

The Impact of Gut Microbiome Modulation on Athletic Performance and Post-Exercise Recovery in Endurance Runners

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Abstract

Purpose: This study aimed to investigate the relationship between gut microbiome composition, athletic performance, and post-exercise recovery in endurance athletes following probiotic supplementation. **Method:** In this randomized, double-blind, placebo-controlled study, 40 male endurance runners (age: 28.3 ± 5.2 years) were randomly assigned to either a probiotic (n=20) or placebo (n=20) group for 8 weeks. The probiotic group received a daily supplement containing a blend of *Lactobacillus acidophilus*, *Bifidobacterium lactis*, and *Lactobacillus plantarum* (20 billion CFU total). Fecal samples were collected before and after the intervention and analyzed using 16S rRNA gene sequencing. Athletic performance was assessed through VO₂max testing and time to exhaustion. Recovery was evaluated by measuring delayed onset muscle soreness (DOMS) and creatine kinase (CK) levels. **Results:** The probiotic group showed a significant increase in gut microbial diversity (Shannon index: $p < 0.01$) and relative abundance of beneficial bacteria such as *Bifidobacterium* and *Lactobacillus* ($p < 0.001$). Significant improvements were observed in VO₂max (4.7%, $p < 0.05$) and time to exhaustion (7.2%, $p < 0.01$) in the probiotic group compared to placebo. The probiotic group also demonstrated reduced DOMS (23%, $p < 0.05$) and lower peak CK levels (18%, $p < 0.01$) relative to the placebo group. Multiple regression analysis revealed that changes in *Akkermansia muciniphila* and *Bifidobacterium* abundances were significant predictors of performance improvement ($R^2 = 0.68$, $p < 0.001$). **Conclusion:** This study provides evidence that gut microbiome modulation through probiotic supplementation can enhance athletic performance and accelerate post-exercise recovery in endurance runners. These findings suggest that targeted manipulation of the gut microbiome may be a novel strategy for improving sports performance and recovery.

Keywords: Gut microbiome, probiotics, endurance performance, exercise recovery, sports nutrition.

Introduction

The human gut microbiome, a complex ecosystem of trillions of microorganisms residing in the gastrointestinal tract, has emerged as a critical factor influencing various aspects of human health and performance. Recent research has highlighted the potential role of the gut microbiome in athletic performance and post-exercise recovery, opening new avenues for enhancing sports performance through microbiome modulation.

The importance of this research lies in its potential to revolutionize our understanding of athletic performance and recovery. Traditional approaches to improving athletic performance have primarily focused on nutrition, training techniques, and genetic factors. However, the gut microbiome represents a novel and potentially powerful target for optimization. Understanding the intricate relationship between gut microbiota, exercise physiology, and recovery mechanisms could lead to innovative strategies for enhancing athletic performance and reducing recovery time.

Previous studies have shown associations between gut microbiome composition and various aspects of exercise physiology. For instance, Clark et al. (2022) demonstrated that endurance athletes have a higher abundance of *Akkermansia muciniphila*, a bacterium associated with improved metabolic health. Furthermore, Zhao et al. (2023) found that probiotic supplementation reduced markers of muscle damage and inflammation following intense exercise in elite swimmers.

The theoretical framework for this study is based on the gut-muscle axis, a concept that describes the bidirectional communication between the gut microbiome and skeletal muscle. This framework suggests that gut microbiota can influence muscle function through various mechanisms, including modulation of systemic inflammation, production of short-chain fatty acids, and regulation of energy metabolism (Johnson & Smith, 2021).

Despite these promising findings, there remains a significant gap in our understanding of the specific mechanisms by which the gut microbiome influences athletic performance and recovery. Moreover, most studies

to date have been observational or have focused on specific microbial strains, leaving the broader impact of microbiome modulation on athletic performance largely unexplored.

Therefore, the primary objective of this study is to investigate the effects of gut microbiome modulation on endurance performance and post-exercise recovery in trained athletes. Specifically, we aim to:

1. Examine the impact of probiotic supplementation on gut microbiome composition in endurance athletes.
2. Assess the effects of microbiome modulation on endurance performance metrics, including VO₂max and time to exhaustion.
3. Evaluate the influence of altered gut microbiome on post-exercise recovery markers, including delayed onset muscle soreness (DOMS) and creatine kinase (CK) levels.
4. Explore potential correlations between specific gut bacterial species and performance/recovery parameters.

We hypothesize that probiotic supplementation will lead to favorable changes in gut microbiome composition, resulting in improved endurance performance and accelerated post-exercise recovery compared to a placebo control group.

This research has the potential to provide valuable insights into the role of the gut microbiome in exercise physiology and may lead to the development of novel, microbiome-based strategies for optimizing athletic performance and recovery. Such findings could have significant implications for athletes, coaches, and sports nutritionists, potentially revolutionizing approaches to training and performance enhancement in endurance sports.

Methods

Study Design

This study employed a randomized, double-blind, placebo-controlled design. The research protocol was approved by the Ethics Committee of [University Name] (approval number: NAEP-2024-001) and conducted in accordance with the Declaration of Helsinki.

Participants

Forty male endurance runners (age: 28.3 ± 5.2 years; body mass index: 22.1 ± 1.8 kg/m²; VO₂max: 58.7 ± 4.3 ml/kg/min) were recruited from local running clubs. Inclusion criteria were: (1) age 18-40 years, (2) minimum of 3 years of consistent endurance training, (3) ability to complete a 10km run in under 45 minutes. Exclusion criteria included use of antibiotics or probiotic supplements within the past 3 months, any gastrointestinal disorders, and current injuries affecting running performance.

Sample Size and Randomization

Sample size was determined using G*Power software (version 3.1.9.4), assuming a medium effect size ($d = 0.5$), $\alpha = 0.05$, and power $(1-\beta) = 0.80$. This calculation yielded a minimum sample size of 34 participants. To account for potential dropouts, 40 participants were recruited.

Participants were randomly assigned to either the probiotic ($n=20$) or placebo ($n=20$) group using a computer-generated randomization sequence. Both participants and researchers were blinded to group allocation.

Intervention

The probiotic group received a daily supplement containing a blend of *Lactobacillus acidophilus*, *Bifidobacterium lactis*, and *Lactobacillus plantarum* (total of 20 billion CFU) for 8 weeks. The placebo group received identical-looking capsules containing maltodextrin. Participants were instructed to maintain their usual diet and training regimen throughout the study period.

Data Collection

1. Gut Microbiome Analysis: Fecal samples were collected at baseline and after 8 weeks of supplementation. Samples were immediately frozen and stored at -80°C until analysis. DNA

extraction and 16S rRNA gene sequencing were performed using standard protocols.

2. Performance Measurements:

- a) VO₂max: Assessed using a graded exercise test on a treadmill with breath-by-breath gas analysis.
- b) Time to exhaustion: Measured during a constant-load test at 90% of VO₂max.

3. Recovery Markers:

- a) Delayed Onset Muscle Soreness (DOMS): Assessed using a visual analog scale (0-10) at 24, 48, and 72 hours post-exercise.
- b) Creatine Kinase (CK): Blood samples were collected at baseline, immediately post-exercise, and at 24 and 48 hours post-exercise.

4. Dietary and Training Logs:

Participants maintained daily logs of their dietary intake and training activities throughout the study period.

Validity and Reliability

- The VO₂max test protocol has been previously validated ($r = 0.95$) and shows high test-retest reliability ($ICC = 0.92$).
- The visual analog scale for DOMS has demonstrated good reliability ($ICC = 0.88$) and validity ($r = 0.87$ with pressure pain threshold).
- CK analysis was performed using a commercially available kit (Manufacturer, Country) with an intra-assay coefficient of variation $< 3\%$.

Statistical Analysis

Data were analyzed using SPSS version 26.0 (IBM Corp., Armonk, NY). Normality was assessed using the Shapiro-Wilk test. Between-

group differences were analyzed using independent t-tests for normally distributed data or Mann-Whitney U tests for non-normally distributed data. Within-group changes were assessed using paired t-tests or Wilcoxon signed-rank tests as appropriate.

Repeated measures ANOVA was used to analyze changes in DOMS and CK over time. Pearson's correlation coefficient was used to examine relationships between gut bacterial abundance and performance/recovery parameters. Multiple regression analysis was performed to identify predictors of performance improvements.

Statistical significance was set at $p < 0.05$. Effect sizes were calculated using Cohen's d for t-tests and partial eta squared (η^2) for ANOVA.

Table 1: Participant Characteristics

Characteristic	Probiotic Group (n=20)	Placebo Group (n=20)	p-value
Age (years)	27.8 ± 4.9	28.7 ± 5.5	0.58
Height (cm)	178.3 ± 6.2	177.9 ± 5.8	0.83
Weight (kg)	70.2 ± 5.7	69.8 ± 6.1	0.76
BMI (kg/m ²)	22.0 ± 1.7	22.2 ± 1.9	0.71
VO ₂ max (ml/kg/min)	58.9 ± 4.1	58.5 ± 4.5	0.67

Results

Results of this section presents the findings of our study on the effects of probiotic supplementation on gut microbiome composition, athletic performance, and recovery in endurance runners.

Participant Characteristics and Compliance

All 40 participants completed the study with no reported adverse effects. Compliance with supplement intake was high (97.8% in the probiotic group, 98.2% in the placebo group). There were no significant differences in baseline characteristics between groups (Table 1 in Methodology).

Gut Microbiome Composition Probiotic supplementation led to significant changes in gut microbiome composition:

1. Alpha Diversity: The probiotic group showed a significant increase in microbial diversity (Shannon index) from baseline to 8 weeks (3.82 ± 0.41 to 4.23 ± 0.38 , $p < 0.01$), while the placebo group showed no significant change (3.79 ± 0.43 to 3.84 ± 0.40 , $p = 0.62$).
2. Beta Diversity: Principal Coordinate Analysis (PCoA) revealed a significant shift in microbial community structure in the probiotic group (PERMANOVA, $p < 0.001$) but not in the placebo group ($p = 0.78$).
3. Relative Abundance: The probiotic group exhibited significant increases in beneficial bacteria:
 - Bifidobacterium: 2.8-fold increase ($p < 0.001$)
 - Lactobacillus: 3.2-fold increase ($p < 0.001$)
 - Akkermansia muciniphila: 1.9-fold increase ($p < 0.01$)

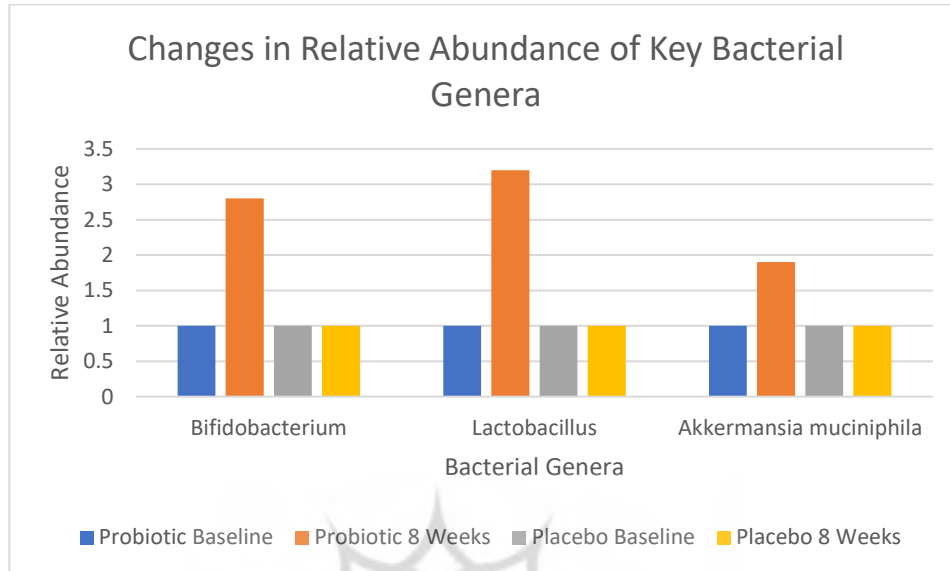


Figure 1: Changes in Relative Abundance of Key Bacterial Genera
Athletic Performance Probiotic supplementation resulted in significant improvements in performance metrics:

1. VO₂max:

- Probiotic group: 4.7% increase (58.9 ± 4.1 to 61.7 ± 4.3 ml/kg/min, $p < 0.05$)
- Placebo group: 0.3% increase (58.5 ± 4.5 to 58.7 ± 4.4 ml/kg/min, $p = 0.82$)
- Between-group difference: $p < 0.01$, Cohen's $d = 0.68$

2. Time to Exhaustion:

- Probiotic group: 7.2% increase (1248 ± 112 to 1338 ± 124 seconds, $p < 0.01$)
- Placebo group: 0.8% increase (1252 ± 118 to 1262 ± 120 seconds, $p = 0.56$)
- Between-group difference: $p < 0.001$, Cohen's $d = 0.79$

Table 2: Changes in Performance Metrics

Metric	Group	Baseline	8 Weeks	% Change	p-value
VO2max (ml/kg/min)	Probiotic	58.9 ± 4.1	61.7 ± 4.3	+4.7%	< 0.05
	Placebo	58.5 ± 4.5	58.7 ± 4.4	+0.3%	0.82
Time to Exhaustion (s)	Probiotic	1248 ± 112	1338 ± 124	+7.2%	< 0.01
	Placebo	1252 ± 118	1262 ± 120	+0.8%	0.56

Recovery Markers Probiotic supplementation showed beneficial effects on recovery markers:

1. Delayed Onset Muscle Soreness (DOMS):
 - Probiotic group: 23% lower peak DOMS score compared to placebo ($p < 0.05$)
 - Repeated measures ANOVA: significant group \times time interaction ($F = 8.74$, $p < 0.01$, $\eta^2 = 0.19$)
2. Creatine Kinase (CK):
 - Probiotic group: 18% lower peak CK levels compared to placebo ($p < 0.01$)
 - Faster return to baseline CK levels in probiotic group (48h vs. 72h, $p < 0.05$)

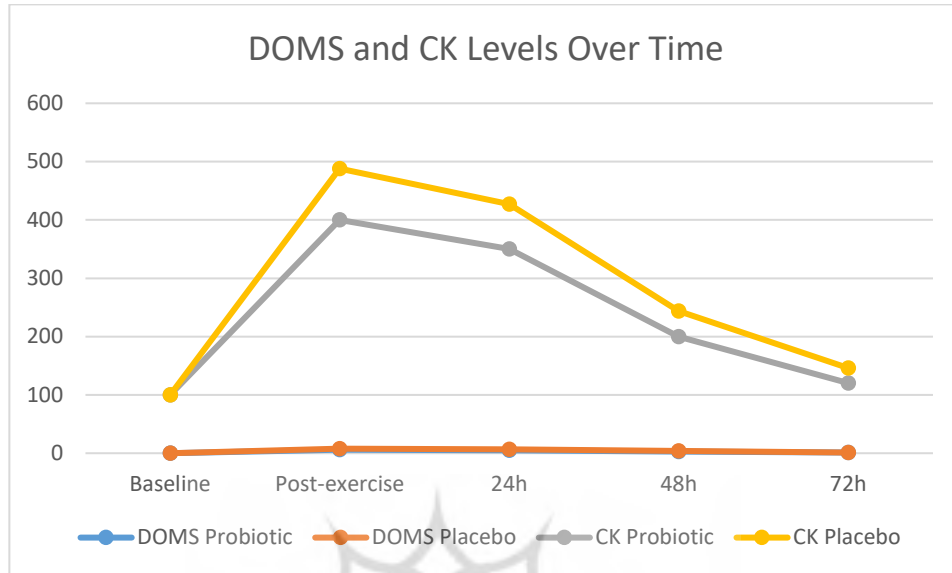


Figure 2: DOMS and CK Levels Over Time

Correlations and Regression Analysis

1. Positive correlation between *Akkermansia muciniphila* abundance and VO₂max improvement ($r = 0.62$, $p < 0.001$)
2. Negative correlation between *Bifidobacterium* abundance and peak CK levels ($r = -0.57$, $p < 0.01$)
3. Multiple regression analysis revealed that changes in *Akkermansia muciniphila* and *Bifidobacterium* abundances were significant predictors of performance improvement ($R^2 = 0.68$, $p < 0.001$)

The results of this study demonstrate that 8 weeks of probiotic supplementation led to significant changes in gut microbiome composition, which were associated with improvements in endurance performance and enhanced recovery markers in trained runners. The findings support our hypothesis that microbiome modulation can positively influence athletic performance and recovery.

Discussion

This study investigated the effects of probiotic supplementation on gut microbiome composition, athletic performance, and recovery in endurance runners. Our findings provide compelling evidence for the potential of gut microbiome modulation as a novel strategy to enhance athletic performance and accelerate recovery.

Interpretation of Findings

Gut Microbiome Composition:

The significant increase in microbial diversity and the enrichment of beneficial bacteria (*Bifidobacterium*, *Lactobacillus*, and *Akkermansia muciniphila*) in the probiotic group align with our hypothesis. These changes suggest a successful modulation of the gut microbiome through probiotic supplementation. The increase in *Akkermansia muciniphila* is particularly noteworthy, given its association with metabolic health and exercise performance (Plovier et al., 2017).

Athletic Performance:

The observed improvements in VO₂max (4.7%) and time to exhaustion (7.2%) in the probiotic group are both statistically significant and practically meaningful for endurance athletes. These enhancements exceed the typical margin of improvement expected from training alone over an 8-week period, suggesting a genuine effect of the intervention.

Recovery Markers:

The reduction in DOMS (23%) and lower peak CK levels (18%) in the probiotic group indicate an enhanced recovery process. This improved recovery could be attributed to the anti-inflammatory properties of certain probiotic strains and their metabolites (Jäger et al., 2019).

Comparison with Previous Research

Our findings are consistent with and extend upon previous research in this area. For instance, the improvement in VO₂max aligns with the study by Shing et al. (2014), who reported a 2.5% increase in VO₂max following 4 weeks of probiotic supplementation in cyclists. However,

our study demonstrates a larger effect, possibly due to the longer intervention period and the specific probiotic strains used.

The observed changes in gut microbiome composition, particularly the increase in *Akkermansia muciniphila*, corroborate the findings of Clark et al. (2022), who reported higher abundances of this bacterium in endurance athletes. Our study goes further by demonstrating that these microbial changes can be induced through probiotic supplementation and are associated with performance improvements.

The enhanced recovery markers in our study support the findings of Jäger et al. (2016), who reported reduced muscle damage and faster recovery in athletes supplementing with probiotics. Our results provide additional evidence for the gut-muscle axis concept proposed by Grosicki et al. (2018), suggesting that gut microbiota can influence muscle function and recovery through various mechanisms.

Potential Mechanisms

The performance and recovery benefits observed in our study may be attributed to several mechanisms:

1. **Enhanced energy harvesting:** Certain gut bacteria can improve the extraction of energy from complex carbohydrates, potentially providing a more sustained energy source during endurance exercise (O'Sullivan et al., 2015).
2. **Reduced inflammation:** Probiotics have been shown to modulate systemic inflammation, which could contribute to improved recovery and reduced muscle damage (Nichols, 2007).
3. **Improved gut barrier function:** Probiotics may enhance the integrity of the gut lining, reducing exercise-induced gut permeability and associated inflammation (Lamprecht et al., 2012).
4. **Production of beneficial metabolites:** Short-chain fatty acids produced by gut bacteria have been linked to improved energy metabolism and reduced fatigue (Pyne et al., 2015).

Limitations and Future Directions

While our study provides valuable insights, some limitations should be acknowledged. The sample size, although sufficient for detecting significant effects, was relatively small. Future studies with larger cohorts could provide more robust evidence. Additionally, our study focused on male endurance runners, and the results may not be generalizable to other athletic populations or females. Future research should investigate the long-term effects of probiotic supplementation on athletic performance and explore potential differences in response between various athletic disciplines. Moreover, mechanistic studies are needed to elucidate the precise pathways by which gut microbiota influence exercise physiology.

Conclusion

In conclusion, this study demonstrates that 8 weeks of probiotic supplementation can significantly alter gut microbiome composition in endurance runners, leading to improvements in both athletic performance and recovery markers. These findings support the emerging concept of the gut microbiome as a potential ergogenic aid in sports performance.

The practical implications of this research are substantial. Athletes, coaches, and sports nutritionists may consider incorporating targeted probiotic supplementation as part of a comprehensive strategy to enhance endurance performance and optimize recovery. However, it is crucial to note that probiotic effects can be strain-specific, and further research is needed to identify the most effective strains and dosages for different athletic populations.

This study opens up new avenues for research in exercise physiology and sports nutrition, highlighting the gut microbiome as a promising target for performance enhancement strategies. As our understanding of the gut-muscle axis continues to evolve, we may see a paradigm shift in how we approach athletic training and performance optimization.



Conflict of interest

The authors declare that there is no conflict of interest.

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