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ORIGINAL ARTICLE

CONTENTS

Dynamics of gut microbiota in qualified female boxers

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ABSTRACT

Aim: To analyze changes in the gut microbiota of qualified female boxers at different stages of the training cycle, influenced by physical activity of different intensity.

Materials and Methods: The study involved nine qualified female boxers, who performed high-intensity training sessions. The study of changes in the state of the microbiota, namely its diversity, was carried out in two phases of the training cycle. A microbiological quantitative method was used, in-depth with species identification and detection of anaerobes, the unit of measurement of which was colony-forming units per gram (CFU/g). The quantitative assessment of microorganisms was performed using arithmetic mean values expressed and log-transformed values ($\log_{10}$ CFU/g).

Results: High-intensity training loads were associated with microbiota changes indicative of dysbiosis. It is noticeable that the concentration of typical pathogenic genera (*Candida albicans*, *Klebsiella pneumoniae, Streptococcus*) significantly increased during the post-competition period. At the same time, classic "beneficial" bacteria (*Lactobacillus and Bifidobacterium*) remained within normal limits, although Bifidobacterium showed some growth. Notably, *Escherichia coli* (a normal representative of the microbiome) exceeded the norm during the post-competition period.

Conclusions: Increased training intensity in qualified female boxers resulted in alterations in gut microbiota composition, most notably a significant rise in *Escherichia coli* and other opportunistic microbes, while the levels of beneficial *Lactobacillus* and *Bifidobacteria* species remained relatively stable. These changes suggest early signs of dysbiosis, consistent with current evidence on the impact of extreme physical exertion on microbial health.

KEY WORDS: microbiota profile, high-intensity training, female athletes

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INTRODUCTION

Recent studies have demonstrated a complex, dose-dependent effect of exercise on gut microbiota composition and function. A meta-analysis of 25 studies including 1044 participants showed that exercise significantly increased gut microbiota alpha diversity, including the Shannon index [1]. A systematic review of 28 studies confirmed that moderate- or vigorous-intensity physical activity performed for 30–90 min, more than 3 times per week (or 150–270 min weekly) for a period of 8 weeks can induce changes in the gut microbiota [2]. Intensive physical activity has been associated with an increase in butyrate- and succinate-producing bacteria, which affects the metabolic homeostasis of the host and has a potentially positive effect on the state of the immune system [4]. Professional soccer players have been shown to have an increased diversity of gut microbiota compared to amateurs, particularly during periods of intensive training.

A study of professional martial artists revealed a significant pattern: higher-level athletes exhibit significantly greater gut microbiota diversity compared to lower-level athletes [5]. Shannon ($p = 0.019$) and Simpson ($p = 0.001$) indices were significantly higher in highly skilled athletes [5]. This is particularly important for female boxers, as increased microbial diversity correlates with better immunity, reduced susceptibility to respiratory infections, and lower BMI values [6]. Gender is an important modulator of the microbiota response to physical exercise. Women tend to show greater changes in Shannon index and observed operational taxonomic units [5].

The gut microbiota may significantly influence the physical performance of female boxers through multiple mechanisms, from energy metabolism to immune regulation. Increased microbial diversity, enrichment with specific beneficial taxa, and optimized microbial function are positively correlated with athletic performance [7].

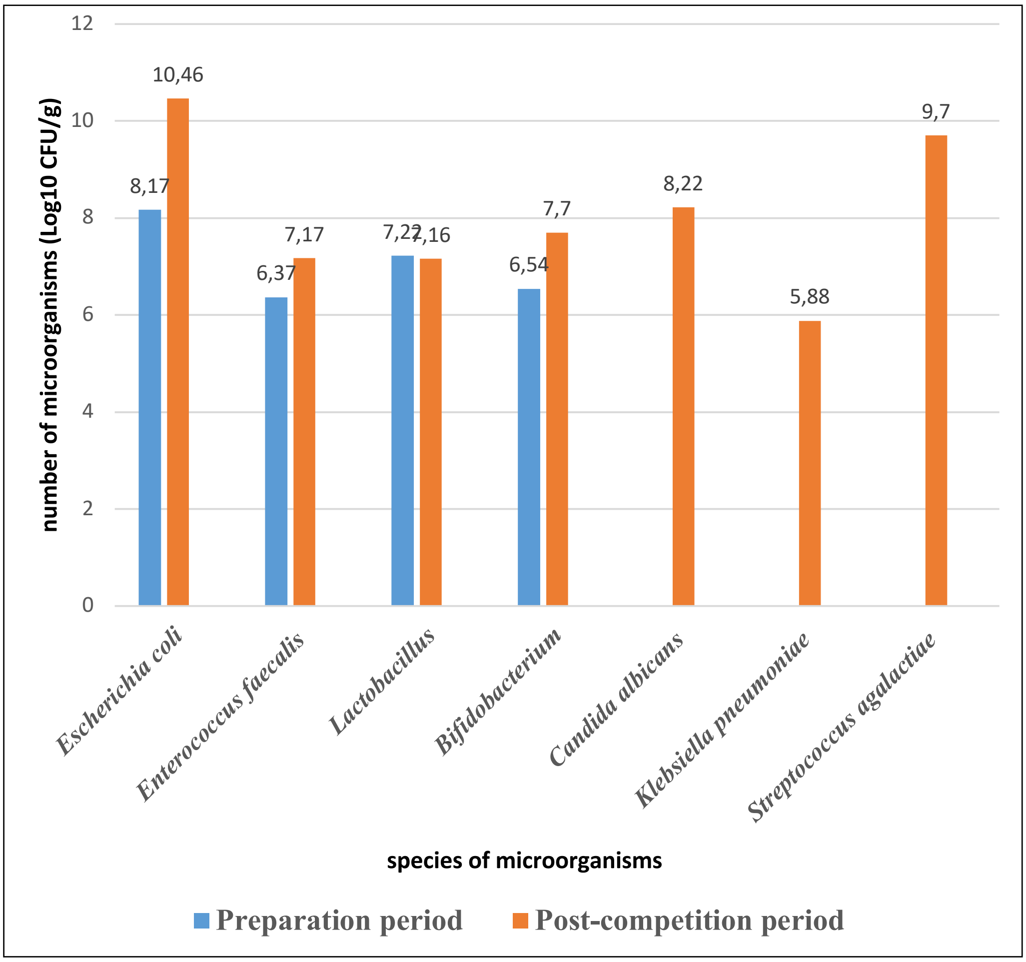

Fig. 1. Average Log10 CFU/g of the main microorganisms in the microbiota of qualified female-boxers
Picture taken by the authors

Although direct studies linking gut microbiota to performance outcomes such as win rate in female boxers are lacking, existing evidence suggests that optimizing microbial health can be an important factor in improving athletic performance and achievement in this sport. Moreover, microbiota profiling may become a tool for predicting athletic performance.

AIM

The aim was to analyze changes in the gut microbiota of qualified female boxers at different stages of the training cycle, influenced by physical activity of different intensity.

MATERIALS AND METHODS

The study involved nine qualified female boxers from the Kyiv City School of Higher Sports Mastery (Kyiv, Ukraine), a specialized sports training center. The average age of the boxers was 22.00 ± 0.89 years. The study was conducted during the preparation period and immediately after the competitive period (post-competition period), during which the athletes performed high-intensity training sessions lasting 90 minutes, 6 times per week [8]. During the scientific study, all recommendations to ethics committee recommendations on biomedical research were followed. All participants provided written informed consent for the use of their data in scientific research. To effectively address the goal of the scientific study, a combination of sport science, microbiological, and medical methods was applied: microbiota research, leukocyte formula analysis to verify the inflammatory process; methods of mathematical statistics. To study the features and diversity of the intestinal microflora in female boxers, an in-depth study of the state of the intestinal microbiota was conducted with the identification of commensal and opportunistic microorganisms to the species with the determination of the diagnostic ratio. For this purpose, a microbiological quantitative method was used, in-depth with species identification (biochemical automated test systems) and detection of anaerobes, the unit of measurement of which was colony-forming units per gram (CFU/g). The quantitative assessment of microorganisms was performed using arithmetic mean values (M±m) expressed and log-transformed values ($\log_{10}$ CFU/g). Statistical significance of differences was

Table 1. Gut microbiota profile of trained female boxers by training intensity, n=9 (M±m), Log_{10}

Microorganism	Preparation period		Post-competition period		Reference range
	Concentration of CFU/g				
	M±m CFU/g	log_{10} CFU/g	M±m CFU/g	log_{10} CFU/g	CFU/g
Escherichia coli, lac+(n=9)	$(1,48±0,98)×10^8$	8,17±1,20	$(2,86±1,55)×10^{10}$	10,46±1,17	$10^6 – 10^8$
t-test	1,84				
P	> 0,05				
Enterococcus faecalis (n=9)	$(2,36±1,32)×10^6$	6,37±1,32	$(1,47±0,14)×10^7$	7,17±0,51	$10^5 – 10^8$
t-test	6,41				
P	< 0,01				
Lactobacillus spp (n=8)	$(1,66±1,14)×10^7$	7,22±1,39	$(1,43±0,14)×10^7$	7,16±0,58	$10^6 – 10^8$
t-test	0,2				
P	> 0,05				
Bifidobacterium spp (n=9)	$(3,49±1.48)×10^6$	6,54±1,05	$(5,03±2,22)×10^7$	7,70±0,62	$10^7 – 10^8$
t-test	2,23				
P	< 0,05				
Candida albicans (n=6)	10^6	-	$(1,67±0,192)×10^8$	8,22±0,91	$<10^4$
Klebsiella pneumoniae (n=4)	10^4	-	$(7,53±2,47)×10^5$	5,88±0,50	$<10^4$
Streptococcus agalactiae (n=2)	10^6	-	$(5,00±5,00)×10^9$	9,70±3,0	$<10^4$

Note: t – Student's test;
p – significance of the difference in different periods of the training cycle
Source: compiled by the authors of this study

determined using Student's t-test. Data analysis was carried out with Statistical 10.0 Software (StatSoft, Inc., USA) and Microsoft Excel.

RESULTS

The study of changes in the state of the microbiota, namely its diversity, was carried out in two phases. The first one was during the preparatory period, during which the athletes engaged in moderate-intensity physical activity. In order to monitor whether changes occur in the microbiota profile during high-intensity training, a follow-up analysis was conducted conducted after 8 weeks, in the post-competition period. The obtained test results are presented in Table 1 and Fig. 1. In the preparation period, the average value of *Escherichia coli* was $(1.48 \pm 0.98)×10^8$ CFU/g (log_{10} 8.17 ± 1.20), and in the post-competition period – $(2.86 \pm 1.55)×10^{10}$ CFU/g (log_{10} 10.46 ± 1.17). Thus, the concentration of E. coli in the post-competition period increased nearly 200-fold. The log_{10} value also increased, but the difference did not reach statistical significance ($p > 0.05$). Both average values exceed the upper limit of the norm (10^8 CFU/g), suggesting excessive colonization – potentially a sigh of intestinal dysbiosis due to intense training load.

Enterococcus faecalis increased approximately 6-fold during the post-competition period. According to the t-test, this increase was statistically significant ($t=6.41$, $p < 0.01$). Specifically its concentration rose from $(2.36 \pm 1.32)×10^6$ CFU/g (log_{10} 6.37 ± 1.32), and in the post-competition period the concentration was $(1.47 \pm 0.14)×10^7$ CFU/g (log_{10} 7.17 ± 0.51). Both values are within the physiological norm. Notably, the decrease in standard error (±0.51 vs ±1.32) indicates a more stable level in the post-competition period. This may reflect an adaptive intestinal response to changes in metabolism or diet during and after the competition.

The concentration of *Lactobacillus spp* was $(1.66 \pm 1.14)×10^7$ CFU/g (log_{10} 7.22 ± 1.39) during the preparation period and $(1.43 \pm 0.14)×10^7$ CFU/g (log_{10} 7.16 ± 0.58) after competition. A slight decrease of approximately $0.2×10^7$ is observed, but this change is statistically insignificant ($t=0.2$, $p > 0.05$). Such a minor reduction during intense training may be a typical sign of dysbiosis, but remained within the normal range and lacked statistical relevance.

Regarding the concentration of *Bifidobacterium spp* in the preparation period the observed value was $(3.49\pm1.48)\times10^6$ CFU/g ($\log_{10}$ 6.54 ± 1.05), and in the post-competition period it increased to $(5.03 \pm 2.22)\times10^7$ CFU/g ($\log_{10}$ 7.70 ± 0.62). The content of bifidobacteria in the post-competition period increased approximately 15 times. The difference is statistically significant ($p < 0.05$). The reference range is approximately ($10^7 - 10^8$ CFU/g), so the values of the preparation period were somewhat underestimated, and in the post-competition period they approached the lower threshold of the reference range. Bifidobacteria are commensal intestinal microorganisms with known anti-inflammatory properties. Their increased abundance following intense physical activity may reflect a beneficial adaptive response.

In the preparation period, a level of 10^6 CFU/g (i.e., which is close to the detection limit) for *Candida albicans* was detected, in the post-competition period it was $(1.67 \pm 0.192)\times10^8$ CFU/g ($\log_{10}$ 8.22 ± 0.91). The normal upper limit for *Candida albicans* is $<10^4$ CFU/g, indicating a pathologically elevated level in the post-competition period. No statistical comparison was performed (it was present in the preparation period in only 1 athlete), but the fact that it appeared in 6 out of 9 people with such a high concentration indicates a significant disturbance of the microbiota.

The presence of *Klebsiella pneumoniae* 10^4 CFU/g in the microbiota of the examined female boxers in the preparation period is also noteworthy. However, in the post-competition period, the level significantly exceeded the normal range $(7.53 \pm 2.47)\times10^5$ CFU/g ($\log_{10}$ 5.88 ± 0.50). *Klebsiella pneumoniae* is a facultative pathogen; its appearance in 4 out of 9 female athletes in significant quantities indicates the presence of dysbiosis. Also, in two female athletes, we observed the appearance of *Streptococcus agalactiae* during the post-competition period $(5.00\pm5.00)\times10^9$ CFU/g ($\log_{10}$ 9.70 ± 3.0) and between competitions 10^6 CFU/g, with an approximate norm of $<10^4$ CFU/g, which represents an unfavorable finding, since *Streptococcus agalactiae* is a potentially pathogenic species, the presence of which indicates the presence of dysbiosis.

High-intensity training loads were associated with microbiota changes indicative of dysbiosis. It is noticeable that the concentration of typical pathogenic genera (*Candida albicans, Klebsiella pneumoniae, Streptococcus*) significantly increased during the post-competition period. This is consistent with the observations that high physiological tension and stress can reduce the barrier functions of the intestine and promote the excessive growth of opportunistic microorganisms. At the same time, classic "beneficial" bacteria (*Lactobacillus* and *Bifidobacterium*) remained within normal limits, although *Bifidobacterium* showed some growth. Notably, *Escherichia coli* (a normal representative of the microbiome) exceeded the norm during the post-competition period. Taken together, these findings indicate a shift in the gut microbiota balance: on the one hand, the number of commensals increased, while on the other, atypical pathogens emerged, a combination that is characteristic of excessive physiological stress. These findings align with previous studies reporting that excessive or extreme physical exertion may lead to a decrease in beneficial barrier bacteria and an overgrowth of potentially pathogenic species, i.e., dysbiosis, reflecting the adverse effects of overly intensive athletic training.

DISCUSSION

A critical consideration in this context is the distinction between moderate and intense physical activity, as they have different effects on the gut microbiota, Clauss M. et al. [3].

Moderate exercise has been associated with reduced inflammation, improved body composition, and favorable changes in gut microbial diversity and composition. In contrast, high-intensity exercise may increase the permeability of the gastrointestinal epithelial barrier and reduce the thickness of the intestinal mucus layer, potentially allowing pathogens to enter the bloodstream and contributing to increased inflammation [3]. In a study of military personnel undergoing intense physical training, intestinal permeability increased by 62±57%, accompanied by alterations in the gut microbiota including increased alpha-diversity and changes in the relative abundance of over 50% of identified genera, Grosicki, G. J. et al. [9]. These findings suggest that sustained physical stress negatively affects intestinal barrier integrity while simultaneously altering microbial composition. Similarly, Barton W. et al. studying elite athletes revealed specific functional adaptations of the gut microbiota. In particular, in marathon runners after the race, an increase in the relative abundance of Veillonella atypica, a bacterium capable of metabolizing lactate as its sole carbon source, was observed [10]. Experimental administration of this strain to mice significantly prolonged treadmill running time to exhaustion, which demonstrates a direct relationship between specific microbes and sports performance. A pivotal discovery was the identification of Veilonella as a bacterium that enhances endurance through a metabolic conversion of exercise-induced lactate into propionate [10]. A study using radiolabeled isotopes showed that serum lactate crosses the epithelial barrier into the intestinal lumen, and intrarectal administration of propionate is sufficient to reproduce increased endurance performance by 133% and reduce muscle fatigue [10].

Short-chain fatty acids (SCFAs) such as propionate, acetate and butyrate are key metabolites that link the gut microbiota to host metabolism. Athletes typically exhibit increased microbial diversity and a compositional shift towards bacterial taxa involved in amino acid biosynthesis and carbohydrate/fiber metabolism, which leads to enhanced SCFA production, Clauss M. et al. [3].

According to Bonomini-Gnutzmann et al., excessively intense or prolonged physical activity can lead to increased intestinal permeability, contributing to exercise-induced gastrointestinal disturbances and systemic inflammation [11]. Paradoxically, among elite athletes, despite a modest increase in diversity, in some cases a decrease in microbiota diversity is observed, compared to individuals performing moderate physical activity Li Y. et al. [12]. However, elite athletes still have high levels of fecal SCFA, which play a crucial role in host energy metabolism. A multi-cohort study of 543 samples from athletes participating in various sports disciplines (aerobics, wrestling, rowing) revealed the presence of sport-specific gut microbiota profiles [12]. Through the application of Latent Dirichlet Allocation (LDA), 10 microbial subgroups were identified and were associated with specific inflammation markers, dietary patterns, and anaerobic performance indicators.

A review of the current literature reveals inconsistent findings regarding the impact of moderate-intensity and vigorous-intensity exercise on gut microbiota diversity and composition. Some studies have reported negative effects of aerobic exercise on gut microbiota, such as elevated intestinal fatty acid–binding protein I-FABP, gastrointestinal discomfort and adverse microbial shifts [11, 13]. However, the majority of evidence supports beneficial outcomes of endurance training on gut microbiota, including increased microbial diversity.

Current research demonstrates a complex, non-linear relationship between exercise intensity and gut microbiota. The "inverted U-curve" model best describes this association: low exercise frequency and duration result in minimal changes, moderate exercise (30-90 minutes 3-5 times a week) causes the greatest positive changes, whereas excessively intense exercise (>90 minutes, >5 times a week) may elicit detrimental effects.

The clinical significance of these findings lies in the possibility of developing personalized training programs that incorporate individual microbiota profiles. Such approaches may not only enhance athletic performance but also support the long-term health of athletes.

Future research should aim to elucidate the mechanisms underlying the bidirectional interactions between physical activity and the gut microbiota. Moreover, standardized methodologies and longitudinal study designs are needed to better understand the sustained effects of various training modalities on microbial health.

CONCLUSIONS

Increased training intensity in qualified female boxers resulted in alterations in gut microbiota composition, most notably a significant rise in *Escherichia coli* and other opportunistic microbes, while the levels of beneficial *Lactobacillus* and *Bifidobacteria* species remained relatively stable. These changes suggest early signs of dysbiosis, consistent with current evidence on the impact of extreme physical exertion on microbial health. To enhance athletes' physical performance and improve competitive outcomes, future interventions may consider probiotic supplementation and the development of personalized nutritional strategies based on individual microbial profiles.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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