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Original article

Effect of different intensity exercise on intestinal microbiota

Ali ÖZKAN, Bilal DEMİRHAN, Ayça GENÇ, Recep AYDIN, Ali Ozan ERKILIÇ, Ferhat GÜDER, Mustafa Alper MÜLHİM

Faculty of Sport Science, Bartin University, Bartin/Turkey

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Abstract

Aim: The aim of this study is to investigate the effect of different intensity exercise on the intestinal microbiota. ***Material and Method:*** Professional footballers (n = 5), amateur footballers (n = 5) and sedentary ones (n = 5), a total of 15 volunteer men whose age were between 18-24 participated in the study. In order to determine the Pie-lou's Evenness Index of all groups, metagenomics analysis was performed using the Next Generation Sequencing method with Illumina MiSeq analyzer by taking stool sample for once. In addition, Food frequency questionnaire was applied to determine participants' eating habits. SPSS 20.0 and Minitab 17 package programs were used in statistical analysis, and the significance level was taken as $p < 0.05$. ***Results:*** In this study, while it was found that amateur footballers (AF) had the most evenness quantitative dis-tribution of types (the difference of Pielou_e was very low ~ 0.01), sedentary ones had the least (Pielou_e has the lowest value of 0.69 - 0.73). In addition, it has been determined that the groups have different eating habits. ***Conclusion:*** Exercise type, intensity and nutrition have an important role in shaping the intestinal microbiota. It can be said that regular exercise with medium intensity has positive effects on evenness index of intestinal micro-biota compared to high intensity exercise and sedentary lifestyle. In addition, we think that the participants' eating habits affect the evenness index, too.

Key Words: Intestinal microbiota, Exercise, Intensity, Nutrition.

Introduction

The genetic diverse microbial population found in the intestines is called the intestinal microbiota and this structure lives in the colon in a colony (1). Intestinal microbiota has different functions such as providing hemostasis, development and promotion of the immune system, protection against pathogens, production and absorption of some vitamins, digestion of some indigestible carbohydrates in the body and play a role in the basis of human health (2,3).

The microbial profile in the intestines is individual and sensitive to the changing endogenous and exogenous factors throughout life. It can be influenced by various factors such as eating habits, exercise, lifestyle, geographic origin, age, birth type, breast milk, past diseases and antibiotic use. The intestinal microbiota contains certain beneficial and pathogenic bacteria. The increase of pathogenic species by decreasing beneficial bacteria is called microbial dysbiosis. Disruption of beneficial / pathogenic bacteria ratio; activating insulin resistance pathways causes obesity, diabetes and atherosclerosis to accelerate the process, causes many other diseases such as allergy, asthma, cancer, inflammatory bowel disease, celiac and dementia (4,5).

Preclinical studies have found that exercise improves microbial diversity, leading to an increase in the population of beneficial bacteria (6,7). Variables such as exercise duration, intensity, and type are effective factors in determining the microbial profile. In a study carried out, while moderate intensity exercise had positive effects on intestinal microbiota, it was concluded that excessive intensity exercise had negative effects on microbiota (8). Another study found that excessive exercise had negative effects on microbial diversity as well as immune and energy metabolism. It has been suggested that regular, medium intensity aerobic exercise and resistance training can be used in the treatment of various gastrointestinal diseases and have a positive effect on microbiota. In addition, it has been said that excessive and long-term endurance exercises may lead to impaired gastrointestinal function, increase intestinal permeability and impair microbial diversity (9).

As much as exercise, proper and healthy eating is also important for microbiota. Nutritional disorders, consumption of frozen foods and additives, unbalanced diets decrease the beneficial species and cause the increase of pathogenic species and degradation of microbiota (10,11,12). The diversity and uniform distribution of the gut microbiota may play an important role in the occurrence, prevention, or treatment of diseases. Therefore, regulation of many factors such as exercise, nutrition, and lifestyle for a healthy microbial profile can have important effects on health. In this study, it was aimed to investigate the effect of different intensity exercise on the intestinal microbiota.

Material and Method

In this study, 15 volunteer men, aged between 18-24 participated. Participants were professional footballers (n = 5), amateur footballers (n = 5) and sedentary ones (n = 5). While professional footballers were practicing 2 hours a day (high-intensity exercise group) 4 days a week, amateur footballers were practicing 2 hours a day 2 days a week (medium intensity exercise group), the sedentary ones did not perform any physical activity.

Criteria that may affect the microbial profile were determined in advance and those who did not meet these criteria were excluded from the study. Criterias were; not to use antibiotics for at least 3 months, not to use pro / prebiotics as supplements, not to have intestinal discomfort and history, and to have at least 5 years or more sports age. Participants were provided with the information about the procedure and clinical side before the study. With obtaining voluntary consent forms, the study started after the ethics committee report numbered 40990478-050.99 and dated 20.06.2018 was received from the Selçuk University Dean of Faculty of Sport Sciences' Interventional Clinical Research Ethics Committee.

In order to determine the Pielou Evenness Index of all groups, stool samples were collected in 2 sterile spoonful stool collection vessels for once. All samples were preserved at -20°C until they were completed, and then delivered to the Medical Microbiology laboratory where analysis would be carried out. In the analysis of the samples, metagenomics analysis was performed with the New Generation Sequencing method using

the Illumina MiSeq analyzer. In addition, the Food frequency questionnaire prepared by the Nutrition and Dietetics Department was applied by face to face interview method to determine the nutritional profiles of the subjects.

Next Generation Sequencing (NGS)

Connector DNA sequences have been added at the 5 'end of the target specific primer pairs for compatibility of the generated library with Illumina index and sequence adapters. Target specific primer-connector sequences specific to 16S rRNA are listed as 5'TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-CCTACGGGNGGCWGCAG-3' for forward primer and 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-GACTACHVGGGTATCTAATCC-3' for reverse primer. The first PCR was applied using "Biospeedy® Proof Reading DNA Polymerase 2x Reaction Mix" and 200 nm from each primer. The following thermal cycle program in the PCR device was as follows; 3 minutes at 95°C; 25 cycles of 30 seconds at 95°C, 30 seconds at 55°C and 30 seconds at 72°C; 5 minutes at 72°C. The PCR product was run on agarose gel to verify its size (~ 550 bp) and then purify it. The second PCR step and binary index and Illumina sequencing adapters were added to the purified first PCR sample using the Nextera XT Index Kit (Illumina, USA). The following thermal cycle program was used: 3 minutes at 95°C; 8 cycles of 30 seconds at 95°C, 30 seconds at 55°C and 30 seconds at 72°C; 5 minutes at 72°C in the second step. The PCR product was then purified. The last library was verified for size (~ 630 bp) using the Bioanalyzer DNA 1000 chip. The last library was diluted to 4 nM using 10 mM Tris pH 8.5 and 5 µl aliquots were mixed to form a library pool. For clustering and preparation for sequencing, the pooled libraries were denatured with NaOH, diluted with hybridization tampon (HT1) and denatured with temperature before MiSeq sequencing. Illumina MiSeq v3 reaction kits were used in the executions. A minimum of 5% PhiX was added to each reaction as an internal control. Unprocessed sequence data (with forward and reverse sequence readings combined) was extracted, reduced and analyzed using Qiime2 2018.11 version (https://qiime2.org/). First index and primer sequences were trimmed and then specific sequences were defined. Unaligned sequences at both ends of the sequences were removed by filtering and error checking was performed. By pre-clustering, pollution is prevented. The built-in UCHIME (Edgar et al., 2011) code was used for chimera elimination. The sequences were classified using the classifier built into Qiime2. After the operational taxonomic unit (OTU) was selected and the taxonomic determination was made according to the Qiime2 database, OTUs were grouped according to their phylotypes. Dendrograms were created by comparing the microbial community profiles obtained using R software. Emperor software was used for the calculation of PCA regulations and subsequent correlation analysis, and the results obtained $p \leq 0.05$ were considered statistically significant.

The following indices were used to determine the microbial evenness profile between groups.

1. Alfa Diversity (α-Diversity)

Diversity in a single ecosystem or sample is the species richness (or operational taxonomic unit- OTU) observed in the sample. Diversity indices provide important information about community composition, species numbers, relative abundance, rarity and partnership of species in the community. The ability to measure diversity in this way is an important tool for understanding the social structure.

a. Pielou's Evenness Index

Evenness of species means how close the number of each species in an environment is. Mathematically, diversity index is defined as a measure of biological diversity that measures how much the community is numerically equal. The closer the number of existence of two different species in a community, it means that the group is more uniform or has evenness. The uniformity of a community can be represented by Pielou's evenness index. The higher the evenness index value of Pielou means the more uniform the distribution of the species in the group (13).

Statistical Analysis

SPSS 20.0 and Minitab 17 package programs were used in statistical analysis, and the significance level was taken as $p < 0.05$. For the microbial community profiles obtained, Minitab 17 software was used and compared with each other and dendrograms that were created. Minitab 17 software was used for the calculation of PCA regulations and subsequent correlation analysis.

Results

The findings of the data obtained from the research are presented in detail in the tables below. According to Table 1, it was found that mean age, height and body weight were respectively; amateur footballers (AF) as 18.00 ± 0.0 years, 181.20 ± 3.9 cm, 74.00 ± 5.7 kg; professional footballers (PF) as 18.80 ± 1.3 years, 182.80 ± 5.2 cm, 73.20 ± 7.9 kg; and sedentary ones (S) s as 21.80 ± 1.3 years, 174.20 ± 2.3 cm, 68.60 ± 10.3 kg.

In the present study, it was found that amateur footballers (AF) had the most evenness quantitative distribution of types (the difference of Pielou_e was very low ~ 0.01). In addition, AF in which the dominant species in groups showed the most evenness distribution compared to other species (Pielou_e dominant species value was very close to both the lowest and highest group values). The group in which the species show the least quantitative level was the S group (Pielou_e has the lowest value of 0.69 - 0.73).

In present study, it was observed that the groups had different eating habits, while the PF group preferred protein- containing foods, AF and S groups preferred more carbohydrate and fiber-containing foods.

Discussion

Exercise appears to be altering intestinal microbial composition and function, and most exercise-related composition changes are beneficial for the host (14). When the alpha diversity index of the mice exposed to intense swimming exercises twice a day for 4 weeks was examined, it was found that they had a lower diversity and smoothness index compared to sedentary mice (15). It was found that short-term, high-intensity interval training does not significantly affect the overall composition of the intestinal microbiome as alpha and beta diversity (16). Another study conducted in rats revealed that intense exercise did not significantly affect microbial diversity at the species level in the gut microbiome, but led to significant changes in the relative abundance of certain members of the fecal microbiota. It was also stated in the same study that intensive

Table 1. Age, body weight, height values of participants

		n	$\bar{x} \pm ss$	Minimum	Maximum
Age (year)	AF	5	18.00±0.0	18	18
	PF	5	18.80±1.3	18	21
	S	5	21.80±1.3	21	24
Body weight (kg)	AF	5	74.00±5.7	68	83
	PF	5	73.20±7.9	65	84
	S	5	68.60±10.3	55	82
Haight (cm)	AF	5	181.20±3.9	176	187
	PF	5	182.80±5.2	175	187
	S	5	174.20±2.3	170	176

AF: Amateur Footballers, PF: Professional Footballers, S: Sedentary

Table 2. Pielou's evenness index graph showing the uniformity of species distribution

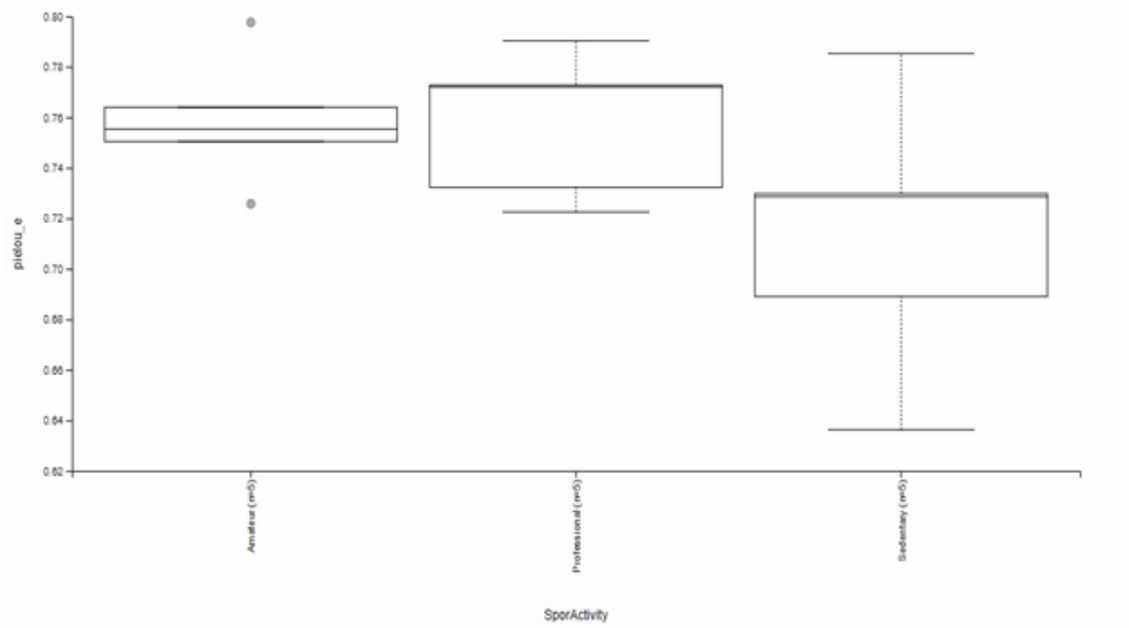

Table 3. Eating habits according to groups

%		Everyday	3-5 times a week	1-2 times a week	Once in 2 weeks	Once a month	Never
PF	**Red meat**		60	20			20
	White meat		80	20			
	Legumes		80	20			
	Vegetable	20	20	40		20	
	Sugar and Pastry		20	40	20	20	
AF	**Red meat**	20	20	20	20		20
	White meat			80		20	
	Legumes		20	60	20		
	Vegetable	20	60			20	
	Sugar and Pastry	20	40	20	20		
S	**Red meat**		40	20	20	20	
	White meat		60	40			
	Legumes	20	20	40		20	
	Vegetable	20	60	20			
	Sugar and Pastry	20	40	20	20		

AF: Amateur Footballers, PF: Professional Footballers, S: Sedentary

exercise could significantly affect the gut microbiota by increasing the abundance of certain potential pathogens. Low-intensity wheel exercise caused an increase in some useful types of rats (17). In this present study, it was determined that amateur footballers had a more uniform microbial profile compared to professional footballers and sedentary ones and groups had different eating habits.

In a study examining the microbial profile between active and sedentary women, it was determined that physical activity performed at a moderate level (max. 3 hours per week) modulated the microbiota profile and made an increase the abundance of bacteria that support health in the microbiota (18). It was determined that the species associated with metabolic health increased and the condition continued in the next 3 months (19). In mice that underwent low and high-intensity wheel turning exercise for 6 weeks, it was found that the low-intensity exercise group had a reduction in bacterial richness and the groups had different evenness index (20).

In a study, it was stated that two-month low moderate exercise did not have a dominant modulating effect in shaping the microbiota in rats subjected to a high-fat diet (21). While high-fiber diets were associated with the presence of Bacteroidetes and Actinobacteria, it was found that the ratio of beneficial bacteria such as Prevotella, Lactobacillus and Bifidobacterium and fecal short chain fatty acid (SCFA) levels in the intestinal microbiota of individuals who had Mediterranean diet (22,23). In studies that conducted with rats, intestinal permeability caused by intense exercise and fermented products were found to have a protective role against impaired microbial profile (24). It has been found that in rats with high fructose diet, their diet negatively affected the benefits of exercise, which may result from reshaping the intestinal microbiota (25). Nutrition is important for players to have an efficient exercise and healthy organs (26). High intensity exercise damages the histological morphology of the intestinal mucosa and integrity of immune function and increases intestinal permeability. There are studies in the literature that intense exercises suppress the immune system of the athlete and suppress their resistance to diseases, while moderate intensity exercises have positive effects on the microbial profile. More studies are needed on the effects of various exer-cise intensities on the gut microbiota.

Conclusion

In our study, it was found that moderate intensity exercise positively affected the evenness distribution of the microbial profile. We think that the nutrition of the amateur footballers containing fiber and carbo-hydrates had an effect on evenness index. In the fu-ture studies, studies which can be conducted on larger populations, different training intensities and nutrition types that are randomized will help to understand the gaps in the literature.

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Correspondence:

Ayça Genç,
Faculty of Sport Science, Bartin University, Bartin/Turkey.
Tel: + 90- 378 501 1000/ 2435
Gsm: +90-5069358401
E-mail: aycagenc@bartin.edu.tr