

Roseburia species in intestinal flora in amateur and professional male football players

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Summary. *Background:* Intestinal flora varies throughout life-long by being affected by many factors such as diet, stress, and exercise. *Purpose:* In this study, it was aimed to examine roseburia intestinal flora in amateur and professional male football players. *Materials and Methods:* A total of 10 male volunteers, aged between 18-21, playing football in the professional league (n=5) and playing football in the amateur league (n=5) participated in the study. A “stool sample” was taken from all subjects and a metagenomic analysis of the Roseburia genus was performed using the IlluminaMiSeq analyzer with the Next-Generation Sequencing method. In addition, a questionnaire was applied to determine participants’ eating and nutrition habits. SPSS 20.0 package program and Minitab 17 software were used in the analysis of the data, the level of significance was taken as $p < .05$. *Results:* It was found that amateur footballers have more Roseburia genus than professional ones, and this difference was statistically significant ($p < .05$). Also, it has been determined that nutrient choices of amateur football players were carbohydrate-based and professional athletes were protein-based. *Conclusions:* Our findings lead us to think that football trainings, which are practiced in amateur and professional level, intensity, and quality, content as well as groups different nutrition styles affect Roseburia species.

Key words: Intestinal Flora, Roseburia, Exercise, Football

Introduction

Microorganismal structures located on the mucous membrane of the digestive system and which have vital functions in the host’s various functions such as digestion, absorption, immune system, production of certain neurotransmitter substances and vitamins, signaling hunger and satiety form our intestinal flora (1). Intestinal flora varies throughout life-long by being affected by many factors such as birth type, breast-fed, diet, stress, and exercise (2). While the gut microbiota reaches the highest level of diversity and number of bacteria in adulthood, this level begins to decrease as we get older. The periods when there is little variety in the intestines are seen as risk factors especially for development of diseases (18). Intestinal flora can

be easily impaired by being affected by various factors such as unconscious use of antibiotics, fast food and food with additives, sedentary lifestyle, stress, smoking, and alcohol (3). As a result of disruption of the intestinal flora, the immune system of individuals is weakened and the rate of observation of diseases such as cardiovascular disorders, cancer types, obesity, depression, diabetes, allergies, Parkinson, Alzheimer, autism increases among them (4).

Intestinal flora consists of useful and pathogenic species; increasing the number and diversity of beneficial bacteria helps prevent diseases (5). One of the useful species, Roseburia; immune system care, showing gram-positive anaerobic properties in rod form, is a part of short-chain fatty acids that affect anti-inflammatory properties and especially common bacteria that

produce butyrate (6). After non-digestible and non-absorbable carbohydrates are fermented by *Enterobacteria*, *Bacteroides*, *Bifidobacterium* *Clostridium* and *Roseburia* strains, they help to synthesize SCFA as butyrate, propionate and acetate, which is an important energy source for the host (7). Butyrate, the primary food source of colonocytes, plays an important role in intestinal homeostasis and gut health (8).

The distribution and consumption of vegetables, fruits and animal-based foods in the geographical region we live in are crucial for nutritional habits and microbiota diversity. There are many studies showing that eating rural context natural products contributes to the increase in bacteria and variety of intestinal flora (9). It is also mentioned that while high protein and low carbohydrate diet decreases the rate of butyrate in the faeces by lowering *Roseburia* and *Eubacterium-rectale* levels in the intestinal microbiota, and since butyrate has an anticarcinogenic effect by increasing the apoptosis of colon cancer cells, it is vital to not to exceed the daily amount of protein need and to add vegetable protein sources to the diet while regulating the intestinal microbiota (10).

Preclinical studies show that physical exercise increases the variety of intestinal flora and increases the population of health beneficial bacteria. It was suggested that regulating the intestinal flora population by changing diet and exercise habits may be a powerful tool in preventing various diseases in the future (11). In addition, moderate exercise has positive effects on intestinal flora, but excessive exercise has negative effects on flora (12).

In present study, it is aimed to search *Roseburia* species in intestinal flora in amateur and professional male football players.

Materials and Methods

In present study, a total of 10 male volunteers, aged between 18-21, playing football in the professional league ($n = 5$) and playing football in the amateur league ($n = 5$) participated. Inclusion criteria were determined: having at least 5 years of sports age, not using antibiotics for at least 3 months, not having any intestinal disorder, not smoking or using alcohol, any

ergogenic or pro/prebiotic supplement and those who have not any of this feature excluded from the study.

After all the subjects were informed about the research procedure and the study, it was directed with the ethics committee report numbered 40990478-050.99 and dated 20.06.2018 that was received from the Ethics Committee of the Faculty of Sports Sciences of Konya Selçuk University in line with the permission obtained from their clubs and themselves.

In this study, stool samples taken with 2 piece in 25 ml volume sterilized spoon-type stool collection containers were first delivered to the Medical Microbiology Laboratory where the analysis procedures will be carried out after all the samples stored at $-20\text{ }^{\circ}\text{C}$ were stored. Then, in the microbiota of all groups, a metagenomic analysis of the *Roseburia* species was carried out with the Next-Generation Sequencing. In addition to determine the nutritional and dietary habits of the participants, a food frequency questionnaire was applied.

Statistical Analysis

SPSS 20 (Statistical Package for the Social Sciences) software and descriptive statistics were used in the analysis and Minitab 17 software were used to analyze the findings of the participants. For the statistical analysis, Mann Whitney U test was used for double comparison from non-parametric tests and the level of significance was taken as $p < 0.05$.

Results

The results obtained from the study are presented below. In present study, the mean age was 18.00 ± 0.00 years, mean height was 181.20 ± 3.96 cm and mean body weight was 74.00 ± 5.78 kg in professional football players, while the mean age was 18.80 ± 1.30 years, mean height was 182.80 ± 5.21 cm and mean body weight was 73.20 ± 7.91 kg in amateur football players.

It was determined that mean sequences of *Roseburia* species were found 7.60 in amateur footballers and 3.40 in professional footballers; amateur footballers participating in the study had more *Roseburia* spe-

cies than professional footballers, and this difference was statistically significant ($p < .05$).

In the study, it was determined that the footballer groups had different eating habits, while professional footballers had choices based on protein, amateur footballers were mostly eating food with carbohydrates.

Discussion

In present study, it was found that amateur football players had higher Roseburia species than professional footballers and this difference was statistically

significant ($p < 0.5$). Matsumoto et al (13) suggested that the butyrate levels in the cecum of physically active rats are the result of compositional changes in butyrate producing bacteria. In a study, individuals with different fitness levels were analyzed to compare stool microbiota with comparable diets, and regardless of diet, VO_2 peak was found to be associated with increased microbial diversity among physically fit participants, increase in Clostridiales, Roseburia, Lachnospiraceae and Erysipelotrichaceae strains and butyrate production. In a rat study, it was concluded that exercise caused an increase in butyrate in fecal samples (14). And in another study, it was determined that there are differences between 2510 bacterial taxa in total compared to immobile rats, and Lactobacillus order is higher in rats performing physical exercise (15). Similarly in a study conducted on male rats, it was found that voluntary wheel turning exercise increased microbial diversity, especially in Actinobacteria, Roseburia and Lactobacillus genus (9). In a study conduct-

Table 1. Footballer groups' mean age, height, body weight

		n	x ±ss	Min.	Max.
Age	Professional Football Player	5	18.00±0.00	18	18
	Amateur Football Player	5	18.80±1.30	18	21
Height	Professional Football Player	5	181.20±3.96	176	187
	Amateur Football Player	5	182.80±5.21	175	187
Body Weight	Professional Football Player	5	74.00±5.78	68	83
	Amateur Football Player	5	73.20±7.91	65	84

Table 2. Comparison of Roseburia species between footballer groups

Groups	n	Mean	Total	u	p
Professional Football Player	5	7.60	38.0	2.00	.03
Amateur Football Player	5	3.40	17.0		

Table 3. Food frequency questionnaire differences among footballer groups

		Food frequency											
		Everyday		3-5 times a week		1-2 times a week		Once in 15 days		Once a month		Never	
Foods		f	%	f	%	f	%	f	%	f	%	f	%
Dairy Foods													
Milk	Professional	-	-	3	60	2	40	-	-	-	-	-	-
	Amateur	2	40	2	40	-	-	1	20	-	-	-	-
Yoghurt	Professional	-	-	4	80	1	20	-	-	-	-	-	-
	Amateur	1	20	1	20	1	20	-	-	1	20	1	20
Cheese	Professional	1	20	4	80	-	-	-	-	-	-	-	-
	Amateur	2	40	2	40	-	-	-	-	-	-	1	20
Meat group													
Red meat	Professional	-	-	3	60	1	20	-	-	-	-	1	20
	Amateur	1	20	1	20	1	20	1	20	1	20	-	-
Meat products (salami, sausage etc.)	Professional	-	-	-	-	2	40	1	20	-	-	2	40
	Amateur	-	-	1	20	-	-	-	-	-	-	4	80
Chicken	Professional	-	-	4	80	1	20	-	-	-	-	-	-
	Amateur	-	-	-	-	4	80	-	-	1	20	-	-
Fish	Professional	-	-	-	-	5	100	-	-	-	-	-	-
	Amateur	-	-	-	-	2	40	2	40	-	-	1	20

Table 3. Food frequency questionnaire differences among footballer groups

		Food frequency											
		Everyday		3-5 times a week		1-2 times a week		Once in 15 days		Once a month		Never	
Egg	Professional	2	40	2	40	1	20	-	-	-	-	-	-
	Amateur	-	-	2	40	1	20	-	-	-	-	2	40
Legumes (lentil, chickpea etc.)	Professional	-	-	4	80	1	20	-	-	-	-	-	-
	Amateur	-	-	1	20	3	60	-	-	1	20	-	-
Nuts (hazelnut, peanut etc.)	Professional	-	-	3	60	2	40	-	-	-	-	-	-
	Amateur	1	20	-	-	2	40	-	-	-	-	2	40
Bread and grains													
White bread	Professional	3	60	1	20	-	-	-	-	-	-	1	20
	Amateur	5	100	-	-	-	-	-	-	-	-	-	-
Rice	Professional	-	-	1	20	2	40	2	40	-	-	-	-
	Amateur	-	-	1	20	4	80	-	-	-	-	-	-
Bulgur	Professional	-	-	2	40	1	20	1	20	-	-	1	20
	Amateur	-	-	-	-	1	20	2	40	-	-	2	40
Pasta	Professional	-	-	3	60	2	40	-	-	-	-	-	-
	Amateur	-	-	1	20	3	60	1	20	-	-	-	-
Bakery	Professional	-	-	1	20	4	80	-	-	-	-	-	-
	Amateur	1	20	-	-	-	-	3	60	-	-	1	20
Fats-sugar-dessert													
Olive oil	Professional	-	-	3	60	2	40	-	-	-	-	-	-
	Amateur	2	40	1	20	1	20	-	-	-	-	1	20
Other oils	Professional	1	20	1	20	2	40	1	20	-	-	-	-
	Amateur	1	20	3	60	1	20	-	-	-	-	-	-
Margarine	Professional	-	-	-	-	4	80	-	-	1	20	-	-
	Amateur	-	-	1	20	-	-	-	-	-	-	4	80
Butter	Professional	1	20	1	20	2	40	-	-	-	-	1	20
	Amateur	3	60	2	40	-	-	-	-	-	-	-	-
Sugar	Professional	1	20	2	40	2	40	-	-	-	-	-	-
	Amateur	3	60	-	-	-	-	-	-	1	20	1	20
Chocolate	Professional	-	-	3	60	1	20	1	20	-	-	-	-
	Amateur	2	40	2	40	-	-	1	20	-	-	-	-
Milk dessert	Professional	-	-	2	40	2	40	1	20	-	-	-	-
	Amateur	-	-	1	20	-	-	-	-	1	20	3	60
Pastry	Professional	-	-	-	-	-	-	-	-	-	-	-	-
	Amateur	1	20	-	-	-	-	-	-	1	20	3	60

ed by military personnel, it was reported that stress caused disruption in the intestinal microbiota due to dysbiosis, and in a 4-day military exercise carried out throughout the winter, there were marked changes in the intestinal microbiota composition and intestinal microbiota-derived metabolites (19).

In addition to exercise, diet affects the diversity of the intestinal flora, too. When the literature is searched, diets that have high-protein and low-carbohydrate reduce Roseburia and Eubacteriumrectale levels in the intestinal microbiota and decrease the rate of butyrate

in the feces, additionally, it is important not to exceed the amount of protein needed daily and to add vegetable protein sources to the diet while regulating the intestinal microbiota due to the anticarcinogenic effect by increasing the apoptosis of colon cancer cells (10). It was determined that decreased carbohydrate intake causes a significant decrease in Roseburia and Eubacteriumrectale and Bifidobacterium in some Clostridium subgroups. High fiber diets were found to be associated with the presence of Bacteroidetes and Actino bacteria (16). In the study comparing the microbiota of European and

African children, the bacterial richness and diversity in the intestines of African children who ate plant based and fiber-rich foods were found higher than European children ate fat-rich and animal-based protein. The amount of Firmicutes and Proteobacteria in the microbiota of children living in Europe was high, and the amount of Prevotella, Xylanibacter and Treponema was higher than other bacteria in children living in Africa. African children have much more SCFA acid than European children, which is one of the signs of beneficial gut flora. The results of the present study reveals the importance of rural eating and nutrition to protect the diversity of the intestinal microbiota from children characterized by modern western diet and rural context nutrition (20). And also in a research it was emphasized that adequate and balanced nutrition is physiologically and psychologically important (21).

A recent study on individuals examined how intensive exercise (4-day cross-country) changed the microbiota composition, compared with the control group, individuals have increased microbial diversity after intensive training, and there was an increased abundance of commensal microbiota that can become pathogenic under certain conditions, and dominant beneficial species. It was found that the abundance of members such as Bacteroides decreased. In addition, a significant relationship was concluded between the bacterial families Clostridiaceae, Roseburia, Bacteroidaceae and Oscillospira and Ruminococcus species and blood lactate accumulation (17).

Conclusion

We think that football training's, which is practiced at the amateur and professional level, intensity, quality and content as well as groups' eating and nutrition habits affect the Roseburia species. Although there are studies in the literature about nutrition and exercise determined effects on the Roseburia species, it is unfortunately limited. In the future studies, researches based on exercise intensity, duration and with a controlled nutrition program may allow us to understand the gaps in the literature.

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