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INVESTIGATION OF THE INTESTINAL FLORA FROM THE RUMINOCOCCACEAE FAMİLY IN SPORTSMEN AND SEDENTARY MEN*

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Abstract: In this study, it was aimed to investigate the intestinal flora from the Ruminococcaceae family in sportsmen and sedentary men.In total, 17 subjects between the ages of 18-24 participated voluntarily in the study including 10 men who had training regularly and played football at an elite level and 7 physically non-active (sedentary) men. The subjects continued their routine eating habits. In the study, "fecal sample" was taken from all the groups once and the Ruminococcaceae family was analyzed metagenomically through the New Generation Sequencing Method and Illumina MiSeq analyzing device. Minitab 17 and SPSS 20.0 package software were used for the statistical analyses of the findings and the significance level was taken as $p<0.05$.It was discovered that elite sportsmen had more intestinal floras from the Ruminococcaceae family compared to the sedentary individuals and this difference was statistically significant ($p<0.05$).It can be said that exercise affects the Ruminococcaceae family positively by causing an increase in this family. In future studies, we suggest the investigation of the factors such as lifestyle, nutritional style and medication, which also influence the internal flora.

Keywords: Intestinal flora, ruminococcaceae, sportsmen, sedentary

SPORCU VE SEDANTER ERKEKLERDE RUMİNOCOCCACEAE CİNSİ BAĞIRSAK FLORASININ İNCELENMESİ

Öz: Bu çalışmada, sporcu ve sedanter erkeklerde Ruminococcaceae cinsi bağırsak florasının incelenmesi amaçlanmıştır. Çalışmaya 18-24 yaş arası düzenli antrenman yapan, elit düzeyde futbol oynayan 10 erkek ve fiziksel olarak aktif olmayan (sedanter) 7 erkek olmak üzere 17 kişi gönüllü olarak katılmıştır. Denekler besin kısıtlamasına girmeksizin standart beslenmelerine devam etmiştir. Çalışmada tüm gruplardan 1 defa "gaita numunesi" alınarak Ruminococcaceae cinsinin, Yeni Nesil Dizileme yöntemi ile metagenomik analizi yapılmıştır. Bulguların istatistiksel analizi için Minitab 17 ve SPSS 20.0 paket programı kullanılmıştır, anlamlılık düzeyi $p<0.05$ olarak alınmıştır. Elit sporcuların sedanter bireylere kıyasla daha yüksek Ruminococcaceae cinsine sahip olduğu ve bu farkın istatistiksel olarak anlamlı olduğu tespit edilmiştir ($p<0.05$). Egzersizin, Ruminococcaceae cinsinde artışa neden olarak bu cinsi pozitif yönde etkilediği söylenebilir. İleride yapılacak çalışmalarda, yaşam tarzı, beslenme stili, ilaç kullanımı gibi bağırsak florasını etkileyen faktörlerin kontrol altına alınmasının alana katkı sağlayacağını düşünmekteyiz.

Anahtar Kelimeler: Bağırsak florası, ruminococcaceae, sporcu, sedanter

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INTRODUCTION

Intestinal flora in the human gastrointestinal tract; is a collection of microorganisms that have important functions in various functions of the host organism, especially digestion (Nazlıkul, 2018). The intestinal flora, which is called as the second brain, is vital because it participates in various physiological activities such as strengthening the immune system, production of

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neurotransmitter substances and vitamins, nutrient absorption, digestion of certain carbohydrates, production of serotonin hormone, signaling of hunger or satiety (Mayer, 2017).

During the period from mother's womb to death; intestinal flora is influenced by many endogenous and exogenous factors such as birth type, nutrition, drug use, stress, exercise, smoking, alcohol, prebiotic/probiotic supplementation and lifestyle. Over time, changes and deteriorations occur in the balance of intestinal flora. Most of the stress hormones in our brain are found in the intestine and factors such as stress, eating disorder and unbalanced diet cause an increase in pathogen species (Çağlayan Tunç, 2019). The deterioration of the intestinal flora weakens the immune system of individuals, the risk of developing diseases such as cardiovascular disorders, cancer, diabetes, depression, allergic reactions, obesity, asthma, Parkinson's and Alzheimer's disease increases (Junger,2018). In particular, disorders such as obesity are closely associated with physiological problems as well as psychological disorders and disruption of the intestinal flora (Genç and Çağlayan Tunç, 2020; Nazlıkul, 2018).

Not all bacterial species in the intestine are identified because some bacteria are not cultured. Although the type and number of bacteria differ from person to person, this numerical ratio is constant for most people (Nazlıkul,2018). Normally, the majority of the flora in the intestine are anaerobic and useful species (Yılmaz,2015). Among the anaerobic species in the intestine, Bacteroides, Clostridium, Fusobacterium, Eubacterium, Ruminococcus, Peptococcs, Peptostreptococcus, Bifidobacterium species are dominant while Escherichia and Lactobacillus species are dominant among the aerobic species (Stojanovic and Vos,2014). Ruminococcaceae is a family of bacteria of the class Clostridia that exhibits anaerobic properties and is known to have beneficial effects on intestinal barrier functions (Starkel et. Al.,2016).

Exercise is very important in increasing the diversity and number of beneficial flora, therefore it contributes to natural immunity against many diseases. While intestinal flora enhances digestion and food absorption for host energy production, in the colon, complex carbohydrates digest SCFAs such as butyrate, acetate and propionate. For this reason, the intestinal flora is thought to play an important role in improving metabolism and energy consumption during severe exercise while controlling oxidative stress (Mach and Botella, 2017). Monda et al. (2017) stated that exercise can increase the number of beneficial microbial species such as Bacteroides, Lactobacillus, Bifidobacterium, Ruminococcaceae, enrich the diversity of microflora and improve the development of commensal bacteria. For 6 weeks, rats were randomly divided into 3 groups as sedentary, voluntary wheel turning (low intensity) and mandatory treadmill exercise (high intensity). It was observed that the microbial community structures of the three groups were clustered separately in the intestinal region and bacterial taxa of rats both wheel spinning and exercising treadmill significantly changed (Allen et al., 2010).

In the literature, there are studies supporting that exercise increases the number of beneficial species found in the intestinal flora, while reducing the number of pathogenic species. The aim of this study was to investigate the intestinal flora of the genus Ruminococcaceae in athletes and sedentary men.

METHOD

Research Model

The research type of our study is observational since it does not contain any medication, treatment and intervention, and the investigated condition is not under the control of the

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researcher. it is also cross-sectional because it involves determining the size of a health problem or an incident by using various diagnostic methods over selected sample groups.

Study Group

A total of 17 volunteers participated in the study, including 10 men who practice regularly at least 4 days a week and play elite football and 7 male who are physically inactive (sedentary). Subjects continued their standard diet without any food restriction. The criteria for inclusion in both groups were determined in advance and subjects that did not meet these criteria were not included in the study. The criteria are as follows; not using antibiotics for at least 6 months, not using probiotics as supplements, not having any bowel disorder and having a sports age of at least 5 years.

All the groups were informed about the procedure and the study a week before the study. Voluntary consent forms from participants and 40990478-050.99 dated and 20.06.2018 numbered ethical board approval report from Konya Selçuk University Faculty of Sport Sciences Deanship Non-interventional Clinical Researches Ethical Board were taken.

Data Collection

"Stool" samples taken from the subjects for one time were kept at -20 ° C until all the samples were completed after the samples were completed, they were delivered to the medical microbiology laboratory where the analysis procedures will be performed. Metagenomic analysis of the genus Ruminococcaceae was carried out by the next generation sequencing method using Illumina MiSeq analyzer.

Analysis of Data

SPSS 20.0 package program was used in the analysis of the data obtained from the study. The distribution of the groups was homogeneous but since the number of subjects was 17, the significance level was taken as $p < 0.05$ by using Mann Whitney U test for paired comparisons from non-parametric tests. The resulting microbial community profile was compared with 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 analyses. The results obtained as $0.05 \geq p$ were considered statistically significant.

RESULTS

Data obtained from the study of the intestinal flora of genus Ruminococcaceae in athletes and sedentary men are presented below;

Table 1. Average age, height, bodyweight values of the groups

		N	$\bar{x}$ ±ss	Min.	Max.
Age (years)	**Athlete**	10	18.50±.654	18	24
	Sedentary	7	19.50±.763	18	23
Height(cm)	**Athlete**	10	177.42±4.65	175	187
	Sedentary	7	178.57±4.16	174	185
Body Weight (kg)	**Athlete**	10	71.55±4.84	68	79
	Sedentary	7	73.80±5.13	67	84

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According to Table 1; The average age of the athletes was 18.50 ± .654 years, the average height was 177.42 ± 4.65 cm and the average body weight was 71.55 ± 4.84 kg. The average age of the sedentary was 19.50 ± .763 years, the average height was 178.57 ± 4.16 cm and the average body weight was 73.80 ± 5.13 kg.

Table 2. Comparison of Ruminococcaceae in sportsmen and sedentary men

Status	N	Row Average	Row Total	U	p
Athlete	10	12.50	125.00	.00	.00
Sedentary	7	4.00	28.00		

***p<0.05**

Table 2 shows that athletes have a higher Ruminococcaceae genus compared to sedentary individuals and this difference was found to be statistically significant.

DISCUSSION AND CONCLUSION

In our study, it was found that elite athletes have significantly higher Ruminococcaceae intestinal flora than sedentary individuals.

In recent studies, a new factor has emerged in which exercise promotes adjunct health effects: intestinal flora modification. The effects of intestinal flora on quality of life and health are quite high. Intestinal flora supported by good exercise and diet contributes to the diversity and development of useful species. The studies in the field of exercise and intestinal flora support this. It has been found that exercise as a homeostatic stimulus diversifies bowel microbiology, which increases the number of benign microbial populations (Codella et al., 2017). In another study, it was found that a sedentary lifestyle was associated with the low richness of intestinal flora (Bressa et al., 2017). In a study on rats, it was found that regular exercise had an effect on BMI as well as a variety of intestinal flora, and that exercise from early ages had positive effects on intestinal flora (Mika and Fleshner, 2016). It was determined that long and intense exercises increase intestinal permeability and cause a change of ischemia and intestinal microbiota and while pathogen species such as E.Coli and Prevotella increase, Lactobacillus and Ruminococcaceae decrease (Wang et al., 2012). In a study conducted in rats, moderate exercise caused an increase in Lactobacillus, Bifidobacterium species and decreased Clostridium family and Enterococcus species (Queipo-Ortuno et al., 2013). In another study conducted on rats, it was found that intensive exercise causes an increase in pathogen species while causing a decrease in bowel movements (Chaves et al., 2018).

In a recent study on human subjects, it was observed that how intensive exercise (4-day cross) changed the composition of the microbiota. After intensive training, it was found that individuals had increased microbial diversity compared to the control group, and increased abundance of commensal microbiota, which could become pathogenic under certain conditions and the abundance of members such as the dominant beneficial species Bacteroides was reduced. In addition, a significant relationship was found among bacterial families Clostridiaceae and Bacteroidaceae and Oscillospira and Ruminococcus genera and blood lactate accumulation (Codella et al., 2017). In the study comparing the intestinal microbiota of sedentary women with moderate regular exercise, Bifidobacterium, Barnesiellaceae, Odoribacter, Paraprevotella, Turicibacter, Clostridiales, Coprococcus and Ruminococceae were found to be significantly different between the two groups, and it was determined that the group doing sports has a higher rate of these species (Bressa et al., 2017).In another study conducted in rats, moderate exercise was found to increase species such as Bacteroidetes and

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Ruminococcus (Panasevich et al., 2016). Liu et al. (2017) found that species such as Prevotella, Helicobacter, and Ruminococcaceae had a positive relationship with cardiac functions and the Parabacteroides genus had a negative relationship between cardiac functions.

In conclusion, it is thought that exercise causes an increase in Ruminococcaceae and affects this genus positively. Undoubtedly, many internal and external factors, including exercise, play a key role in shaping the intestinal flora. Therefore, planning of new studies that will be discussed in future studies on factors such as lifestyle, diet style and different exercise severity may contribute to obtaining more healthy information on this subject.

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