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The alteration of oral microbiota before and after training in swimmers

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ABSTRACT

We aimed to analyze the effect of acute exercise on oral microbiota in regularly trained swimmers. As environmental factors may affect the oral microbiota; we also aimed to analyze the short-duration effect of swimming training on the oral bacteria relative difference in swimmers. Saliva samples of 20 swimmers both before and after the training were used for the oral microbiota metagenesis. The next-generation sequencing method targeting 16S rDNA gene fragments was used for genotyping. The Wilcoxon signed-rank test was used for the statistical evaluation of the taxons. The alfa diversity comparisons were assessed with the One-Way ANOVA, and the Kruskal-Wallis test was used to determine bacterial diversity. Decayed- Missed- Filled total (DMF-T) scores were the indicators of oral hygiene. A comparison of the before and after exercise microbiota of the swimmers gave rise to a statistically significant difference for Firmicutes (p=0.014) and Bacteroidetes *(p=0.007)* phylum; Clostridia *(p=0.006)* and Bacilli *(p=0.048)* classes; Clostridiales *(p=0.004)*, Entomoplasmatales *(p=0.009)* and Bacillales *(p=0.006)* for ordo; *Lachnospiraceae (p=0.001)* family and *Stenotrophomonas (p=0.013)* genus. Although there were some differences within the other taxa of the bacteria, all were statistically insignificant. *Streptococcus*, *Pseudomonas* and *Rothia mucilaginosa* showed a correlation with the DMF-T values in swimmers. This study was the first in Turkish swimmers to investigate the relative abundance of oral microbiota. We showed that exercise within the pool water changed the oral bacteria's relative abundance. To confirm our results and clarify the effect of pool water on oral bacteria relative abundance, more studies on dietary intake should be carried out.

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Introduction

Microbiota is generally ecological communities of commensal, symbiotic and pathogenic microorganisms living in various organs of multicellular organisms, and microbiome refers to the genomic community of all microorganisms. Microorganisms found in the human oral cavity are defined as the oral microflora, or more recently as the oral microbiome (1) It is the second largest and most diverse microbiome after the gut, with more than 700 species including bacteria as well as some fungi, viruses, and protozoa (2).

The composition of the oral microbiome varies among microhabitats, and each individual has a 'microbial identity' consisting of a distinct microbial community (3, 4). While the oral microbiome differs between individuals, it is the main function that remains the same. It may affect each individual's physiological, nutritional, and defensive development. Interactions between the microbiome and the host, and the physiological characteristics of the oral cavity play a large role in shaping the oral microbiome (2). The oral cavity provides the oral microbiome a stable ecological niche as an evolutionary partner, and in turn, the oral microbiome maintains host health locally through the formation of symbiotic biofilms, which prevent pathogen growth and maintain pH balance, as well as systematically enhance physiological processes within the body, including cardiovascular homeostasis(5,6,7). Therefore, the oral microbiome, like the microbiome in other parts of the body, has been recognized as a critical organ in maintaining human health, rather than a causative agent of the disease, and has recently been the focus of much research. However, many endogenous and exogenous factors may influence the ecological balance by altering the composition, structure, and metabolic processes of oral bacteria; thus, affecting disease susceptibility. These factors are related to drug use, environment, host conditions, and genetics as well as daily lifestyles such as diet and oral hygiene (5, 8, 9).

Recently, information about the relationship between competitive sports and human microbiomes has been circulating in both the dental and sports worlds. It has been reported that physical exercise has favorable effects on the structure of the gut microbiome and metabolite production in sedentary subjects (10, 11). Therefore, a new concept known as "athletic microbiome" has emerged.

When we consider the interaction between the oral and gut microbiomes, we can speculate that the effect of exer-

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cise on the gut microbiome might be similar for the oral as well. Recent studies showed that both microbiomes are related since approximately 45% of the bacteria in the large intestine and oral cavity overlap. This may be related to nutrition, or the saliva swallowed. The bacteria swallowed may tolerate the extreme pH of the stomach and can reach the gastrointestinal tract and localize there. Studies have suggested that exercise has an intense effect on the alteration of microbiota. Long-term exercise can cause harmful effects on the microbiota and the general health of athletes whereas low levels of continuous physical activity can increase the diversity of microbiota, and improve the metabolic profile, and immunological responses of individuals (12). Exercise affects the sympathetic nervous system and in response to those effects, salivary ions such as sodium (Na+), potassium (K+), and hydrogen (H+) cause temporary changes in the salivary pH (13,14). In addition to ionic changes, high-intensity exercise also increases the lactate concentration (15, 16). Also, high, intense exercise increases the amount of proinflammatory cytokines that inhibit the activity of the innate immune cells (17). These immune disorders may lead to the deterioration of oral microbiota due to intense exercise; therefore, it is important to have information about the oral microbiota changes before and after exercise.

There is, however, limited information on the effect of prolonged competitive swimming in chlorinated pool water on the oral microbiota. Hence, the current study is designed to fill in this knowledge gap by determining the microbiome composition before and after swimming exercise depending on the 16S rDNA gene. The literature indicates that professional swimmers can experience tooth erosion caused by chlorinated compounds. These findings suggest that swimming can adversely affect dental health Additionally, Decayed- Missed- Filled Teeth (DMF-T) scores were also determined in terms of oral hygiene.

Materials and Methods

Sample group

A total of 20 professional swimmers (14 males and 6 females), aged between 15-30, enrolled in the study. Our study groups (before -training) were used as an internal control for each participant since we aimed to determine the change in the relative abundance of the oral microbiota after- training. Inclusion criteria for the players were as follows: 1) being a professional swimmer, 2) not smoking, 3) agreement to an oral hygiene examination and biological analyses of oral microbiota, 4) not being under any medication that may affect oral microbiota within the last 3 weeks, and 5) not having any kind of genetic disease. All the swimmers performed a regular training program with a 6-day/ week and minimum of 90 min./training.

The study was approved by the Clinical Research Ethics Committee of Marmara University, Faculty of Medicine (09.2020.296). Consent forms giving detailed information about the study were also signed by all the swimmers.

Saliva collection

Oral microbiota sampling was carried out from saliva samples. Saliva samples were collected from swimmers in the morning before and after- training (between 8:30 am and 10:00 am). Sampling was completed just before and after- training sessions and 2 ml of saliva was collected and placed directly into sterile tubes. The subjects were not allowed to drink or eat 2 h before the sampling. To prevent contamination, the subjects brushed their teeth for 30 min. before sampling, and cleaned their mouths with sterile water for 1 minute.

Swimming pool water oroperties

The swimming pool's water was found to have an average daily temperature of 26.4°, 1.6 ppm of free chlorine, and an average pH value of 7.5.

Determination of DMF-T index

The DMF-T index was used to determine oral hygiene according to the World Health Organization (WHO) criteria. The DMF-T index was evaluated as 0-1 good, 2-4 moderate acceptable, 5-10 bad, and 10 and above very bad.

Molecular analysis oral microbiota

400µl saliva samples were used for DNA isolation. Isolation was performed using the TANBead Nucleic Acid Extraction Kit–Gram Bacteria DNA Auto Plate protocol kit (REF M61GA46, Taoyuan City 330, Taiwan (R.O.C.). DNA quantification was performed with Qubit (Thermo Fisher Scientific, Waltham, MA, USA). 16S Universal Eubacterial primers with 16S forward, and reverse primers were used to amplify the 16S rDNA V3-V4 regions in each sample. During the library preparation, 2-step PCR with 25 cycles was performed separately for each sample using the KAPA HiFi HotStart ReadyMix (2X)* KK2602 (Roche, Cape Town, South Africa). In the first PCR step, denaturation for 3 min at 95°C, followed by 95°C for 30 sec, 55°C for 30 sec and 72°C for 30 sec were followed for 25 cycles; and finally, a single cycle at 72°C for 5 min. In the second PCR application, Nextera XT Index Primer 1 and Nextera XT Index Primer 2 sets (Illumina, CA, USA) were used to add the Illumina index and adapter sequences. In this PCR step, denaturation for 3 min at 95°C, followed by 95°C for 30 sec, 55°C for 30 sec and 72°C for 30sec for 8 cycles; and a final single cycle at 72°C for 5 min. were performed. After both PCR procedures, purification was performed with the Agencourt AMPure XP (Beckman Coulter) kit. The sequencing application was used with Gen-Era (Gen-Era Diagnostics, TR) on the Illumina iSeq 100 next-generation sequencing platform (Illumina, CA, USA), duplex (2x150bp) reading with the iSeq 100 i1 Reagent kit, following the manufacturer's instructions. The sequences of 16S Universal Eubacterial primers encoded 16S forward and reverse for the amplification of 16S rDNA V3-V4 regions are given in Table 1.

Table 1. Sequences of 16S Universal Eubacteria primers.

Primer	Primer sequences
Forward	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGWGCAG
Reverse	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC

Bioinformatics analysis

The FastQC3 program was used in the quality control steps after the sequencing application. For the taxonomic profiling, reads were aligned to target organisms based on the Greengenes database using the Ribosomal Database Project (RDP) Classifier developed by Wang et al.(18). After the alignments were completed, the OTU groups in each sample were determined. R scripts were used in the data reporting, statistical analysis, and data visualization steps. Shannon index 6 was used to calculate diversity. R:vegan 7 package was used to calculate the indices.

Statistical analysis

Within the scope of the study, all the taxonomic units were statistically compared for each sample with BT (Before-Training) and AT (After-Training) data. The Wilcoxon Signed-Rank Test using SPSS Statistics was used for comparisons.

Results

The demographic properties of the participants were as follows: the mean age of the females was 18.83 ± 2.23, and the mean height was 177 ± 0.062 cm and the mean body weight was 67 ± 6.75 kg. For the male swimmers, the mean age, height and body weight were 19.28 ± 4.32, 183 ± 0.048 cm and 75.3 ± 5.66 kg, respectively (Table 2).

A total of 29 phyla, 51 classes, 89 orders, 220 families, 610 genera, and 877 species were determined in terms of the analysis. The highest 20 bacterial strains that were detected in our cohort are shown in Figure 1. A total of 29 phyla were detected in our cohort and are listed in Figure 2. The relative abundances in the phylum, class, order, family, genus, and species levels were statistically compared among the samples.

The before-training and after-training microbiota concentrations of the phylum were compared in the swimmers in our study cohort (Table 3). The microbiota densities of the Bacteroidetes *(p=0.007)* and Firmicutes (*p*=0.014) before and after training showed a statistically significant difference. In the Bacteroidetes phylum, the microbiota density was found to be lower after training (0.08±0.10) than before training (0.34±0.27). The microbiota density of the Firmicutes phylum was lower after training (0.27±0.11) compared to before training (0.36±0.12). The bacterial density of the phylum Proteobacteria was lower before the training (0.36±0.12) compared to after the training (0.40±0.26); The Actinobacteria phylum bacterial density after training (0.18±0.14) compared to before-training (0.10±0.12) was found to be higher. No significant differences were detected between the microbiota densities of the Proteobacteria (*p=0.709*), Actinobacteria (*p=0.073*), and Candidatus (*p=0.167*) species before and after -training *(p>0.05)*.

The microbiota densities were compared in before and after-training classes shown in Table 4. Bacilli *(p=0.048)* and Clostridia *(p=0.006)* classes only showed a statistically significant difference in the microbiota densities before and after-training. In the Bacilli class, the microbiota density was found to be lower after-training (0.22±0.10) compared to before-training (0.28±0.09). After-training (0.31±0.29) compared to before-training (0.28±0.30) in the Gammo proteobacteria class, before-training (0.12±0.09) compared to after-training (0.19±0.14) in the Actinobacteria class higher rates were determined. The microbiota densities before and after-training were Actinobacteria *(p=0.232);* Flavobacteria *(p=0.940);* Bacteridia *(p=0.126),* and no statistically significant difference was found in the classes (*p>0.05).*

As a result of the analysis, six ordos (Clostridiales, Entomoplasmatales, Bacillales, Lactobacillales, Pseudomodales, Actinomycetales) were determined the most. The microbiota densities before and after-training showed a statistically significant difference in Clostridiales *(p=0.004),* Entomoplasmatales *(p=0.009)* and Bacillales *(p=0.006)* ordo (Table 5). Clostridiales (0.05±0.03 versus 0.02±0.01); Entomoplasmatales (0.07±0.03 versus 0.04±0.02); Bacillales ordo (0.02±0.01 versus 0.01±0.01) microbiota density was found to be lower after-training

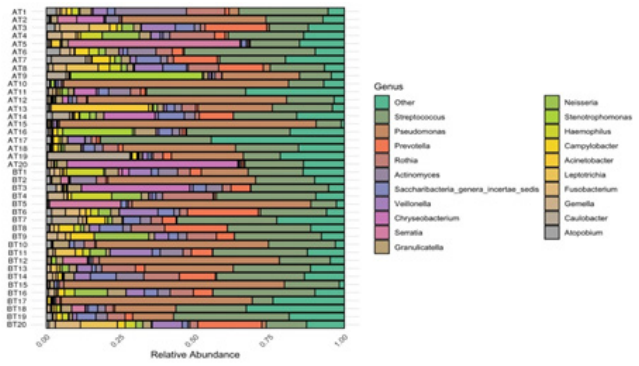

Figure 1. 20 bacterial strains with the highest rate (AT: After training, BT: Before training).

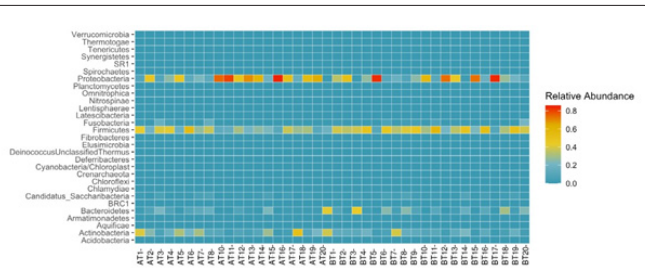

Figure 2. The graph of the first 29 phyla with the highest rate (BT: Before-training; AT: After-training).

Table 2. All the demographic properties of the participants.

Study cohort (n)		x̄ ±ss	Minimum	Maximum
Female swimmers	Age	18.83 ± 2.23	17	22
	Height (cm)	177 ± 0,062	171	186
(n=6)	Body weight(kg)	67 ± 6,75	60	73
Male swimmers	Age	19.28 ± 4.32	15	30
	Height (cm)	183 ± 0.048	177	192
(n=14)	Body weight (kg)	75.3 ± 5.66	67	83

Table 3. Relative abundances (%) and statistical comparison of the most common bacterial phyla.

		Firmicutes		Bacteroidetes	
		BT	**AT**	**BT**	**AT**
1	**Female**	0.38	0.43	0.08	0.02
2	**Male**	0.20	0.20	0.01	0.08
3	**Male**	0.38	0.40	0.43	0.22
4	**Male**	0.48	0.42	0.03	0.08
5	**Male**	0.11	0.18	0.0007	0.01
6	**Male**	0.46	0.54	0.25	0.08
7	**Male**	0.34	0.27	0.05	0.13
8	**Female**	0.40	0.37	0.25	0.16
9	**Male**	0.46	0.13	0.01	0.003
10	**Male**	0.31	0.15	0.01	0.002
11	**Male**	0.57	0.30	0.10	0.08
12	**Male**	0.21	0.21	0.002	0.03
13	**Male**	0.35	0.26	0.03	0.01
14	**Male**	0.48	0.23	0.16	0.23
15	**Female**	0.23	0.11	0.001	0.0004
16	**Male**	0.53	0.37	0.11	0.02
17	**Female**	0.10	0.29	0.01	0.02
18	**Male**	0.32	0.31	0.30	0.02
19	**Female**	0.50	0.11	0.04	0.02
20	**Female**	0.39	0.18	0.23	0.42
Mean±SD		**0.36±0.12**	**0.27±0.11**	**0.34±0.27**	**0.08±0.10**
Median (IQR)		**0.38 (0.17)**	**0.26 (0.19)**	**0.26 (0.45)**	**0.02 (0.10)**
P value		***0.014**		***0.007**	

(*is statistically significant).

Table 4. Relative abundances (%) and statistical comparison of the most common bacterial classes.

		Bacilli		Clostridia	
		BT	**AT**	**BT**	**AT**
1	**Female**	0,25	0,35	0,03	0,06
2	**Male**	0,22	0,18	0,01	0,12
3	**Male**	0,34	0,18	0,09	0,03
4	**Male**	0,40	0,34	0,05	0,08
5	**Male**	0,34	0,18	0,09	0,03
6	**Male**	0,31	0,52	0,012	0,04
7	**Male**	0,28	0,21	0,05	0,06
8	**Female**	0,32	0,23	0,05	0,08
9	**Male**	0,34	0,12	0,006	0,08
10	**Male**	0,28	0,12	0,03	0,02
11	**Male**	0,37	0,28	0,02	0,07
12	**Male**	0,20	0,20	0,01	0,005
13	**Male**	0,28	0,23	0,03	0,02
14	**Male**	0,36	0,20	0,03	0,10
15	**Female**	0,21	0,01	0,01	0,022
16	**Male**	0,44	0,35	0,02	0,05
17	**Female**	0,07	0,23	0,02	0,03
18	**Male**	0,27	0,28	0,02	0,02
19	**Female**	0,42	0,09	0,01	0,07
20	**Female**	0,18	0,17	0,01	0,10
Mean±SD		**0,28±0,09**	**0,22±0,10**	**0,025± 0,02**	**0,05±0,03**
Median (IQR)		**0.28 (0.15)**	**0.20 (0.13)**	**0,02(0,03)**	**0,05 (0,08)**
P value		*** 0.048**		***0.006**	

(*is statistically significant).

Table 5. Relative abundances (%) and statistical comparison of the most common bacterial ordos.

		Clostridiales		Entomoplasmatales		Bacillales	
		BT	**AT**	**BT**	**AT**	**BT**	**AT**
1	**Female**	0,06	0,03	0,001	0,0006	0,03	0,011
2	**Male**	0,12	0,01	0,001	0,0004	0,002	0,003
3	**Male**	0,03	0,09	0,001	0,0002	0,02	0,02
4	**Male**	0,12	0,01	0,001	0,0004	0,002	0,003
5	**Male**	0,006	0,01	0,0002	0,00013	0,0004	0,01
6	**Male**	0,04	0,013	0,001	0,001	0,05	0,034
7	**Male**	0,06	0,05	0,001	0,001	0,014	0,008
8	**Female**	0,08	0,05	0,001	0,0005	0,004	0,012
9	**Male**	0,08	0,01	0,001	0,0003	0,04	0,01
10	**Male**	0,02	0,03	0,0004	0,0002	0,003	0,002
11	**Male**	0,07	0,01	0,001	0,0002	0,003	0,003
12	**Male**	0,010	0,01	0,0003	0,0004	0,022	0,01
13	**Male**	0,02	0,03	0,0005	0,0005	0,012	0,002
14	**Male**	0,10	0,03	0,0008	0,0005	0,021	0,01
15	**Female**	0,02	0,008	0,0003	0,0002	0,01	0,002
16	**Male**	0,05	0,02	0,0014	0,0006	0,02	0,006
17	**Female**	0,03	0,02	0,0004	0,0004	0,001	0,002
18	**Male**	0,02	0,02	0,0003	0,0001	0,0114	0,004
19	**Female**	0,07	0,01	0,001	0,0002	0,003	0,003
20	**Female**	0,010	0,01	0,0003	0,0004	0,022	0,01
Mean±SD		**0,05±0,03**	**0,02±0,01**	**0,07±0,03)**	**0,04±0,02**	**0,02±0,01**	**0,01±0,01**
Median(IQR)		**0.05(0.06)**	**0,02(0,02)**	**0.01 (0,01)**	**0.01(0,01)**	**0.01 (0,02)**	**0,01(0,01)**
P value		***0.004**		***0.009**		***0.006**	

(*is statistically significant).

than before-training. The microbiota densities before and after-training were Pseudomonadales *(p=0.391),* Actinomycetales *(p=0.247)*, Lactobacillales *(p=0.079),* and no statistically significant difference was found in ordos *(p>0.05)*.

The microbiota density of the family, which was most common in our before and after-training cohort, is presented in Table 6. In the Lachnospiraceae family (*p=0.001*) only microbiota density showed a statistically significant difference. The bacterial density before training (0.04±0.03) was higher than after-training (0.02±0.01). The Veillonellaceaa, Pseudomonadaceae, Staphylococcaceae, and Xanthomonadaceae families were more common than other bacteria; however, no statistical significance was detected (p>0.05). Statistical rates were found as Lachnospiraceae (*p=0.00255)*, Veillonellaceaa (*p=0.3),* Pseudomonadaceae *(p=0.495),* Staphylococcaceae *(p= 0.271)*, and Xanthomonadaceae *(p= 0.117)*.

The microbiota densities of the genus before and after-training were compared in Table 7. The genus *Stenotrophomonas* (p=0.013) only before training and after-training microbiota density showed a statistically significant difference. In the *Stenotrophomonas* genera microbiota density was found to be higher after-training (0.037±0.11) compared to before training (0.001±0.003). While the bacterial density of the *Stenotrophomonas* genus was not observed in some individuals, it increased or decreased in some after-training. *Streptococcus* was significantly higher in the before-training (0,24±0,08), compared to after-training (0,21±0,09). The *Pseudomonas* genus was detected at a higher rate before-training (0.22±0.27) than after-training (0.20±0.28). The bacterial densities of the genus

Table 6. Relative abundances (%) and statistical comparison of the most common bacterial family.

		Lachnospiraceae	
		BT	**AT**
1	**Female**	0,04	0,025
2	**Male**	0,096	0,005
3	**Male**	0,023	0,003
4	**Male**	0,06	0,024
5	**Male**	0,003	0,003
6	**Male**	0,03	0,01
7	**Male**	0,05	0,03
8	**Female**	0,08	0,04
9	**Male**	0,06	0,005
10	**Male**	0,012	0,03
11	**Male**	0,07	0,02
12	**Male**	0,003	0,01
13	**Male**	0,02	0,024
14	**Male**	0,08	0,023
15	**Female**	0,02	0,01
16	**Male**	0,03	0,015
17	**Female**	0,03	0,01
18	**Male**	0,02	0,015
19	**Female**	0,03	0,004
20	**Female**	0,07	0,004
Mean±SD		**0,04±0,03**	**0,02±0,01**
Median(IQR)		**0,03(0,06)**	**0,01(0,02)**
P value		***<0.001**	

(*is statistically significant).

Table 7. Relative abundances (%) and statistical comparison of the most common bacterial genus.

		Stenotrophomonas	
		BT	**AT**
1	**Female**	0,0005	0,00003
2	**Male**	0,0000	0,00005
3	**Male**	0,0000	0,00012
4	**Male**	0,0000	0,00040
5	**Male**	0,0001	0,00020
6	**Male**	0,0006	0,00000
7	**Male**	0,0000	0,00004
8	**Female**	0,0000	0,00000
9	**Male**	0,0000	0,44100
10	**Male**	0,0000	0,01200
11	**Male**	0,0000	0,00100
12	**Male**	0,0154	0,00500
13	**Male**	0,0000	0,00010
14	**Male**	0,0000	0,03400
15	**Female**	0,0000	0,00100
16	**Male**	0,0000	0,23414
17	**Female**	0,0000	0,00000
18	**Male**	0,0007	0,00500
19	**Female**	0,0000	0,00140
20	**Female**	0,0000	0,00000
Mean±SD		**0,001±0,003**	**0,037±0,11**
Median(IQR)		**0.00 (0.00)**	**0.01 (0.01)**
P value		*** 0.013**	

(*is statistically significant).

Serratia were determined equally with the mean value of (0.03±0.05) both before and after-training. While the mean values of the relative densities of the *Chryseobacterium* genus were determined as (0.01±0.08) before- training, they increased by (0.04±0.13) after-training. *Streptococcus (p=0.232), Pseudomonas* (p=0.526), *Serratia (p=1.000), Chryseobacterium (p=0.372), Rothia (p=0.052)* genera microbiota densities before and after- training were not statistically significantly different *(p>0.05).*

Although 877 species were identified, only those species with the highest BT and AT microbiota densities were specified. For example, showed a large increase in the relative abundance of *Granulicatella adiacens* (0,10±0,06 in AT vs 0,09±0,08 in BT), *Pseudomonas deceptionensis* (0,03±0,10 in AT vs 0,02±0,07 in BT. *Acinetobacter_lwoffii* (AT 0.03±0.13 vs. BT 0.02±0.01) and *Prevotella pallens* species (AT 0.03±0.05 vs BT 0.02±0.03) were detected to be higher. A decrease was observed in the species of *Rothia mucilaginosa*, normally found in the oral flora (BT 0.16±0.08 versus AT (0.12±0.09). The *Veionella rogosae* bacterial species (BT 0.02±0.02 versus AT 0.02±0.03) was determined. Although an increase was observed in the microbiota densities of the species in both groups, no statistically significant difference was observed *(p>0.05).*

DMF-T oral hygiene assessment

Bacterial genera determined extensively in the oral microbiota of the swimmers in our cohort were compared with DMFT indexes (Table 8).

The total bacterial count and oral microbiota diversity (between 5-10 according to the DMFT index) were the highest. *Streptococcus* genus was seen in 9 individuals in total. DMFT index between 0-2 0.299±0.03; between 5-10, 0.279±0.07; 10 and above 0.343±0.00 bacterial abundance was determined. In addition, *Rothia mucilaginosa* species were also observed in some individuals with *Streptoccocus*. In 7 people detected with *Pseudomonas*, the mean value of the density between 0-2 in the DMFT index was 0.604; was found to be 0.595±0.20 between 5-10. DMFT index of the genus *Prevotella* the mean value is 0.226±0.02 between 5-10, 0.364±0,00 in the 0-2 DMFT index of *Chryseobacterium* genus; the genus *Elizabethkingia* is 0.247±0,00. Moreover, we show an increase in the abundance of each genus of *Streptococcus* in between DMFT index (Table 8), in particular in genus implicated caries. In addition, the relative densities of bacterial genera and species determined at the highest rate in individuals and the DMFT index comparison are stated in Table 9.

Alpha diversity analysis of oral microbiota

The diversity index (also called phylogenetic indices or phylogenetic metrics), how many different species (such as species) are present in a dataset (a community) is also a quantitative measure that reflects phylogenetic relationships (co-distribution, species proximity, species richness) among distributed individuals. The Shannon diversity index is an index used to characterize species diversity in a community. The higher the index value, the greater the diversity. The values in the Simpson diversity index range from 0 to 1. If the Simpson dominance index is high, the dominance is high in that ecosystem, on the contrary, the species diversity is low. The Inverse Simpson index is the effective number of species obtained when using the weighted arithmetic mean to measure the average proportional abundance of the species in the relevant dataset.

The alfa diversity comparisons in the oral microbiota analysis were assessed with a One-Way Anova, and the Kruskal-Wallis test was used to determine the bacterial diversity. The level of significance was accepted as $p<0.05$. When compared in terms of alpha diversity in our cohort, the Shannon index bacterial abundance and diversity increased to 3.05 ± 0.54 in AT versus 2.92 ± 0.40 in BT in

Table 8. Comparison of the average values of the most intensive bacteria genus and DMF-T analysis.

Genus	**n=20**	**0-2 DMFT ındex**	**2-4 DMFT ındex**	**5-10 DMFT ındex**	**>10 DMF-T ındex**
Streptococcus	**n=9**	0,299 ± 0,03	-	0,279±0,07	0,343±0,00
Pseudomonas	**n=7**	0,604±0,00	-	0,595±0,20	-
Prevotella	**n=2**	-	-	0,226±0,02	-
Chryseobacterium	**n=1**	0,364±0,00		-	-
Elizabethkingia	**n=1**	-	0,247±0,00	-	-

* (data as mean± SD)

Table 9. Relative abundance (RA) (%) of the bacterial genus and species detected and DMF-T Index.

Swimmer	GENUS				SPECIES				DMFT INDEX			
	Before Training	**RA**	**After- Training**	**RA**	**Before-Training**	**RA**	**After –Training**	**RA**	**D**	**M**	**F**	**T**
1	***Pseudomanas***	*0.6*	***Pseudomanas***	*0.8*	***Granulicatella adiacens***	*0,4*	***Rothia muciaginosa***	0,2	4	4	0	**8**
2	***Streptococus***	*0.3*	***Streptooccus***	*0.2*	***Rothia muciaginosa***	*0,4*	***Rothia muciaginosa***	0,4	1	1	0	**2**
3	***Streptococus***	*0.3*	***Streptooccus***	*0.2*	***Granulicatella adiacens***	*0,2*	***Prevotella pallens***	0,1	4	1	0	**5**
4	***Prevotalla***	*0.2*	***Streptococcus***	*0.4*	***Gemella haemolysansi***	*0,2*	***Rothia mucilaginosa***	0,2	4	5	0	**9**
5	***Pseudomanas***	*0.6*	***Serratia***	*0.6*	***Serratia liquefaciens***	*0,5*	***Serratia liquefaciens***	0,5	9	0	0	**9**
6	***Streptococus***	*0.2*	***Streptococus***	*0.3*	***Rothia muciloginosa***	*0,5*	***Rothia muciloginosa***	0,5	1	8	0	**9**
7	***Chryseobacterium***	*0.4*	***Prevotella***	*0.2*	***Granulicatella adiacens***	*0,3*	***Prevotella pallens***	0,4	2	0	0	**2**
8	***Pseudomonas***	*0.5*	***Pseudomonas***	*0.5*	***Rothia muciloginosa***	*0,3*	***Granulicatella adiacens***	0,2	0	0	0	**0**
9	***Streptococus***	*0.2*	***Streptococcus***	*0.3*	***Haemophilus parainfluenza***	*0,4*	***Rothia muciloginosa***	0,5	0	5	1	**6**
10	***Prevotella***	*0.2*	***Chryseobacterium***	*0.6*	***Prevotella pallens***	*0,2*	***Granulicatella adiacens***	0,3	2	3	0	**5**
11	***Streptococcus***	*0.4*	***Caulobacter***	*0.4*	***Rothia mucilaginosa***	*0,5*	***Rothia mucilaginosa***	0,2	5	2	0	**7**
12	***Elizabethkingia***	*0.2*	***Pseudomonas***	*0.4*	***Pseudomonas deceptionensis***	*0,4*	***Pseudomonas deceptionensis***	0,3	3	1	0	**4**
13	***Pseudomonas***	*0.6*	***Streptococcus***	*0.4*	***Rothia mucilaginosa***	*0,1*	***Actinosynnema mirum***	0,2	1	6	0	**7**
14	***Streptococcus***	*0.3*	***Streptococcus***	*0.3*	***Neissera flavescens***	*0,2*	***Granulicatella adiacens***	0,3	2	9	0	**11**
15	***Pseudomonas***	*0.9*	***Pseudomonas***	*0.7*	***Rothia mucilaginosa***	*0,5*	***Veionella rogosae***	0,3	3	3	0	**6**
16	***Streptococcus***	*0.3*	***Streptococcus***	*0.2*	***Rothia mucilaginosa***	*0,4*	***Prevotella salivae***	0,2	4	0	0	**4**
17	***Pseudomonas***	*0.3*	***Acinetobacter***	*0.3*	***Rothia mucilaginosa***	*0,5*	***Acinetobacter woffii***	0,6	4	5	0	**9**
18	***Pseudomonas***	*0.6*	***Pseudomonas***	*0.7*	***Pseudomonas deceptionensis***	*0,2*	***Pseudomonas deceptionensis***	0,5	4	9	2	**15**
19	***Streptococcus***	*0.3*	***Streptococcus***	*0.3*	***Neisseria perflava***	*0,2*	***Rothia mucilaginosa***	0,3	0	0	0	**0**
20	***Streptococcus***	*0.3*	***Stenotrophomonas***	*0.4*	***Neisseria perflava***	*0,5*	***Rothia mucilaginosa***	0,5	1	4	0	**5**

the male swimmers, significantly decreased in BT (3.12 ± 0.39) compared to AT (2.99 ± 0.22) in the female swimmers. The Shannon index diversity statistical value was determined as *(p=0.54)* (Figure 3). The Simpson index bacterial abundance and diversity decreased in (0.88 ± 0.09) in AT versus (0.89 ± 0.05) in BT in the male swimmers, significantly decreased in BT (0.89 ± 0.05) compared to AT (0.88 ± 0.04) in female swimmers. The Simpson index was used to determine the correlation *(p= 0.784)* (Figure 4). The Inverse Simpson index bacterial abundance and diversity increased to (11.61 ± 5.25) in AT versus (10.51 ± 4.64) in BT in the male swimmers, and it significantly decreased in BT (10.90 ± 4.43) compared to AT (9.02 ± 2.78) in the female swimmers. The inverse Simpson index was calculated as (p=0.747) (Figure 5).

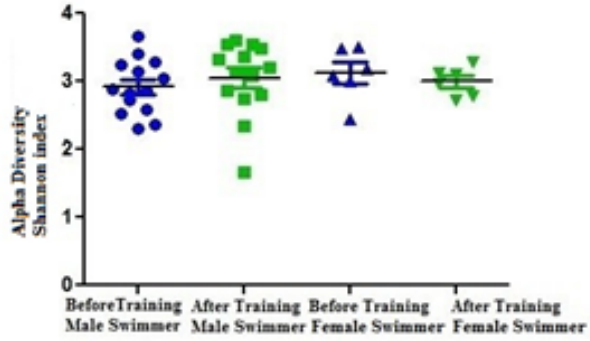

Figure 3. Diversity and richness in the Shannon index (p=0.54).

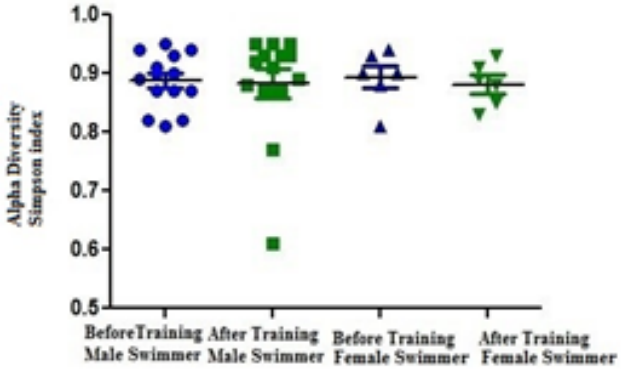

Figure 4. Diversity and richness in the Simpson index (*p=0.784).*

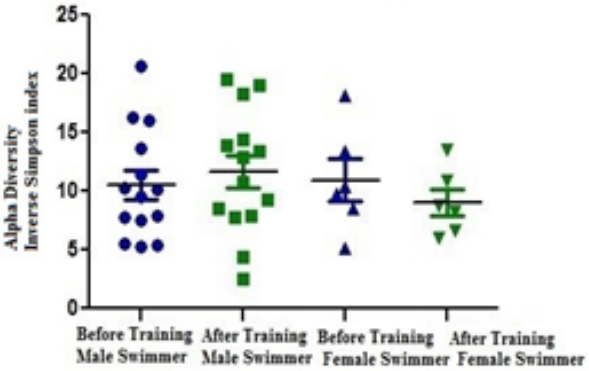

Figure 5. Diversity and richness in the Inverse Simpson index (*p=0.747).*

Discussion

Studies to date have attempted to prove links between the microbiome, disease, and human health, and disease, and are gaining considerable importance. As different microbiota diversity is found in different organisms, each organism having its own microbiota has been the subject of discussion today. It is stated that factors such as the healthy microbiota of the athlete, and energy metabolism, especially in endurance sports where the exercise period is long and intense positively affect critically important metabolisms such as oxidative stress and hydration status (19). Although there is not much information on the contribution of the genomic content of the microbiota to one's athletic performance, there are a limited number of studies on athletes' microbiota.

The microbiota densities and differences of a total of 20 swimmers, both before and after training were compared. Firmicutes and Bacteroidetes phylum; Clostridia and Bacilli class; Clostridiales, Entomoplasmatales and Bacillales ordos, Lachnospiraceae family and *Stenotrophomonas* genus were found to be statistically significant. No statistically significant difference was detected between the other taxa. An increase in the density of Proteobacteria and Actinobacteria phylum and the Clostridia class was observed in AT groups. Moreover, *Stenotrophomonas* genus density increased, and this increase was statistically significant. However, the relative abundance of the *Streptococcus*, *Pseudomonas*, *Serratia, Chryseodomodales*, and *Rothia* genera was higher than the other detected genera. When we compared the species in both groups, the relative abundance of *R. mucilaginosa* was reduced, and the *Granulicatella adiacens, Pseudomonas deceptionensis* and *Prevotella pallens* increased in the AT group. When we compared the DMFT and microbiota, *Streptococcus* and *Pseudomonas* genera and *R.* the *mucilaginosa* species were found to be high in the individuals with the highest DMFT index. *Rothia* and *Streptococcus* were the most commonly detected genera in the oral microbiota and their degradation may cause local and systemic diseases (20). The relative abundance of the *Rothia* species and *Streptococcus* species in individuals with a high DMFT index suggests that they may be associated with oral hygiene.

Recently, there has been an increased diversity in the Firmicutes phylum (including *Faecalibacterium prausnitzii,* species of *Oscillospira* genus, *Lachnospira* genus, and *Coprococcus* genus) and this diversity was considered to contribute to a healthier intestinal environment in individuals who exercise regularly (21). In a study conducted on cyclists; professional versus amateurs, the Prevotella family and *Methanobrevibacter smithii,* both of which are involved in energy and carbohydrate metabolism, were found to be relatively high (22).

Bressa et al. (23) compared the gut microbiota profiles of women athletes who regularly do physical activity for a maximum of 3 hours a week and reported that physical activity modulates the microbiota profile and increases the amounts of beneficial bacteria such as *Faecalibacterium prausnitzii*, *Roseburia hominis*, and *Akkermansia muciniphila*. In a study on marathon runners, an increase in the relative abundance of Veillonella was observed after the marathon, and the authors considered the association between members of the *Veillonella* genus and exercise performance (24). In rowers and ultra-marathon runners, Veillonella strains were found to be abundant (25). The detected bacteria species use lactate as their only carbon

source and are responsible for the conversion of lactate to pyruvate.

The Firmicutes species were detected to be higher in a football player cohort (26). This suggests that exercise may have an effect on performance development by causing an increase in this species in the Firmicutes genus, which is dominant in the intestine. As a result of physical activation, studies are showing that microbial diversity and members of the Firmicutes phylum in the intestine increase as well as studies showing that microbial diversity decreases in swimmers. The reduction in exercise volume has been associated with the loss of taxa associated with Firmicutes (27). In our study, there was a decrease in Firmicutes bacteria density after- training.

Most of the studies have primarily focused on the gut microbiome; however, given the interaction between the mouth and gut microbiomes, the effect of exercise on the gut microbiome may be similar to the oral microbiome. Concerning information on the uniqueness of the healthy human oral microbiota, most studies have focused on diseases (oral cancer, oral dysbiosis), DMFT, survey studies (plaque index due diligence, gingival index), sports injuries, and the effect of nutrition on the oral health of athletes (28, 29).

When the studies on the effect of sports on oral health are examined, those who do physical activity at the recommended level, and who follow a healthy diet program have a 40% lower risk of developing periodontal disease compared to sedentary and overweight individuals. Needleman et al. (30) examined the oral health of athletes who participated in the 2012 London Olympics and indicated poor oral hygiene. Solleveld et al. (31) reported the association between oral health problems and risk of injury in a survey study. Studies have shown that poor oral health affects both the oral health-related quality of life and the training process of athletes. Moreover, it has been reported that poor oral health affects athletic performance and may also cause systemic damage.

Chlorine, which is used as a microbial agent in swimming pools, can lower the pH and can cause tooth erosion as a change in saliva chemistry. Therefore, the salivary flow rate of swimmers may differ, and the long duration of the training session may affect oral microbiota. D'ercole et al. (32) determined that tooth stains and tooth erosion were higher in swimmers compared to non-competitive swimmers. Bissar et al. (33) detected the average DMFT as 2.3 in athletes, whereas Eken et al. (34) detected it as 4.73 in sprinters and as 2.77 in distance runners. Bağlar et al. (35) found that there was a strong correlation between the athletic performance results of athletes with a DMFT index value of <3 in their study conducted on 10-20-30 m short-distance runners. In the current study, we detected it as 6.15. The reason why DMFT values differ in different cohorts is hard to explain, but in swimmers, it may be explained by the water pool chemistry.

Oral health is an important element of general health and well-being and is important for improving the quality of life in sedentary people and improving athletic performance in athletes. There are very few studies on oral health and microbiota in athletes. In the study by Minty et al. (36), they compared the taxonomic analysis of the oral microbiota with the analysis of the Caries, Missing and Filled (DMF) tooth index on 24 professional rugby players and 22 controls. The DMF index and incidence of gingivitis significantly increased in players when compared to the controls. *Streptococcus* species like *S. mutans*, *S. thermophilus*, *S. sobrinus* and *S. gordonii* species were detected to be high in the players. Moreover, a linear regression analysis showed that the abundance of *Streptococcus* genus in the oral microbiota was significantly and negatively correlated with oral microbiota diversity according to the Shannon index. *Fusobacterium* abundance was significantly and positively correlated with oral microbiota diversity. Also, *Rothia* and *Streptococcus* abundances were significantly and positively correlated with body weight. Our results were in agreement with the results of the study. However, it is hard to explain the reasons for the similarities as the *Streptococcus* genus was dominant in the oral cavity.

Prevotella, Lachnospiraceae, Haemophilus, Leptotrichia and *Streptococcus* bacteria types were detected as being high in the oral cavity of soccer players when compared to sedentary individuals. Higher amounts of Lachnospiraceae and *Streptococcus* species were statistically significant between the groups ($p<0.05$). The Haemophilus species were detected at a higher rate than the other groups, but this was not statistically significant. The authors stated that exercise may be the reason for the high amount of the Lachnospiraceae family and Streptococcus (29). The *Streptococcus sobrinus* species were detected to be higher in swimmers (32).

Physical activity increases the amounts of bacteria mainly in the Clostridiales order and Lactobacillus, Prevotella, Bacteroides, and *Veillonella* species, and their numbers may vary depending on the sport type that the individual is involved in. These bacteria are mostly abundant in athletes whose sports activity requires high VO_2 max (37). Physical activity has been shown to have an effect on the qualitative and quantitative differentiation of the microorganisms capable of producing butyrate such as Clostridiales or Firmicutes (38).

In conclusion, our study was the first oral microbiota study conducted on Turkish swimmers. The present study aimed to analyze the bacterial density and diversity of the oral microbiota of acute exercise in regularly trained swimmers. Gram-positive Pseudomonadales and Entomoplasmatales ordo, *Streptococcus,* and *Pseudomonas* species increased after the training. There are several reasons for the increase. Rather than dividing, the increase in the ratio can be explained by the decrease in the other species.

Increased *Streptococcus* in the oral microbiota may be a risk factor for the development of dental caries. In fact, some opportunistic bacteria for caries development, such as *Streptococcus mutans,* have a specific virulence factor that allows them to invade dental tissue and subsequently induce caries (39). In addition, it was concluded that the Prevotella, Lactobacillus, Dialister and *Filifactor* genera may be associated with the pathogenesis and progression of dental caries (40). In our study, high levels of *Rothia*, *Prevotella, Streptococcus* and *Pseudomonas* bacteria were detected in swimmers. These pathogenic bacteria, with a high carbohydrate diet and poor oral hygiene, may cause caries in the swimmers. As a result, studies with higher data are needed to further clarify the relationship of the examined oral microbiota with exercise and oral health.

We have some limitations in our study and those should be considered in further studies. The first is the small num-

ber of athletes recruited for the study. The reason for this is that we wanted to evaluate the results of the oral microbiota as an independent variable by inviting swimmers who implemented the same training program as our study. The second limitation was the lack of comprehensive data on the habitual diets of swimmers before training, and the third was the structure of the study group as it was not a homogeneous group, with different heights, weights, and ages.

Interest conflict
The authors declare no conflict of interest within the publication of the study

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The author read and proved the final manuscript for publication.

Authors' Contribution
Conceptualization: BFE + TK + KU; Data curation: BFE + OA + İY + KU; Formal analysis: BFE + KU; Investigation: BFE + IY + OA; Methodology: BFE + KU Project administration: TK + KU; Resources: BFE + TK + KU; Supervision: SCA + IY + TK; Roles/Writing: BFE + SCA + TK + KU - original draft: BFE + SCA + OA; Writing: BFE; review & editing: TK + KU

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