EXAMINATION OF THE EFFECTS OF EXERCISE AND NUTRITION ON THE ESCHERICHIA COLI SPECIES IN THE INTESTINAL FLORA IN SEDENTARY AND ELITE ATHLETES

Erkut Tutkun
Faculty of Sports Science, Bursa Uludağ University, Turkey

ABSTRACT

Aim*: The aim of this study was to examine the relationship of the Escheria coli (E. coli) species in the intestinal flora through exercise and dietary habits in elite athletes and sedentary people.*

Materials and method*: A total of 16 volunteers aged 18-20 participated in the study, 8 of them were male handball players at an elite level and 8 of them were sedentary males. In this study, metagenomic analysis of the E. coli species of intestinal flora was made by taking one stool sample from each case. Moreover, a questionnaire was applied to determine the subjects' nutritional habits. For the statistical analysis of the findings, SPSS 20.0 and Minitab 17 software were used and $p<0.05$ was taken as significance level.*

Results*: It was determined that elite athletes had lower levels of E. coli in the intestinal flora than sedentary individuals ($p<0.05$). Moreover, in the questionnaire form that was applied to determine nutritional habits, it was revealed that the groups had different dietary habits and that the athlete group's diet consisted predominantly of proteins, whereas the sedentary group's diet was mainly consisted of carbohydrates.*

Conclusion*: Sedentary individuals have higher levels of E. coli bacteria than elite handball players. Nutrition and exercise play a pivotal role in determining the levels of E. coli bacteria displaying pathogenic characteristics.*

Keywords: *Escherichia Coli, Exercise, Nutrition, Athletes, Sedentary.*

DOI: 10.19193/0393-6384_2020_1_47

Received *September 30, 2019;* ***Accepted*** *November 20, 2019*

Introduction

Notwithstanding everyone is born in a germ-free environment, people begin a symbiotic relationship with various microorganisms when these enter the body due to the interaction with their mothers and their surroundings. In this process, the first place where colonisation occurs is the gastrointestinal system[1]. The intestinal flora, created by the colonisation of bacteria living in the gastrointestinal system, is a community of microorganisms that performs important duties in the metabolic, physiological and immune system of each human being[2]. The intestinal flora begins to form at the time of birth and is affected throughout life by many factors, such as nutrition, medicines, prebiotics, tobacco, probiotics, alcohol, stress, depression and exercise and also differs from person to person[3].

These microorganisms living in the human gastrointestinal canal have an important impact on sickness and health and under the effects of the above mentioned factors and environmental pollution in the course of time may cause dysbiosis of the intestinal flora[4], followed by dysfunction of the intestinal mucosal barrier and increase in intestinal epithelial permeability. In this way, pathogens and toxic substances can easily pass through the intestinal wall and trigger the formation of irritable bowel syndrome or inflammatory intestinal disorders, metabolic syndrome, obesity, diabetes, cardiovascular disease, different types of cancer, depression, allergies, autoimmune disorders and a number of unexpected illnesses[2, 5, 6].

Under normal conditions, 98% of the intestinal flora in a healthy person consists of beneficial bacteria.

Escheria coli (E. coli), which displays pathogenic characteristics, occurs in low densities in healthy individuals. The abundance of this bacteria group increases with age and makes up about 1% of the total gastrointestinal microbiota in elderly people[7-9]. It is reported in the literature that nutritional preference plays an important role in the formation of bacteria living in the gut and that bad and unbalanced nutritional preferences lead to a reduction in the number of beneficial bacteria and an increase in pathogenic bacteria in the intestinal flora[2].

The main sources of dysfunction or negative changes (increase of pathogenic bacteria) in the structure of bacterial colonies come from chemically processed or frozen foods, products with additives, fast food, sugar, gluten, canned foods, pasteurised products and even chlorine in water, all of which cause dysfunction of the intestinal microbiota[8]. A healthy diet contributes to the growth of beneficial bacteria in the intestinal flora. The natural fermentation products, high-fibre foods and rural-style foods that are consumed in a balanced diet have a prebiotic effect by stimulating beneficial bacteria in the intestinal flora, thereby preserving the variety and health of the gut microbiota[3, 10].

In a study in which different dietary habits and intestinal floras of different geographical areas were examined, it was found that in countries where western-style dietary habits (fatty and fast food) were prevalent, a greater amount of pathogens were active, whereas in rural areas (where vegetables and natural foods were more consumed), there was a more beneficial microbiota profile[11]. It was determined that a Mediterranean-style diet resulted in high rates of Prevotella, Lactobacillus and Bifidobacterium types of bacteria and high levels of faecal Short Chain Fatty Acids (SCFA), while densities of E. coli and its derivatives were low[12]. In gluten-free diets, limited intake of polysaccharides will limit the number of carbohydrate components reaching the colon, as a result of which, since saccharolytic fermentation does not take place, short chain fatty acids are not formed and beneficial probiotic bacterial populations that produce butyrate, such as Bifidobacterium and Lactobacillus, are reduced, while pathogenic bacterial populations such as E. coli and Entero-bacteriaceae increase[13].

It is reported in clinical studies that together with nutrition, exercise is also important for the modification of the intestinal flora, that exercise enriches the variety of microflora by increasing the number of beneficial microbial species and that it can prevent the development of pathogenic bacteria[14]. In another study examining intestinal microbiota profiles of active and sedentary women, it was revealed that even low doses of regular physical activity (max. 3 hours per week) modulated the microbiota profile and increased the number of bacteria that support healthy microbiota. It has also been reported that the presence of microbiota richness is low with a sedentary lifestyle, however extensive research into which types of exercise or which exercise parameters may increase microbiota diversity still needs to be intensified[15].

Allen et al. in a study with rats, reports that high intensity exercises caused a decrease in Turicibacter spp. and E. Coli, which have strong relationships with intestinal function and intestinal disorders[16]. In another study, it was determined that exercise had positive effects on absorption of nutrients in the intestinal flora, energy distribution and immunity[14] and that intensive exercise resulted in weakening of antioxidant enzyme activity by disturbing the mesenteric redox environment and that disruption of the epithelial barrier in the gut increased the detection of TLR-mediated intestinal commensal bacteria[16].

Findings exist in the studied literature that support the idea that exercise habits carried out together with diet can be an effective strategy for regulating intestinal microbiota populations and for preventing and treating various diseases. Although studies are found in the literature aimed at the effects of the E. coli species on the intestinal flora, there are few studies that examine the relationship between this species and both nutrition and regular exercise, therefore the aim of this study is to examine the relationship between the E. coli species of intestinal flora with exercise and dietary habits in elite athletes and sedentary individuals.

Materials and method

A total of 16 volunteers, 8 males whom were doing 2 hours of regular training 4 days per week and playing handball at an elite level and 8 male whom were physically inactive (sedentary), took part in the study. In this study, the average age of the elite athletes was 18.50±0.755 years, their average height was 179.62±5.55 cm, and their average body weight was 73.75±5.84 kg. The average age of the sedentary individuals was 19.00±0.755 years, their average height was 179.75±4.46 cm, and their average body weight was 73.75±5.84 kg.

The athlete group of the study was made up of sportsmen who had played active sports for at least 5 years, who had not used antibiotics, prebiotic supplements or probiotic supplements for at least 6 months and who did not have any kind of intestinal disorder. In the study, subjects were informed about the procedures one week before samples were taken. In accordance with the Helsinki Declaration, an informed consent form was taken from all participants prior to participation to the study.

To determine the nutritional and dietary habits of the participants, a food consumption frequency questionnaire was applied[17-20]. For the participants' microbiota analysis, a stool sample was taken once, the samples were kept in -200 C storage conditions and after they had all been completed, they were transported to a medical microbiology laboratory, where the metagenomic analyses were carried out. The samples sent to the laboratory were weighed in 200 mg portions and separated into 1.5 mL microcentrifuge tubes. To prevent any changes occurring in the microbiota quantification results, DNA isolation was carried out without keeping the samples standing for max within 48 hours. In the study, metagenomic analysis of the E. coli species was performed in all groups with new generation sequencing.

For the microbial community profiles obtained, comparisons with each other were made and dendrograms were created by using Minitab 17 software (Minitab, UK). For calculating the PCA ordinations and the correlation analyses that were performed following these, Minitab 17 software was again used. The obtained data were assessed with SPSS 20 (Statistical Package for the Social Sciences) software and descriptive statistics were used in the analysis of the data. In the study, percentage frequency distribution was used in the examination of age, height and weight. For the statistical analysis of the E. coli species, since the distribution of the groups was homogeneous but the subject group consisted of 16 people, Shaphiro-Wilk test was performed for the control of normal distribution because the individuals in different groups were different and randomly selected, according to the results of the data does not have a normal distribution ($p = 0.038$).

Therefore, the Mann Whitney U test was applied to evaluate differences between groups, the significance level was set at $p < 0.05$.

Results

Examination of Table 1 reveals that there is a significant difference between elite athletes and sedentary individuals in terms of E. coli bacteria ($p<0.05$). With regard to mean ranks, it is seen that E. coli bacteria, which display pathogenic characteristics, were observed more in sedentary individuals than in elite athletes.

Variables	n	Mean Rank	Rank Total	U	p
Elite Male athletes	8	4.63	37.00	1.00	.00
Sedentary Males	8	12.38	99.00		

Table 1: Comparison of E. Coli species in elite male athletes and sedentary males.

Table 2 shows that 50% of the elite athletes who took part in the study consumed milk and dairy products every day, while 50% of the sedentary individuals consumed milk and dairy products once or twice a week. It was revealed that while 50% of the elite athlete group consumed red meat 3-5 days a week and 37.5% of them consumed white meat 3-5 days a week, 50% of the sedentary group consumed red meat once in 15 days and 37.5% of them consumed white meat once a month. It was determined that legumes were consumed once or twice a week by 50% of the elite athletes and by 37.5% of the sedentary individuals. It was seen that the highest frequency of vegetable consumption for both groups of participants was 3-5 times per week. 37.5% of both the elite athletes and the sedentary individuals consumed vegetable 3-5 times per week. It was revealed that the sedentary group consumed more rice than the elite athlete group; 50% of the former group consumed rice every day, while 37.5% of the latter group consumed rice once or twice per week. It was observed that 50% of the sedentary group consumed pasta 3-5 times a week, whereas 50% of the athlete group consumed pasta once or twice a week. 50% of the elite athletes consumed edible oils and butter every day, while 75% of the sedentary individuals consumed edible oils every day and 37.5% of them consumed butter every day. The table shows that the sedentary individuals consumed more sugar and pastry than the elite athlete group. While 75% of the sedentary individuals consumed sugar and pastry every day, 37.5% of the elite athletes consumed these once in 15 days.

Variables (%)		Every-day	3-5 times a week	1-2 times a week	Once every 15 days	Once every month
Athletes*	Milk products[1*]	25.0	25.0	50.0	-	-
	Red meat[2*]	-	12.5	12.5	50.0	25.0
	White meat[3*]	12.5	12.5	12.5	25.0	37.5
	Legumes[4*]	12.5	12.5	37.5	12.5	25.0
	Vegatables[5*]	37.5	37.5	12.5	12.5	-
	Rice[6*]	50.0	25.0	25.0	-	-
	Pasta[7*]	25.0	50.0	25.0	-	-
	Edible oils[8*]	75.0	25.0	-	-	-
	Butter[9*]	37.5	25.0	25.0	12.5	-
	Sugar & pastry[10*]	-	33.3	-	66.7	-
Sedantary**	Milk Products[1**]	25.0	-	75.0	-	-
	Red meat[2**]	-	12.5	12.5	50.0	25.0
	White meat[3**]	12.5	12.5	12.5	25	37.5
	Legumes[4**]	12.5	12.5	37.5	12.5	25.0
	Vegatables[5**]	66.7	33.3	-	-	-
	Rice[6**]	25.0	50.0	12.5	12.5	-
	Pasta[7**]	25.0	50.0	25.0	-	-
	Edible oils[8**]	66.7	33.3	-	-	-
	Butter[9**]	37.5	25.0	25.0	12.5	-
	Sugar &pastry[10**]	16.7	16.7	33.3	33.3	-

*-**(X^2: 8,000, sd: 4, p: 0,92) [1-1*], (X^2: 16,000, sd: 6, p: 0,014) [2-2*], (X^2: 12,000, sd: 8, p: 0,15) [2-3*], (X^2: 13,000, sd:9, p:0,163) [3-2*], (X^2:16,000, sd:12, p:0,191) [3-3*], (X^2:12,000, sd:8, p:0,151) [4-4*], (X^2:15,111, sd:9, p:0,088) [5-5*], (X^2:12,000, sd:6, p:0,062) [6-6*], (X^2:16,000, sd:4, p:0,003) [7-7*], (X^2:2,667, sd:1, p:0,102) [8-8*], (X^2:9,000, sd:6, p:0,174) [9-9*], (X^2:4,444, sd:4, p:0,349) [10-10*]

Table 2: Frequency percentage of food consumption of athletes and sedantaries.

Sedentary individuals and athletes have a similar food consumption pattern. It was found that the biggest difference in food consumption was between fats and sugar/pastry consumption, and red meat, while white meat, legumes, vegetables, rice, pasta and butter consumption rates were similar (Table 3).

Groups	Foods									
	Milk Products	Red Meat	White Meat	Legumes	Vegatables	Rice	Pasta	Edible Oils	Butter	Sugar & Pastry
Sedantaries vs. Athletes	0.07*	0.000*	0.003*	0.005*	0.002*	0.007*	0.000*	0.134	0.003*	0.114

Table 3: Food consumption differences between athletes and sedantaries.

Discussion and conclusion

In this study, it was determined that elite athletes had lower levels of E. coli intestinal flora than sedentary individuals. It has been revealed that regular exercise increases microbial diversity; that athletes' microbiotas may be related to dietary protein content and that their exercise capacity may be affected by the presence of various microbiotas; that high-fat diets increase inflammation of the intestines and that exercise decreases this inflammation and enhance the development of commensal bacteria[14, 21]. In a study conducted with 4 male rowers over a period of 33 days, in which metagenomic analysis was performed on stool samples taken before, during and after a 5000-m rowing race, it was determined that although ultra-endurance-type exercises were included, microbial diversity increased in the subjects and that this adaptation of species showing positive correlation with health also continued during the following 3 months[22]. In another study, however, it was revealed that long and intensive exercises resulted in changes in gut microbiota by increasing intestinal permeability through ischaemia and that while an increase was observed in species displaying pathogenic characteristics such as E. coli and Prevotella, a decrease in species such as Lactobacillus was also evinced[23]. In a study examining the relationship between intestinal microbiota and exercise performance in pathogen-free mice, germ-free mice and mice with Bacteroides fragilis, it was found that among the 3 groups, endurance swimming time was longer in pathogen-free mice and mice with Bacteroides than in germ-free mice and that weight of the liver, the muscles, brown adipose and epididymal fat pads was higher in pathogen-free mice and mice with Bacteroides than in germ-free mice[24].

In a study conducted on elderly individuals aged 65-80, it was determined that E. coli was a primary producer of trimethylamine and inflammation in the human gut, that in patients with a reduced exercise capacity, the abundance of this bacteria increased and that E. coli could play an important role in decreasing exercise capacity in elderly patients with hypertension[25]. In a study carried out on athletes, Clarke et al.[26] reported that the athletes consumed more protein than the control group, that they had a higher diversity of gut microorganisms representing 22 different phyla which were positively correlated with creatine kinase, that exercise had a positive effect on beneficial microbiota species and played an important role in the relationship between host immunity and host metabolism, but that microbiota diversity is a complex topic that also needs to take diet into account[26].

In the present study, according to the data obtained in the food consumption questionnaire, it was determined that the groups had different dietary styles and that while the elite athletes' diet consisted predominantly of proteins, the sedentary group's diet was mainly composed by carbohydrates. An examination of the literature reveals that there are studies supporting the idea that bad and unbalanced dietary habits upset the balance of the intestinal flora, resulting in a decrease in the number of beneficial bacteria and hence an increase in the number of pathogenic bacteria[2]. It is accepted that gut microbes play a role in the absorption of monosaccharides and that generally, carbohydrate fermentation results in beneficial effects on life due to the formation of SCFA. In one study, it was revealed that excessive carbohydrate consumption resulted in an increase in species such as Bifido-bacteria, E. Rectale and E.coli[27]. It was determined that a high-fat diet decreased gut microbial diversity and that while causing an increase in Bacteroides, Alistipes and Bilophila, high-fat diets resulted in a decrease in fecal SCFA acid concentration and the number of Bifidobacteria. It was emphasised that for regulation of the microbiota, the amount of fat as well as the type of fat were important[28].

In conclusion, it was observed that sedentary individuals had higher levels of the E. coli species than elite handball players did. It is considered that the different distributions of the E. coli species, which displays pathogenic characteristics, between groups is due to the positive effects of exercise and the different dietary styles of the groups. Studies in which dietary habits of different genders and sport groups are examined can contribute to future knowledge in the field of gut microbiota.

References

1) Kataoka K. The intestinal microbiota and its role in human health and disease. J Med Invest. 2016; 63(1-2): 27-37.10.2152/jmi.63.27
2) Nazlikul H. Duygusal beyin:bağırsak: Destek Yayınları; 2018.
3) Paoli A, Mancin L, Bianco A, Thomas E, Mota JF, et al. Ketogenic Diet and Microbiota: Friends or Enemies? Genes (Basel). 2019; 10(7).10.3390/genes10070534
4) Junger A. Temiz bağırsak: Pegasus Yayınları; 2018.
5) Yetkin İ, Satış H, NK S. Bağırsak mikrobiyotasının insülin direnci, diabetes mellitus ve obezite ile ilişkisi. Turkish Journal of Diabetes and Obesity. 2017; 1: 1-8
6) Bianco A, Pomara F, Thomas E, Paoli A, Battaglia G, et al. Type 2 diabetes family histories, body composition and fasting glucose levels: a cross-section analysis in healthy sedentary male and female. Iran J Public Health. 2013; 42(7): 681-90
7) Rajilic-Stojanovic M, de Vos WM. The first 1000 cultured species of the human gastrointestinal microbiota. FEMS Microbiol Rev. 2014; 38(5): 996-1047.10.1111/1574-6976.12075
8) Perlmutter D, Loberg K. Beyin ve bağırsak Pegasus Yayınları; 2017.
9) Murray P. Temel tıbbi mikrobiyoloji: Güneş Tıp Kitabevleri; 2018.
10) Genç A, Tutkun E, Acar H, Zorba E. Investigation of Relation Between Clostridium Colonization and Nutrient Consumption in Intestinal Flora in Athletes and Sedentary Men. Progress in Nutrition. 2019; 22: 1-9.10.23751/pn.v22i2.8292
11) Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, et al. Human gut microbiome viewed across age and geography. Nature. 2012; 486(7402): 222-7.10.1038/nature11053
12) De Filippis F, Pellegrini N, Vannini L, Jeffery IB, La Storia A, et al. High-level adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome. Gut. 2016; 65(11): 1812-21.10.1136/gutjnl-2015-309957
13) Sanz Y. Effects of a gluten-free diet on gut microbiota and immune function in healthy adult humans. Gut Microbes. 2010; 1(3): 135-7.10.4161/gmic.1.3.11868
14) Monda V, Villano I, Messina A, Valenzano A, Esposito T, et al. Exercise Modifies the Gut Microbiota with Positive Health Effects. Oxid Med Cell Longev. 2017; 201: 3831972.10.1155/2017/3831972
15) Bressa C, Bailen-Andrino M, Perez-Santiago J, Gonzalez-Soltero R, Perez M, et al. Differences in gut microbiota profile between women with active lifestyle and sedentary women. PLoS One. 2017; 12(2):e0171352.10.1371/journal.pone.0171352
16) Goldsmith F, O'Sullivan A, Smilowitz JT, Freeman SL. Lactation and Intestinal Microbiota: How Early Diet Shapes the Infant Gut. J Mammary Gland Biol Neoplasia. 2015; 20(3-4): 149-58.10.1007/s10911-015-9335-2
17) Bianco A, Mammina C, Paoli A, Bellafiore M, Battaglia G, et al. Protein supplementation in strength and conditioning adepts: knowledge, dietary behavior and practice in Palermo, Italy. J Int Soc Sports Nutr. 2011; 8(1): 25.10.1186/1550-2783-8-25
18) Bianco A, Thomas E, Bellafiore M, Martines F, Messina G, et al. Mediterranean diet and dietary protein supplementation as possible predicting variables of weight management: An update of the protein project. Acta Medica Mediterranea. 2015; 31(6): 1265-70
19) Bianco A, Mammina C, Thomas E, Ciulla F, Pupella U, et al. Protein supplements consumption: a comparative study between the city centre and the suburbs of Palermo, Italy. BMC Sports Science, Medicine and Rehabilitation. 2014; 6(1): 29
20) Bianco A, Mammina C, Thomas E, Bellafiore M, Battaglia G, et al. Protein supplementation and dietary behaviours of resistance trained men and women attending commercial gyms: a comparative study between the city centre and the suburbs of Palermo, Italy. J Int Soc Sports Nutr. 2014; 11: 30.10.1186/1550-2783-11-30
21) Campbell SC, Wisniewski PJ, 2nd. Exercise is a Novel Promoter of Intestinal Health and Microbial Diversity. Exerc Sport Sci Rev. 2017; 45(1): 41-7.10.1249/

JES.0000000000000096
22) Keohane DM, Woods T, O'Connor P, Underwood S, Cronin O, et al. Four men in a boat: Ultra-endurance exercise alters the gut microbiome. J Sci Med Sport. 2019.10.1016/j.jsams.2019.04.004
23) Wang F, Li Q, Wang C, Tang C, Li J. Dynamic alteration of the colonic microbiota in intestinal ischemia-reperfusion injury. PLoS One. 2012;7(7):e42027.10.1371/journal.pone.0042027
24) Hsu YJ, Chiu CC, Li YP, Huang WC, Huang YT, et al. Effect of intestinal microbiota on exercise performance in mice. J Strength Cond Res. 2015; 29(2): 552-8.10.1519/JSC.0000000000000644
25) Yu Y, Mao G, Wang J, Zhu L, Lv X, et al. Gut dysbiosis is associated with the reduced exercise capacity of elderly patients with hypertension. Hypertens Res. 2018; 41(12): 1036-44.10.1038/s41440-018-0110-9
26) Clarke SF, Murphy EF, O'Sullivan O, Lucey AJ, Humphreys M, et al. Exercise and associated dietary extremes impact on gut microbial diversity. Gut. 2014; 63(12): 1913-20.10.1136/gutjnl-2013-306541
27) Lopez-Legarrea P, Fuller NR, Zulet MA, Martinez JA, Caterson ID. The influence of Mediterranean, carbohydrate and high protein diets on gut microbiota composition in the treatment of obesity and associated inflammatory state. Asia Pac J Clin Nutr. 2014; 23(3): 360-8.10.6133/apjcn.2014.23.3.16
28) Özdemir A, Demirel Z. The Relation Between Diet and Microbiota. Journal of Biotechnology and Strategic Health Research. 2017; 1: 25-33

Corresponding Author:
Erkut Tutkun
Bursa Uludağ University
Email: erkuttutkun@gmail.com
(Turkey)