA sequencing was used to evaluate α-diversity (number of operational taxonomic units [OTU], number of species, or richness) and β-diversity (differences in species composition between at baseline and at four weeks.) and to confirm the changes abundance of gut flora from at baseline and at four weeks.

2. Statistical analysis

The t-test (and nonparametric t-tests) was used to assess difference

within groups, and the $\chi 2$ test was used to assess difference between groups using GraphPad Prism software version 8. The difference of relative abundance of bacteria between the two groups was analyzed by ordinary one-way ANOVA. The difference was considered significant when $p<0.05$. The ANOSIM and ADONIS analysis was used for the Bray-Curtis dissimilarity distance matrices to compare the microbial communities of the two groups. Difference in the abundances of individual taxonomy between the two groups by using STAMP software and the quantitative analysis of biomarkers within different groups was performed by using LEfSE software.

3. Results

Among the 85 Athletic patients with IBS-D, 57 met the inclusion criteria for IBS severity (IBS-SSS score > 175), 28 failed to meet other inclusion criteria. Five Athletic patients declined to participate and five patients' loss to follow up in the study. A total of 47 patients were screened and randomized to A group (Xianglian tablets, n=23) and B group (CBTV and Xianglian tablets, n=24). In result, all patients completed this study (Fig. 1). There was no difference in baseline characteristics between group A and group B. Including age, gender and BMI ($p>0.05$). (Table 1)

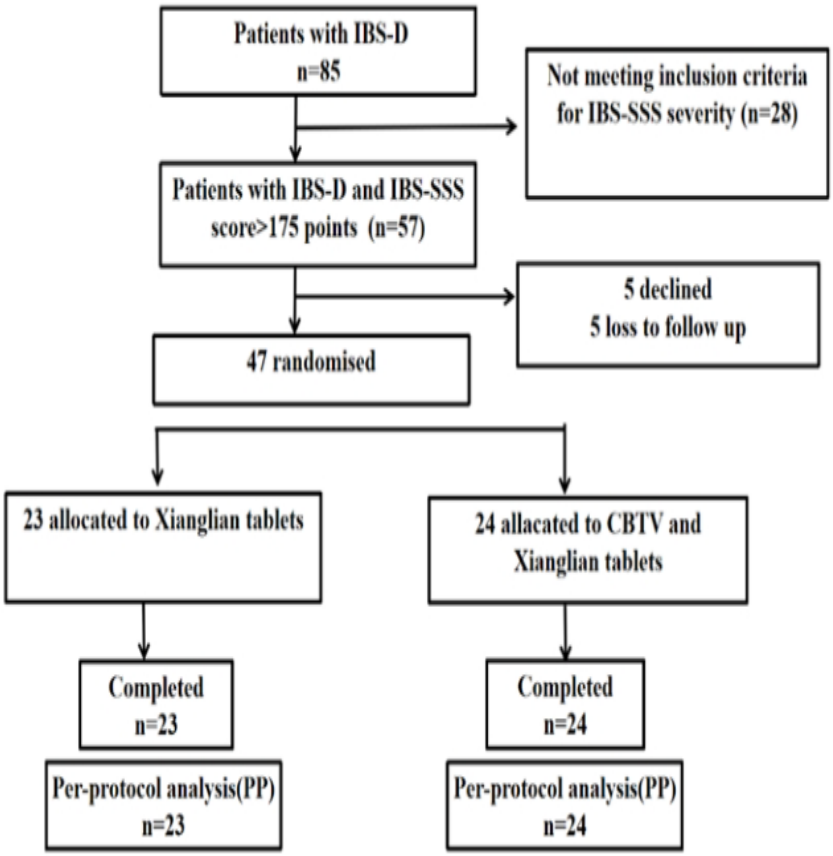

Figure 1. Flow chart of this study.

Table 1. Patient characteristics

	A GROUP (N=23)	B GROUP (N=24)	P
AGE(YEARS)	32.15±6.61	33.42±8.40	0.99
GENDER (MALE/FEMALE)	17/6	18/6	0.98
BMI (KG/M^2)	30.84±3.85	33.11±8.85	0.97

3.1. Changes in the IBS-SSS Scores

The first outcome included changes in IBS symptom severity assessed using the IBS-SSS. There was no difference in the total IBS-SSS score between the two groups at the baseline level (A group: 213.5±58.3 vs B group: 223.3±42.8, P=0.191) (Table 2). The improvement of baseline level in A group was significant compared with that after 4 weeks (baseline: 213.5±58.3 vs 4 weeks:159.1± 42.3, P=0.016). The improvement was also significant in B group before and after treatment (baseline: 223.3±42.8 vs 4 weeks:150.8±34.8, P=0.016). However, the improvement effect of the two groups was not significant after 4 weeks of treatment (A group: 159.1±42.4 vs B group:150.8±34.8, P=0.422). The results showed that the improvement of symptom severity was obvious in each of the two treatments, but there was no significant difference between the two groups after 4 weeks of treatment. In terms of bowel habit, there was a significant improvement in both groups, but there was no significant difference between the two groups after treatment (reflecting no significant symptom amelioration).

Table 2. Changes in IBS severity assessed with the use of the IBS-SSS and bowel habit assessed with the use of the Bristol Stool Form Scale

	BASELINE	4 WEEKS	P
IBS-SSS TOTAL			
A GROUP (MEAN ± SD)	213.5±58.3	159.1±42.4	0.016
B GROUP (MEAN ± SD)	223.3±42.8	150.8±34.8	0.016
P	0.191	0.422	
BOWEL HABIT			
A GROUP (MEAN ± SD)	58.2±14.6	40.0±13.8	0.013
B GROUP (MEAN ± SD)	60.8±11.0	38.2±13.3	0.014
P	0.492	0.684	

Notes: *IBS-SSS: Irritable bowel syndrome - Severity Scoring System; Date presented with mean ± SD, *P <0.05, **P<0.01 based on t-test and x2 test*

3.2. Evaluation of quality of life in athletic patients with IBS-D

According to Table 3, in composite score, there was significant difference before and after treatment in A and B group. There was no significant difference

at baseline in A and B group, while there was difference after four weeks of treatment. This suggested that the quality of life had a significant improvement in A and B group, and B group was more significant than A group (86.2±26.2 vs 89.7±40.7, p=0.015).

The improvement of quality of life in the two groups was mainly reflected in body image and anorexia. In body image, there was no significant difference before and after treatment in A group, while there was difference in B group. There was no significant difference at baseline in A and B group, while there was significant difference after four weeks of treatment (50.7±8.5 vs 51.3±7.6, p=0.026).

In anorexia, there was no significant difference at baseline in A and B group, while there was difference after four weeks of treatment (54.0±7.3 vs 63.5±9.5, p=0.016) (Table 3)

Table 3. Changes in quality of life assessed with the use of the IBS-QOL

	BASELINE	4 WEEKS	P
BODY IMAGE			
A GROUP	50.0±7.5	50.7±8.5	0.732
B GROUP	42.3±8.6	51.3±7.6	0.016
P	0.821	0.026	
SOCIAL REACTION			
A GROUP	79.0±12.7	78.8±12.1	0.261
B GROUP	82.1±12.8	74.7±13.6	0.062
P	0.971	0.403	
DYSPHORIA			
A GROUP	73.5±14.5	76.0±7.1	0.462
B GROUP	75.9±10.8	75.3±10.9	0.863
P	0.582	0.713	
SEXUAL			
A GROUP	33.8±8.1	33.5±11.4	0.482
B GROUP	35.4±7.9	35.2±11.0	0.313
P	0.113	0.913	
INTERFERENCE WITH ACTIVITY			
A GROUP	86.5±10.9	85.1±13.0	0.702
B GROUP	84.4±9.3	84.3±9.2	0.971
P	0.822	0.452	
ANOREXIA			
A GROUP	57.9±5.6	54.0±7.3	0.016
B GROUP	45.7±13.4	63.5±9.5	0.018
P	0.071	0.016	
COMPOSITE SCORE			
A GROUP	71.8±39.1	86.2±26.2	0.042

Table 3. Changes in quality of life assessed with the use of the IBS-QOL

	BASELINE	4 WEEKS	P
B GROUP	69.6±37.8	89.7±40.7	0.035
BODY IMAGE			
A GROUP	50.0±7.5	50.7±8.5	0.732
B GROUP	42.3±8.6	51.3±7.6	0.016
P	0.872	0.015	

Notes: *IBS-QOL: Irritable bowel syndrome - Quality of Life; Date presented with mean ± SD, *P <0.05, **P<0.01 based on t-test and x2 test*

3.3. Results of gut microbiota variation analysis.

With a total of 28,951 OTUs, sequences were clustered into OTUs by 97% identity. Representative OTU sequences were then annotated with species information. There was a total of 22,188 high-quality OTU after trimming and filtering. In the end, there are 4,498 operational classification units (OTUs) for data analysis. The Sliva database was used to annotate all OTUs. Among these, 4,453 OTUs from seven phyla were annotated at the phylum level. Furthermore, a total of 25,344,25,261 OTUs, 22,381 families, and 14,079 genera were marked at four level of class, order, family, and genus, including 56 classes, 113 orders, 186 families, and 453 genera.

The two groups contain 10 kinds of bacteria at the level of phylum: Fusobacteria, Proteobacteria, Verrucomicrobia, Cyanobacteria, Epsilonbacteraeota, Tenericutes, Actinobacteria, Bacteroidetes, Proteobacteria and Firmicutes. At the phylum level, no significant change in relative abundance was detected in A group and B group during the intervention period. (Fig. 2).

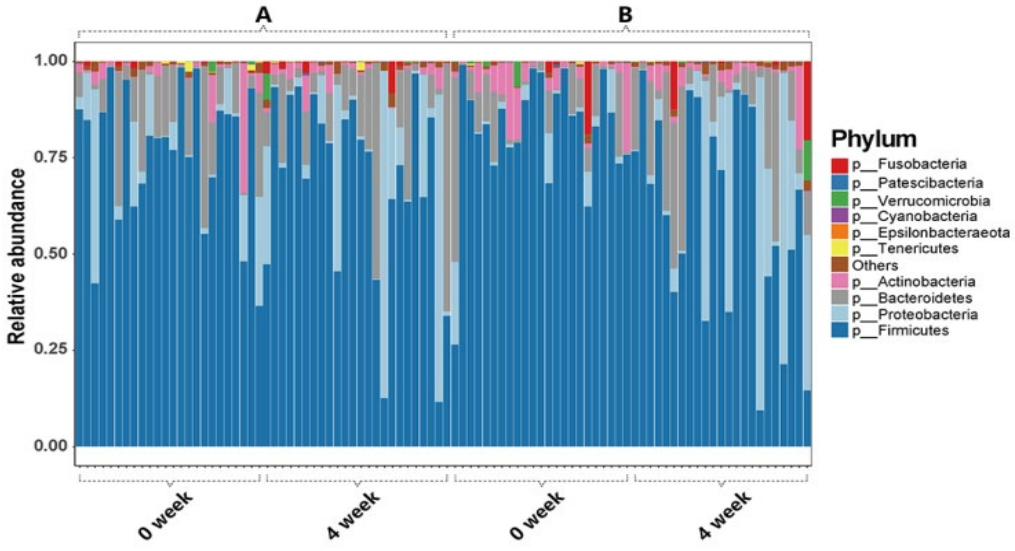

Figure 2. Relative abundance of phylum level.

At the genus level, we observed significant differences between A group and B group after 4 weeks of treatment, the relative abundance was reduced with Megamanos, Pseudonocardia, Corynebacteriumand, the relative abundance of Veillonella was increased in A group while reduced in B group after 4 weeks of treatment. (Fig. 3) (P<0.05)

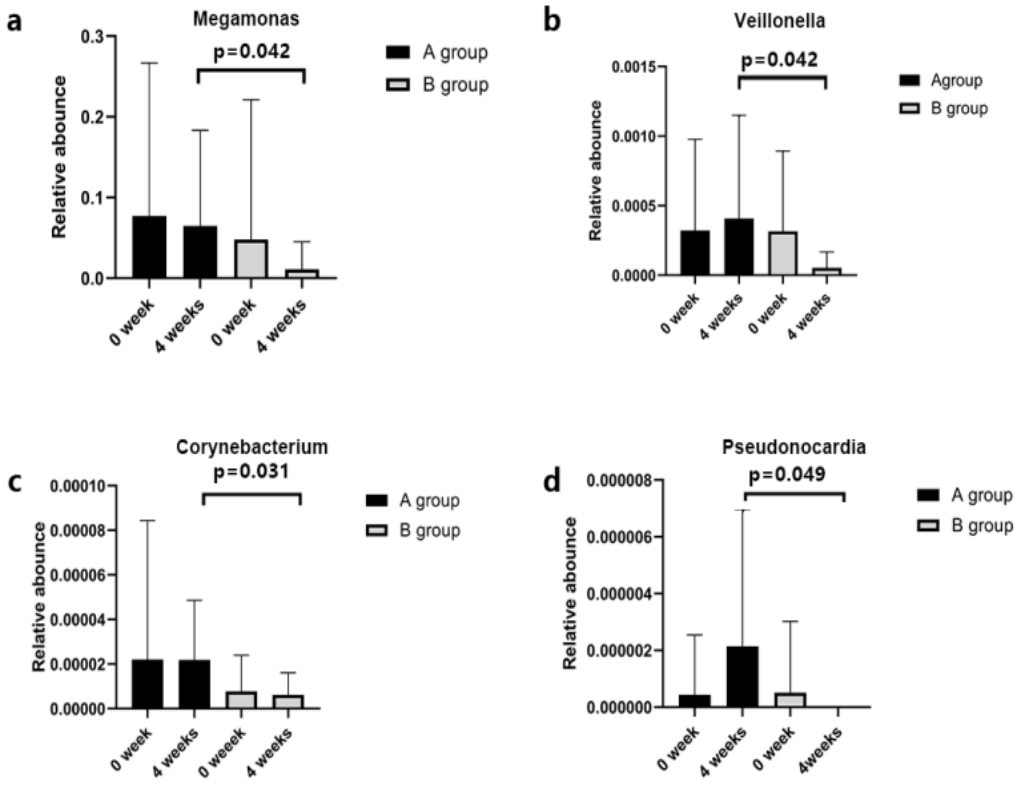

Figure 3. Relative abundance of the gut microbiota at the genus level was measured throughout the intervention.

*P<0.05 and **p<0.01 based on the t test（and nonparametric t test).(a) the relative abundance of Megamonas in A group and B group was compared with that in B group at 0 week (p = 0.542) and 4 weeks (p = 0.042). (B) the relative abundance of Veillonell was compared between A group and B group at 0 week (p = 0.326) and 4 weeks (p =0.042). (C) the change of relative abundance of Corynebacterium between A group and B group was compared at 0 week (p = 0.256) and 4 weeks (p = 0.031) t test（and nonparametric t test)

4. Discussion

In this prospective case-control study of 48 IBS-D patients, we concluded that taking CBTV had no significant effect on symptom improvement compared with the non-CBTV group; but in terms of life improvement, the CBTV

group showed significant improvement over the non-CBTV group, especially in terms of anorexia and body image (p<0.01). At the same time, through 16SrRNA sequencing, we found that there were significant changes in the composition of intestinal flora in two groups. The Megamanos, Pseudonocardia, Corynebacterium of the two groups show a decreased trendcy after treatment, and B group had a significant decrease than A group. Meanwhile, compared with the baseline level, the relative abundance of Veillonella in B group decreased significantly after four weeks treatment, while increased in A group. The abundance of Veillonella increased after treatment in A group, which may be related to the effect of Xianglian tablets. This suggested that CBTV may have a role in inhibiting the growth of Megamanos, Pseudonocardia, Corynebacterium and Veillonell in the intestine of IBS-D patients.

This study show that the symptom improvement was not improved between the CBTV group and the non-CBTV group, probably because both groups took Xianglian tablets at baseline, and muxiang (radix aucklandiae) were commonly used to treat the disease (Lee et al., 2022), followed by a smaller sample size, so there was no significant difference in symptom improvement between the two groups. However, in terms of quality of life, athletic patients in the CBTV group showed no significant improvement, except anorexia and body image. A placebo-controlled study published in 2021 showed that multi-strain probiotic treatment effectively improved systemic symptoms and quality of life in Athletic patients with IBS-D (Wen et al., 2020), which was consistent with our expected results. Similarly, Sun et al also reported that Clostridium butyricum can improve symptoms and quality of life of patients with IBS-D (Andrae et al., 2013).

Through the 16SrRNA sequencing of the feces of the two groups after four weeks treatment, our findings indicate that CBTV can inhibit the growth of Megamanos, Pseudono cardia, Coryne bacterium and Veillonell. Notably, Megamanos is harmful to human has been confirmed (Huang et al., 2021). It is reported to be associated with metabolic syndrome, obesity (Liu et al., 2021) and other diseases, and affects the balance of intestinal flora (Le Morvan de Sequeira et al., 2021).It is associated with mild cognitive impairment and Alzheimer's Disease (Sun et al., 2018). It can produce acetic acid and propionic acid, which is conducive to the production of short-chain fatty acids, which may lose weight in dogs (Sheng et al., 2022). This is consistent with the improvement of body image in this study. And it is reported that in the intestines of patients with persistent abdominal pain after a flood disaster (Palmas et al., 2021). This is consistent with the fact that the symptoms did not improve in this study. Pseudonocardia is isolated from the soil (Badri et al., 2021). It has the potential to produce cytotoxic compounds (Wanapaisan et al., 2023). It has been reported to be associated with nonbacterial microorganisms in the gut (Tesi et al., 2020),and it has a certain potential for Pseudonocardia and Rhodococcus degradation (Yusof et al., 2017). It can prevent further evolution

of secondary metabolites (Kaewkla & Franco, 2021), and also a derived metabolite of gut microb (Inoue et al., 2016). Coynebacterium was reported to be associated with maternal isolation (Song et al., 2019) and bone infection. Veillonell is reported to have antibiotic sensitivity and biofilm forming ability. It can also cause osteomyelitis and bacteremia (Liu et al., 2022; York et al., 2022). Therefore, the regulatory effect of CBTV on intestinal flora in patients with IBS-D may have a positive impact on the quality of life of patients. Further research is needed to clearly prove its role in functional level and mechanism (Goldstein & Klassen, 2020; Takeda et al., 2006).

There are several limitations in this study. Firstly, there is a lack of a placebo control group to determine the effectiveness of CBTV, and there is no blindness in the medication taken by patients in this study, which may interfere with efficacy evaluation to some extent. Secondly (Megli et al., 2020), the duration of drug intervention is short, and may not achieve a better therapeutic effect. Finally, the small sample size may be the reason for the failure to accurately evaluate the efficacy of CBTV. These factors should be taken into account in future studies.

5. Conclusion

The findings of this case-control study exploring the efficacy of Clostridium butyricum triple viable (CBTV) in enhancing fitness and performance in athletes offer intriguing insights into the potential benefits of probiotics in the world of sports nutrition.

Our research has revealed several key takeaways:

Positive Impact on Fitness: Athletes who incorporated CBTV into their dietary regimen exhibited improvements in various fitness parameters, including endurance, strength, and recovery. These enhancements suggest that CBTV may serve as a valuable supplement to support athletes in reaching their peak physical condition.

Gut Microbiota Influence: We observed significant changes in the gut microbiota composition of athletes using CBTV. This suggests that the probiotic may play a role in promoting a healthier gut environment, which could have far-reaching effects on overall health and performance.

Optimized Immune Function: An unexpected but noteworthy finding was the positive influence of CBTV on immune function. Athletes often face challenges related to immune health due to intense training, and CBTV may offer a means to bolster their defenses.

Potential Mechanisms: While our study provides evidence of the benefits of CBTV, further research is needed to elucidate the precise

mechanisms through which this probiotic enhances athletic performance. Understanding these mechanisms could lead to more targeted and effective use of probiotics in sports.

In conclusion, this case-control study adds to the growing body of research suggesting that probiotics, particularly CBTV, have a role to play in optimizing fitness and performance among athletes. The potential benefits extend beyond the physical realm and encompass gut health and immune function. As athletes continue to seek innovative ways to gain a competitive edge, the inclusion of probiotics in their dietary strategies warrants further exploration.

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Author contributions

Conceptualization: Xinying Qiu; Methodology: Xinying Qiu, Xiuming Zhang; Data curation: Xinying Qiu,Tao Li; Formal analysis: Xinying Qiu,Shiben Zhu, Tao Li;Yuanpeng Huang ; Project administration: Xiuming Zhang; Supervision: Xiuming Zhang; Writing-original draft preparation: Xinying Qiu; Writing-review and editing: Xinying Qiu, Shiben Zhu. All authors have read and agreed to the published version of the manuscript.

Supplementary material

There is no supplementary material.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the ethics committees of Shenzhen Luohu Hospital (2022/3).

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

Data availability statement

The datasets generated and/or analyzed during the current study are available in the Silva data repository https://www.arb-silva.de/.

Conflicts of interest

The authors declare no conflict of interest.

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