Progress in Nutrition 2020; Vol. 22, N. 2: 440-448 DOI: 10.23751/pn.v22i2.8292

Original article

Investigation of relation between Clostridium colonization and nutrient consumption in intestinal flora in athletes and sedentary men

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Summary. The aim of this study is to investigate the relation between clostridium colonization and frequency of nutrient consumption in intestinal flora of athletes and sedentary men. Fifteen volunteer males aged between 18-24 participated in the study and the participants were divided into 3 groups; the first group (n = 5); consisted of athletes who play football in the professional league and have regular training, the second group (n=5); amateur league football players and regular training athletes and the third group (n=5); 5 men who were physically inactive (sedentary). In this study, 1 stool sample was taken from all subjects and metagenomic analysis was analyzed using the Illumina MiSeq analyzer with the New Generation Sequencing method. In addition, "Nutrition Consumption Frequency" questionnaire was applied to determine the eating habits of the subjects. For statistical analysis of the findings, the package program of Minitab 17 and SPSS 24 was used and the significance level was taken as $p<0.05$. It was determined that meat, eggs, clumps (protein group) and yoghurt, milk, ayran (dairy product) were the most consumed food group while the bread, cereals (carbohydrate group), sweet and fatty foods were the least consumed food group by the Professional football players. The dessert, fatty foods, bread and cereal products (carbonhydrate group) were the most consumed food group while the meat, eggs, legume (protein group) and yoghurt, milk and ayran (dairy product) were the least consumed food group by the amateur football players. The most consumed food group of the sedentary individuals was the group of vegetables and fruits and bread, the cereals (carbohydrate group), while the least consumed foods are milk, yogurt, ayran (group of dairy products) and meat, eggs, rind (protein group). Although there are differences in nutrient consumption among professional and amateur footballers and sedentary individuals, no statistically significant difference was found in clostridium genus intestinal flora ($p>0.05$). It was determined that professional football players have lower clostridium intestinal flora than amateur football players and sedentary group. We think that this difference is caused by the difference between the nutrition habits together with exercise, and that exercise supported by the healthy nutrition program will increase the development of beneficial flora in the intestine and contribute to the suppression of pathogen species.

Keywords: Intestinal flora, clostridium, nutrition, athlete, sedentary

Introduction

From birth to death, different microbial communities including bacteria, viruses, fungs and protozoa live our gastrointestinal system as a host and form our intestinal flora (1). The intestinal flora is a collection of microorganisms which live in the digestive system mucosa and have various functions especially digestive system of the host organism. %98 of bacteria which is located in a healthy intestinal flora are beneficial (2). In the formation of intestinal flora, the type of birth, breast milk, nutrition, drug use and exercise play a key role (3). The nutritional habits from infancy to death provide the formation of intestinal flora. It is known

that maternal milk increases the number of beneficial flora in the intestine thanks to rich oligosaccharides, lactoferrin, antibodies, prohiotic and prebiotic (4). Our feeding behavior and habits are very important for bacteria living in the intestine. Malnutrition disrupts the balance of the intestinal flora by increasing in the pathogen bacteria and decreasing in the beneficial bacteria (2).

In time, our intestinal flora deteriorates due to some reasons such as the use of antibiotics, cortisone, painkiller, hormone, antidepressant drugs, consumption of fast food and convenience food, climate change, smoking, alcohol, sedentary lifestyle, stress and emotional pressure. As a result of deterioration of intestinal flora, individuals' immune system is weakened and the risk of contracting diseases such as cardiovascular system, cancer types, obesity, depression, diabetes, asthma, allergic reactions, Parkinson, Alzheimer, autism and similar diseases increase (5).

The boundary between beneficial and pathogenic flora in the intestine is not clear. The ratio of the races to each other and the general diversity of species in this boundary may lead to a differentiated rate of intestinal microorganisms into some pathogens, even if they have positive effects on health (6). One of the dominant species in our intestine, clostridium microorganisms is a species that can show pathogenic properties which are anaerobic and gram-positive (7). Such bacteria are highly resistant to antibiotics. As a result of the decrease of useful bacteria after the use of antibiotics increases in colonization and it causes diarrhea and many diseases due to toxic substances (6). Some subspecies of the genus Clostridium separate complex sugars to produce the acetate, are very effective in healing bowel diseases (eg, eubacterium limosum) and are stimulated by prebiotics (8).

In many studies, it has been found that the nutrition is the main reason of many changes which comprise of in our microbiota and has been the center of interest for researchers due to being arrangable factor (9). The studies are in existence which show natural products and rural feeding style cause variety of intestinal flora and rising the number of it. In the study which is investigated the long-term nutritional habits and intestinal flora of different populations who live in different geogrophies, it is determined that there are significant differences in beneficial and pathogenic microbiota profiles of African and South Americans who are living in rural areas where vegetarian nutrition is dominant the US and European union countries where western-style nutrition model is widespread with fatty and fast food nutrition (10). In another study which is comparing microbiotas of African and European children, the bacterial richness and diversity in the intestine of African children who are nourished with fibrous and herbal protein were found to be higher than those of the European children who are fed with oil rich and animal-derived protein. It was confirmed that African children had more SCFA (short chain fatty acid) than European children and this indicates beneficial intestinal flora. This study supports the knowledge that the nutrition of the countyside protects the diversity of the intestinal microbiota (11). In a study examining the effects of a high protein diet, it was found that there was a decrease in the bifidobacterium genus in the intestine and an increase in the genus bacterium and clostridium in the period when consumption of red meat was high [8]. In a study on mice, high fat diet was found to be positively associated with low lactobacillus and high amounts of clostridium, bacteroides and acetate producing species (12). In another study in which carbohydrate intake was restricted, it was found that there was a significant decrease in some clostridium subgroups (roseburia and eubacterium rectale) and bifidobacterium (13). In another study, it was determined that vegetarian diet caused a decrease in the genome of clostridium microorganisms in the intestinal structure (14).

Beside nutrition, there are studies that exercise is highly effective in the modification of the intestinal flora. Cerda et al. (2016), found in their study that regular exercise increased microbial diversity, resulting in increase of useful bacterial populations (15). Campbell et al. (2017), found in their study that exercise increases microbial diversity without diet, the microbioli of athletes may be related to protein consumption in the diet, exercise capacity may be affected by the presence of various microbials and high fat consumption in diet increases intestinal inflammation and regular exercise reduces intestinal inflammation (16). In another study which was made by athletes, it was found that they had a higher diversity of microorganisms which rep-

resent 22 different phyla which correlated positively with protein consumption and creatinine kinase, and the exercise played a crucial role in the relationship between host immunity and host metabolism. However, the microbiota diversity is a complex issue including diet (17). In the study which compared professional rugby players, obese and sedentary healthy control subjects, it was determined that exercise increased the SCFA (short chain fatty acid) production by intestinal flora, and in this way, it cured your intestine (18). The intestinal flora augments digestion and nourishment absorption to produce energy in the host and in the column, complex carbohydrates digest carbohydrate elaeosteraics (SCFAs) such as butyrate, acetate and propionate. For this reason, intestinal flora is thought that it plays an essential role in controlling oxidative stress and improving metabolism and consumption of energy during severe exercise (19). In the study on mice, it was determined that kefir supplementation could change the composition of the intestinal microbiota and increase the performance and combat physical fatigue (20). Even in the high-fat diet, exercise can reduce inflammatory trickle and preserve integrity with the morphology of the intestine. Exercise can enrich the variety of microflora and enhance the body weight, pathologies which are related to obesity and the rate of bacteroidetes-firmicutes that may contribute to the reduction of gastrointestibal disorders. The obesity, which can modulate mucosal immunity and increase barrier functions, may stimulate the reproduction of bacteria which resulting in a reduction in the incidence of metabolic diseases and may contribute to the increase of bacteria which can produce preservative substances against gastrointestinal diseases and colon cancer (eg. SCFA) (5). Therefore nutrition and exercise can be used as a treatment to maintain the balance of the microflora or to rebalance its final dysbiosis to improve health status (21). It has been determined that nutrition and exercise have effects on intestinal flora. Although there are studies in the literature on the effects of nutrition on clostridium genus intestinal flora, there are few studies examining the relationship of this species with both nutrition and exercise. The aim of this study is to investigate clostridium colonization and frequency of food consumption in intestinal flora of athletes and sedentary men.

Materials and Methods

In this study, there were 15 volunteer participants who aged 18 to 24. This study included the professional football players who have regular training at Gençlerbirliği Sports Club (n=5), the amateur football players who are premier amateur league in Ankara and have regular training at Çınar Gençlik Sports (n=5), and sedentary men.

The criterias which could affect the study were determined in advance and the subjects who did not adapt the criterias were excluded from the study. The criteria for inclusion in the study was determined that not to use antibiotics for at least 3 months, not to diet, not to use probiotic and prebiotic supplements, not to use ergogenic subsidiary and supplementary food, not to smoke and drink alcohol, any intestinal disorder or bowel cancer and having at least 5 years sports experience for athletes.

The measurements of the study were taken during the competition periods in which they were more active for the athlete group. The control and the athlete groups were informed about the procedure and study before a week. After the voluntary consent documents were received from the participants and their clubs, they were approved by taking ethics committee report, which is in accordance 40990478-050.99 and dated 20.06.2018, from Ethics Committee of Non-Intervential Clinical Researches Diaconate of Faculty of Sport Sciences in Konya Selçuk University.

In order to determine the nutrition and nutrition habits of subjects, food consumption frequency questionnaire was applied. For the microbiota analysis, two samples of 25 ml volumes of sterile spotted feces were taken to the Medical Microbiology laboratory where the samples were stored before storage at -20 ‘C and the analysis was carried out. Samples which were sent to the laboratory were weighed to 200 mg and divided into 1,5 mL micro centrifuge tubes. In order to prohibit any changes in the microbiota quantitation results, the DNA isolation was carried out without wasting too much. The DNA isolation was performed according to procedures and metagenomic analysis was used with new generation sequencing in all groups in the clostridium.

Statistical Analysis

The resulting microbial community profile was compared to each other using Minitab 17 software (Minitab, UK) and dendrograms were generated. Minitab 17 software was used for the calculation of PCA ordinations and subsequent correlation analyzes. The data obtained were evaluated in SPSS 24 package program and descriptive statistics were used in the analysis of the data. Descriptive statistics were used for analysis of data. As the distribution of the groups was homogeneous in the intestinal flora analysis, the One-Way Anova test was used for multiple comparisons and the significance level was taken as $p<0.05$.

Results

The data obtained in the study are presented below.

According to Table 1; it was determined that the Professional football players' age average was 18.00±0.00 years, height average was 181.20±3.96 cm and body weight was 74.00±5.78. It was found that the amateur football players' age average was 18.80±1.30 years, height average was 182.80±5.21 cm and body weight average was 73.20±7.91 kg. In addition to these, it was confirmed that the sedentaries' age average was 21.80±1.30 years, height average was 174.20±2.38 cm and body weight average was 68.60±10.31 kg.

Table 1. Age, height and body weight values of the groups

	Group	n	$\bar{x}$ ± ss	Min.	Max.
Age (year)	Professional football player	5	18.00±0.00	18	18
	Amateur football player	5	18.80±1.30	18	21
	Sedentary	5	21.80±1.30	21	24
Height (cm)	Professional football player	5	181.20±3.96	176	187
	Amateur football player	5	182.80±5.21	175	187
	Sedentary	5	174.20±2.38	170	176
Body weight (kg)	Professional football player	5	74.00±5.78	68	83
	Amateur football player	5	73.20±7.91	65	84
	Sedentary	5	68.60±10.31	55	82

Table 2. Comparison of clostridium values between groups

Group	n	$\bar{x}$ ±ss	Min.	Max.	p
Professional	5	3,59±,736	2,45	4,51	
Amateur	5	4,41±,1,00	2,97	5,67	,16
Sedentary	5	4,89±1,25	3,61	6,98	
p<0.05					

According to Table 2; There is no statistically significant difference among the averages of professional, amateur and sedentary groups of Clostridium genus bacteria. (p> 0,05).

It was found that the participants consumed more milk and their products and consumed meat, eggs and legumes 3-5 times a week. 65% of the professional athletes, 20% of amateur athletes and 15% of sedentaries have consumed their milk and products 3-5 times a week. 53% of professional athletes, 17% of amateur athletes and 30% of sedans were consumed meat, eggs and cloves 3 to 5 times a week. It was found that in the study; 37% of professional athletes, 14% of amateur athletes and 49% of sedentaries consume fresh vegetables and fruits 1-2 times a week and 30% of professional athletes, 25% of amateur athletes and 45% of sedentaries consume bread and cereals 3-5 times a week (Table 3).

According to Table 4, it was found that the participants consumed fat, sugar and sweet 1 to 2 times a week when they consumed the most. 45% of professional athletes, 26% of amateur athletes and 29% of sedentaries were consumed, fat, sugar, sweet foods 1 to 2 times a week.

Discussion

In the study, the results of measuring the food consumption frequency of clostridium intestinal flora of professional group (n=5), amateur footballers group (n=5) who have at least 5 years of sports age and the sedentary group (n=5) who did not perform any regular physical activity were discussed below.

In our study, it was determined that there was no significant difference between the groups despite the fact that professional football players had lower clostridium type than amateur footballers and sedentary group.

Table 3. The distribution of the food consumption of the participants according to the groups

Frequency of food consumption													
Nutritions		**Every day**		**3-5 times a week**		**1-2 times a week**		**Once every 15 days**		**Once a month**		**Never**	
Dairy product		**f**	**%**	**f**	**%**	**f**	**%**	**f**	**%**	**f**	**%**	**f**	**%**
Milk	Professional	-	-	3	60	2	40	-	-	-	-	-	-
	Amateur	2	40	2	40	-	-	1	20		-	-	-
	Sedentary	2	40	1	20	1	20	-	-	1	20	-	-
Yoghurt	Professional	-	-	4	80	1	20	-	-	-	-	-	-
	Amateur	1	20	1	20	1	20	-	-	1	20	1	20
	Sedentary	-	-	-	-	2	40	1	20	-	-	2	40
Cheese	Professional	1	20	4	80	-	-	-	-	-	-	-	-
	Amateur	2	40	2	40							1	20
	Sedentary	1	20	2	40	1	20					1	20
meat, egg, legume													
Red meat	Professional	-	-	3	60	1	20	-	-	-	-	1	20
	Amateur	1	20	1	20	1	20	1	20	1	20	-	-
	Sedentary	-	-	2	40	1	20	1	20	1	20	-	-
Chicken	Professional	-	-	4	80	1	20	-	-	-	-	-	-
	Amateur	-	-	-	-	4	80	-	-	1	20	-	-
	Sedentary	-	-	3	60	2	40	-	-	-	-	-	-
Fish	Professional	-	-	-	-	5	100	-	-	-	-	-	-
	Amateur	-	-	-	-	2	40	2	40	-	-	1	20
	Sedentary	-	-	-	-	1	20	2	40	2	40	-	-
Egg	Professional	2	40	2	40	1	20	-	-	-	-	-	-
	Amateur	-	-	2	40	1	20	-	-	-	-	2	40
	Sedentary	4	80	1	20	-	-	-	-	-	-	-	-
Legume (white beans, chick pea, lentil)	Professional	-	-	4	80	1	20	-	-	-	-	-	-
	Amateur	-	-	1	20	3	60	-	-	1	20	-	-
	Sedentary	1	20	1	20	2	40	-	-	1	20	-	-
Fresh vegetable and fruit													
Green-leafy vegetables	Professional	1	20	1	20	2	40	-	-	-	-	1	20
	Amateur	1	20	3	60	-	-	1	20	-	-	-	-
	Sedentary	2	40	1	20	1	20	-	-	1	20	-	-
Other vegetables	Professional	1	20	1	20	2	40	-	-	-	-	1	20
	Amateur	1	20	3	60	-	-	1	20	-	-	-	-
	Sedentary	-	-	-	-	4	80	-	-	1	20	-	-
Potato	Professional	-	-	1	20	4	80	-	-	-	-	-	-
	Amateur	-	-	1	20	2	40	2	40	-	-	-	-
	Sedentary	-	-	-	-	5	100	-	-	-	-	-	-
Citrus	Professional	1	20	3	60	1	20	-	-	-	-	-	-
	Amateur	1	20	1	20	1	20	2	40	-	-	-	-
	Sedentary			2	40	2	40	-	-	1	20	-	-
Other fuits	Professional	1	20	3	60	1	20	-	-	-	-	-	-
	Amateur	-	-	1	20	3	60	-	-	-	-	1	20
	Sedentary	-	-	-	-	4	80	1	20	-	-	-	-

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Table 3. The distribution of the food consumption of the participants according to the groups

Frequency of food consumption													
Nutritions		**Every day**		**3-5 times a week**		**1-2 times a week**		**Once every 15 days**		**Once a month**		**Never**	
Dairy product		**f**	**%**	**f**	**%**	**f**	**%**	**f**	**%**	**f**	**%**	**f**	**%**
Bread and cereal													
White bread	Professional	3	60	1	20	-	-	-	-	-	-	1	20
	Amateur	5	100	-	-	-	-	-	-	-	-	-	-
	Sedentary	3	60	2	40	-	-	-	-	-	-	-	-
Whole-wheat bread	Professional	1	20	2	40	-	-	1	20	-	-	1	20
	Amateur	-	-	-	-	-	-	-	-	2	40	3	60
	Sedentary	-	-	-	-	1	20	-	-	1	20	3	60
Rice	Professional	-	-	1	20	2	40	2	40	-	-	-	-
	Amateur	-	-	1	20	4	80	-	-	-	-	-	-
	Sedentary	-	-	1	20	4	80	-	-	-	-	-	-
Bulgur	Professional	-	-	2	40	1	20	1	20	-	-	1	20
	Amateur	-	-	-	-	1	20	2	40	-	-	2	40
	Sedentary	-	-	1	20	4	80	-	-	-	-	-	-
Pasta	Professional	-	-	3	60	2	40	-	-	-	-	-	-
	Amateur	-	-	1	20	3	60	1	20	-	-	-	-
	Sedentary	-	-	1	20	4	80	-	-	-	-	-	-
Bakery products	Professional	-	-	1	20	4	80	-	-	-	-	-	-
	Amateur	1	20	-	-	-	-	3	60	-	-	1	20
	Sedentary	-	-	1	20	3	60	-	-	1	20	-	-
Breakfast cereals (oatmeal, cornflakes)	Professional	-	-	1	20	2	40	-	-	1	20	1	20
	Amateur	-	-	1	20	1	20	-	-	-	-	3	60
	Sedentary	3	60	-	-	-	-	-	-	1	20	1	20

Taniguchi et al. (2018), in a study of the effects of nutritional habit and 5-week endurance exercise on intestinal microbiota in elderly Japanese men, they were determined that the abundance of clostridium decreased in the experimental group significantly and in addition to this, the endurance exercise caused a decrease in the toxic substance produced by the clostridum defficile in the intestine (22). Codella et al. (2017), they found that the intense exercise (4-day cross-country) changed the composition of the microbiota and increased the microbial diversity in the experimental and control groups after the training. Under certain conditions, they found an increased abundance of commensal microbiota that could become pathogenic, as well as decreased abundance of members such as bacteroides from the dominant beneficial species (23). In a study performed in rats, moderate exercise resulted in an increase in lactobacillus and bifidobacterium species, while it decreased in clostridium and enterococcus species (24). In another study on rats, it was determined that intense exercise caused a decrease in intestinal motility besides the increase in lactobacillus and clostridium species (25).

Matsumoto et al., (2008), found that a decrease in clostridium type on the rats who had normal diet with wheel exercise (26). In a study which was made with groups who had a high fat or normal diet with exercise and sedentary group, they established that there was an increase in types of allobaculum spp. Veclostridiales (27). It was found that the 4-week treadmill exercise in rats increased the relative abundance of butyricimonas and akkermansia in the experimental group and also increased the species such as clostridiales and lactobacillus (28). The rats divided into four groups which each containing two dietary conditions. These are High-intensity interval training (HIIT),

Table 4. The distribution of the food consumption of the participants according to the groups

Nutritions	Groups	Frequency of food consumption											
		Every day		3 to 5 times a week		1 to 2 times a week		Once every 15 days		Once a week		Never	
		f	%	f	%	f	%	f	%	f	%	f	%
Olive oil	Professional	-	-	3	60	2	40	-	-	-	-	-	-
	Amateur	2	40	1	20	1	20	-	-	-	-	1	20
	Sedentary	-	-	1	20	3	60	-	-	-	-	1	20
Oil	Professional	1	20	1	20	2	40	1	20	-	-	-	-
	Amateur	1	20	3	60	1	20	-	-	-	-	-	-
	Sedentary	2	40	2	40	-	-	-	-	-	-	1	20
Margarin	Professional	-	-	-	-	4	80	-	-	1	20	-	-
	Amateur	-	-	1	20	-	-	-	-	-	-	4	80
	Sedentary	2	40	-	-	-	-	-	-	2	40	1	20
Butter	Professional	1	20	1	20	2	40	-	-	-	-	1	20
	Amateur	3	60	2	40	-	-	-	-	-	-	-	-
	Sedentary	1	20	3	60	-	-	1	20	-	-	-	-
Sugar	Professional	1	20	2	40	2	40	-	-	-	-	-	-
	Amateur	3	60	-	-	-	-	-	-	1	20	1	20
	Sedentary	5	100	-	-	-	-	-	-	-	-	-	-
Chocolate	Professional	-	-	3	60	1	20	1	20	-	-	-	-
	Amateur	2	40	2	40	-	-	1	20	-	-	-	-
	Sedentary	1	20	1	20	2	40	1	20	-	-	-	-
Milky desserts	Professional	-	-	2	40	2	40	1	20	-	-	-	-
	Amateur	-	-	1	20	-	-	-	-	1	20	3	60
	Sedentary	-	-	-	-	2	40	2	40	1	20	-	-
Pastry	Professional	-	-	-	-	-	-	-	-	-	-	-	-
	Amateur	1	20	-	-	-	-	-	-	1	20	3	60
	Sedentary	-	-	-	-	3	60	-	-	2	40	-	-

low-intensity interval training (LIT), sedentary and normal group. high-fat, high-fructose diet (HF) and standard diet (SC) were implemented them. There was no significant difference in the microbiota among the activity conditions in the rats which fed with HF diet however, changes in LIT and HIIT groups were also observed in the absence of phylotypes. An increase occured both HIIT and LIT groups in the types of clostridium (29).

The high fat diet was found to cause disruption of the intestinal flora and a change in the clostridia, enterobacteriales, bifidobacterium species (30). In another study, 3 weeks of soy protein supplementation in rats, bacteriodates species decreased, while bifidobacteriaceae, clostridiales species increased (31). In rats with controlled diet and insulin supplementation, a decrease in the prevalence of clostridium XI has been found (32).

In the literature, nutrition and exercise play an important role in shaping the intestinal flora, including the type of clostridium. While the nutritional model which is rich in fat and carbohydrates causes the proliferation of pathogen species; the consumption of fruit in abundant fiber vegetables contributes to the development of useful flora. Exercise is effective on many species including clostridium in intensity and severity. Moderate exercise is recommended for microbial health because the exercise of high intensity affects the microbial diversity negatively in the intestinal flora.

As a result of our study; it was determined that professional athletes mostly fed protein and amateur

athletes and sedentaries mainly fed carbohydrates. In addition, it is found that, although professional football players has a lower clostridium genus than amateur footballers and sedentary group, there was no significant difference between the groups. Low clostridium genus intestinal flora in professional athletes may be associated with protein-weighted nutrition and intense exercise. For this reason, new studies with different exercise severity and protein-based controlled nutrition programs and different nutritional programs may provide more healthy information.

Acknowledgements

After the voluntary consent documents were received from the participants and their clubs, they were approved by taking ethics committee report, which is in accordance 40990478-050.99 and dated 20.06.2018, from Ethics Committee of Non-Intervential Clinical Researches Diaconate of Faculty of Sport Sciences in Konya Selçuk University.

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