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The effect of six days open water swimming activity on salivary, oxidative stress and cortisol levels on elite master swimmers

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Abstract

Open water swimming is a sport and recreational activity in which individuals swim in natural bodies of water. When the body is under stress, stress hormones, and reactive oxygen metabolite production may increase. The study aims to evaluate the effect of swimming stress on oxidation formation, antioxidant response, and cortisol levels in whole saliva samples of long-distance open-water swimmers taken before and after swimming. Lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and sialic acid (SA) were determined in the whole saliva of the 12 swimmers before and after swimming. Also, cortisol levels were measured from whole saliva collected in 10 laps, before and after each swim. The salivary LPO level of the swimmers significantly increased, and SOD and CAT activities and SA levels decreased significantly after swimming compared to the baseline. After swimming, the salivary cortisol levels of the swimmers significantly increased in the 1st, 2nd, and 3rd laps of the swim compared to each one's baseline levels. It was also observed that swimming activity caused an increase in total cortisol levels in all laps compared to baseline swimming. Swimming in open water can cause stress on the organism, leading to metabolic adaptations for prevention.

Keywords: Antioxidants, open water swimming, oxidation, saliva, stress

Altı günlük açık suda yüzme aktivitesinin elit yüzücülerde tükürük, oksidatif stres ve kortizol düzeyleri üzerine etkisi

Öz

Açık suda yüzme, bireylerin doğal su kütlelerinde yüzdüğü bir spor ve eğlence aktivitesidir. Vücut stres altındayken stres hormonları ve reaktif oksijen metabolit üretimi artabilir. Bu çalışmada, 12 uzun mesafe açık deniz yüzücüsünden yüzme öncesi ve sonrası alınan tükürük örneklerinde, yüzme stresinin oksidasyon oluşumu, antioksidan tepki ve kortizol düzeyleri üzerindeki etkisinin değerlendirilmesi amaçlanmıştır. Yüzücülerde yüzme öncesi ve sonrası tam tükürüğünde lipit peroksidasyonu (LPO), süperoksit dismutaz (SOD), katalaz (KAT), glutatyon (GSH) ve sialik asit (SA) miktarları belirlendi. Ayrıca, her yüzme aktivitesinden önce ve sonra 10 turda toplanan tükürükte kortizol seviyeleri ölçüldü. Yüzme sonrasında yüzücülerin tükürük LPO düzeyi önemli ölçüde artarken, SOD ve CAT aktiviteleri ve SA düzeyleri başlangıca göre önemli ölçüde azaldığı tespit edildi. Yüzme sonrasında yüzücülerin tükürük kortizol seviyeleri, yüzmenin 1. 2. ve 3. turlarında başlangıç seviyelerine göre önemli ölçüde arttı. Ayrıca yüzme aktivitesinin, yüzme öncesine kıyasla tüm turlarda toplam kortizol düzeylerinde artışa neden olduğu gözlenmiştir. Açık suda yüzmek vücutta strese neden olabilir ve bu da vücudun kendini koruması için geliştirdiği metabolik adaptasyonlara yol açabilir.

Anahtar Kelimeler: *Açık suda yüzme, antioksidanlar, oksidasyon, stres, tükürük*

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Genişletilmiş Türkçe Özet makalenin sonunda yer almaktadır.

INTRODUCTION

Swimmers, especially those who swim in the open water, can experience various sources of stress related to their sport. It can be stressful for athletes who may feel pressure to perform well and meet expectations in aquatic conditions (Pearson et al., 2020). Besides, long-distance swimming can also be stressful as it requires significant physical and mental endurance to carry out the effort during a performance (Casado et al., 2021). Open water swimming is a sport and recreational activity in which individuals swim in natural bodies of water. It offers a unique and challenging experience, as swimmers encounter a variety of conditions, including waves, currents, tides, and temperature fluctuations, exercise training, weather. Although they adapt to these conditions during training, it can be stressful for some individuals cause anxiety, and increase oxidative stress levels (Bladassarre et al., 2017). When the body is under stress, it produces stress hormones such as cortisol and adrenaline, which can increase the production of reactive oxygen metabolites (ROM) in the body. Antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and other antioxidant molecules, such as glutathione (GSH) can neutralize ROM and prevent damage. However, in conditions where performance is required and some factors force the body to stress, the antioxidant defenses can become overwhelmed by oxidative stress (Torres et al., 2004).

Oxidative stress is a physiological imbalance that occurs when there is an excess of ROM and other free radicals in the body that are not adequately neutralized by the body's natural antioxidant defenses (Katerji et al., 2019). ROM are highly reactive molecules that can damage cells and tissues by reacting with important biological molecules such as proteins, lipids, and DNA. Some ROM are produced naturally by the body as part of normal metabolic processes, while others are produced in response to external stressors such as pollution, radiation, and toxins (Alhayaza et al., 2020). Stress also can cause an increase in the production of ROM through several mechanisms, including activation of the hypothalamic-pituitary-adrenal axis and sympathetic nervous system. This can lead to the accumulation of ROM in cells and tissues, which can damage lipids, proteins, and DNA (Jakubczyk et al., 2020).

Sialic acids (SAs) are present in various tissues throughout the body and are found in glycoproteins, glycolipids, or gangliosides. They are located at the end of sugar chains on the surface of cells or soluble proteins (Yang et al., 2021). Additionally, SAs play a role in various biological processes, including maintaining the cell structure, affecting cellular adhesiveness and antigenicity, facilitating the transport process, regulating some hormones, and influencing the catalytic properties of enzymes (Varki et al., 2007).

Cortisol is a hormone produced by the adrenal glands in response to stress. It is often referred to as the "stress hormone" because its production increases during times of stress (Doan et al., 2007). It plays an important role in the body's stress response. It helps to mobilize glucose and other energy sources from the body's stores, increase heart rate and blood pressure, and suppress the immune system. These responses are designed to help the body deal with acute stressors, such as physical danger or a sudden threat. However, chronic or prolonged stress can lead to excessive cortisol production, which can negatively affect the body (Brosschot et al., 2006).

Saliva is a valuable body fluid because it is easily accessible and can be used as a non-invasive diagnostic material for monitoring biochemical parameters. Furthermore, the salivary glands produce various components that come from both serum and cells, thus saliva may be preferred instead of blood to examine changes and parameters in the body caused by physical exercise (Papacosta & Nassis, 2011; Ligtenberg et al., 2016).

Stress may occur in the body as a result of various factors that develop while swimming in the open water, and this may lead to excessive radical production and increased oxidative stress. Frequent formation of radicals in indivudual who swim regularly can cause damage to the body. This study evaluates the effect of swimming on oxidation formation, antioxidant response, and cortisol levels in whole saliva samples of long-distance open-water swimmers taken before and after swimming.

METHOD

Participants

The study consists of 12 long-distance open water swimmers (aged 30-50 years, 9 men and 3 women) participating in the 320-kilometer distance Sochi (Russia)-Giresun (Turkey) course. The study was approved by the Ethical Committee of Marmara University (no: 09.2023.478).

Each participant was monitored for their training regimen for three months before the event. As per the group's swimming schedule (1 hour of swimming every 11 hours for 6 days), each participant was required to swim for one hour in a predetermined sequence. The swimmer's distance is measured using smart swimming watches and a GPS system onboard the cruise, which is managed by the official swimming referees for synchronization. Each swimmer's average swimming and stress data is given in Table 1.

Table 1. Participants average swimming and stress data.

Swimmer	Distance (m)	Pre-swim fatigue	Post-swim fatigue	Lap difficulty	Lap stress	Cal. (kcal)	Stroke/ min	Pulse (min)
1	4038.2	3.5	5	5.2	4.4	713.8	31.2	145.8
2	3400	3.3	5.3	6.5	3.5	846.4	39.6	153.4
3	3667.1	4.7	5	6.4	2.4	822.7	39.3	154.5
4	3104.5	3.5	5.5	6	3.6	670	45.1	146.6
5	2911.8	5.8	8.1	8	7.8	643.7	47.3	143.2
6	3259.1	5.5	5.6	5.8	5	1090.5	32.6	140.1
7	3665.5	3.4	7.4	6.8	4.1	609.5	32.8	137.1
8	3521.4	3.6	5.3	6.1	3.6	810	29.6	118.3
9	3414.6	4.1	5.7	5.8	3.8	838	24.2	142
10	3491.2	4.2	5.2	5.2	1	809.7	42.5	135.5
11	3338.5	3.1	6.2	6.8	3.2	792.8	64	133.5
12	3214.8	2.1	4.4	4.6	3.6	670.5	47.6	153.2

M: meters; Cal: Calorie; Min: Minute; Fatigue/difficulty/stress value 0: minimum, 10: maximum

Collection of saliva samples

The biochemical analysis of swimmers was examined by the collected whole saliva before and after every swimming round for each swimmer. As the swimmers were getting ready for the race, their unstimulated saliva samples were collected after the mouth had been rinsed with distilled water using sterilized Falcon tubes and a funnel. The samples were immediately frozen at a temperature of -20°C and kept until the analysis.

Exclusion criteria included the following: Having a systemic problem, treatment for any disease, having any medication in the last 6 months, and smoking. Individuals were not allowed to brush their teeth, chew gums, or eat before sampling saliva.

Analyses

Biochemical parameters related to oxidative stress; Malondialdehyde (MDA), a product of lipid peroxidation (LPO), (Aebi, 1984), SOD (Walter & Schült, 1974), CAT (Beutler, 1975), and GSH (Warren, 1959) and inflammation; SA (Sureda et al., 2005), were determined in whole saliva of the swimmers before and after swimming. Also, cortisol levels, as a stress parameter were measured by ELISA kit (Salimetrics LLC, 101 Innovation Boulevard, Suite 302, State College, PA 16803) from the whole saliva collected in 10 laps, before and after each swim.

Statistical analysis

The statistical analyses were performed using the Graph Pad 9.0 Prism program and data of the study were presented as the mean ± standard deviation. One-way analysis of variance (ANOVA) analysis was used, and followed by Tukey's multiple comparison tests to compare groups and a p value <0.05 was set as significant.

FINDINGS

The saliva LPO level, SOD and CAT activity, and GSH and SA levels of the swimmers are given in Figure 1. The LPO level of the swimmer significantly increased compared to the initial level ($p<0.05$). Additionally, salivary SOD and CAT activity decreased significantly after swimming compared to the initial ($p<0.01$, $p<0.05$, respectively). A slight decrease in GSH salivary level was observed in post-swimming saliva samples of the swimmers compared to the initial level but, the result was insignificant. A significant decrease was found in SA level in post-swimming saliva samples compared to the initial ($p<0.05$).

The salivary cortisol levels of the swimmers are given in Figure 2. The whole saliva of the swimmers was taken in a total of 10 laps, before and after each swimming. Cortisol levels significantly increased in the 1st, 2nd, and 3rd laps of the swim compared to each one's initial levels ($p<0.001$) (Figure 2A). Also, swimming leads to an elevation in total cortisol levels of all laps compared to the pre-swimming level ($p<0.001$) (Figure 2B).

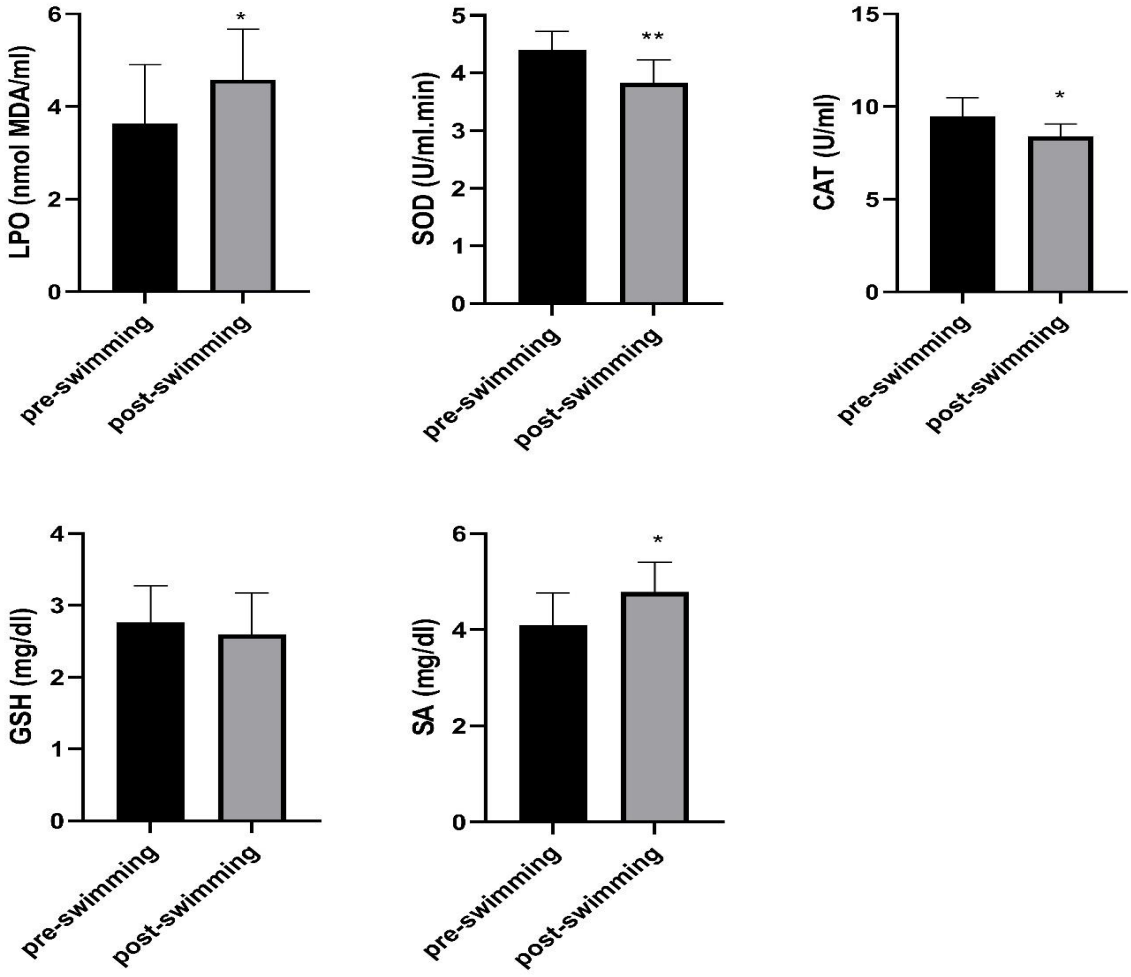

Figure 1. The saliva LPO level, SOD and CAT activity, and GSH and SA levels of the swimmers

Values are given as mean±standard deviation. LPO: Lipid peroxidation; SOD: Superoxide dismutase; CAT: Catalase; GSH: Glutathione; SA: Sialic acid. *p<0.05, **p<0.01 significantly different from pre-swimming.

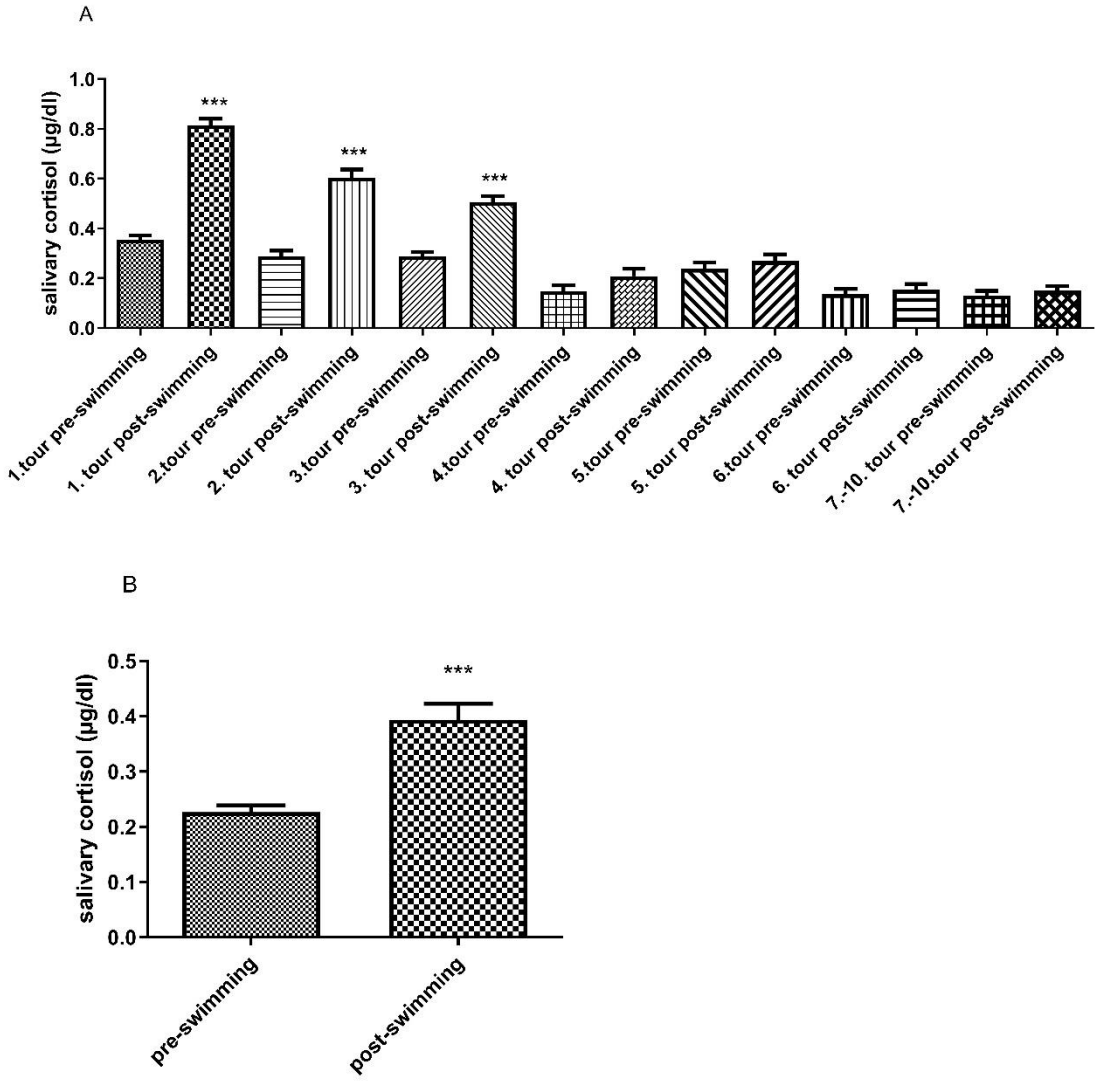

Figure 2. The salivary cortisol levels of the swimmers

Values are given as mean±standard deviation. ***p<0.01 significantly different from pre-swimming.

DISCUSSION AND CONCLUSION

Free radicals play a crucial role in several biological processes, but their uncontrolled production can cause damage to cells and tissues. Previous research has shown that engaging in high-intensity physical exercise is associated with elevated generation of ROM due to increased metabolic processes by elevated oxygen consumption, which can result in oxidative

stress. (Powers et al., 2016; Powers et al., 2020). During extreme exercising and in conditions that take place under stress, free radicals can form due to elevated levels of xanthine oxidase and catecholamine, buildup of lactic acid, and formation of inflammation. The utilization of oxygen may increase by up to 10 times for extra performance. This increase is linked to a rise in the generation of mitochondria, as well as the metabolic leak of superoxide and hydrogen peroxide (Radak et al., 2013).

Similar to the results of previous studies designed with different sports disciplines (McIntosh & Sapolsky, 1996; Sahin & Gümüslü, 2004; Haleagrahara et al., 2009), in the present study, an increased LPO level and decreased SOD and CAT activity were observed in the post-swimming whole saliva of the swimmers compared to the baseline level. Increased LPO level is in accordance with an increase in oxidative stress, which suggests that the oxidative defenses were overwhelmed by the swimming performance. The increase in free-radical production due to swimming performance may be mediated through various pathways in addition to electron leakage. When the sympathetic-adrenal system is activated due to stress, it can increase catecholamine levels. This increase can lead to auto-oxidation, a process that generates electrons, which can produce ROM (Mehdi et al., 2012). Besides, the rapidly increasing radical production may have exceeded the antioxidant capacity and the depletion of SOD and CAT of the body. Therefore, this may lead to a decrease in SOD and CAT activity in the post-swimming saliva compared to the pre-swimming level. However, Cavas et al. (2005) found increased antioxidant enzyme activities after the post-exercise in the judoists, also, Andrade-Lima (2021) pointed that walking training leads an elevation in blood SOD and CAT activities, which may be due to the fact that SOD and CAT are sufficient for the radicals formed as a result of different athletes and different training. In the present study, a slight decrease was found in the GSH level of the post-swimming saliva samples compared to the baseline, but the result was insignificant. It's possible that the body's antioxidant defense system, specifically glutathione, was able to handle the oxidative stress and prevent a significant decrease in GSH levels.

Salivary SA is suggested as a marker of oxidative stress (Haneishi et al., 2007; Oktay et al., 2020). Compared with the baseline, we detected increased SA levels in the swimmer's whole saliva after the performance. This may result from the cell's protection mechanism from damage that may occur due to increased metabolic processes during the performance. In another study conducted in a different sports branch (Cavas et al., 2005), the SA level was found to increase after training, and the authors suggested that salivary SA also increased due to increased oxidative stress.

Since swimming in the open water is an event where maximum power is spent under difficult conditions, it affects metabolic pathways and also induces hormonal responses. In different sports with players, the same results were found with our cortisol levels and it was suggested that increased cortisol response can be attributed to forcing the body for more performance (Lehmann et al., 1998; Elloumi et al., 2003; Doan et al., 2007). Psychological stress caused by several factors, such as environment, competition, and difficulty, can be attributed to an increase in cortisol levels during a performance. Elloumi et al. (2003) reported that cortisol levels increase significantly after a match, however, when competition is eliminated, cortisol levels during a physiologically strenuous activity did not reach the same size. Our study found that post-swimming cortisol values increased during the 1st, 2nd, and 3rd laps of swimming compared to the values before the swim, while no significant increase was observed in the following laps. This may be due to adaptation to conditions such as getting used to water temperature, fighting waves, and marine environment. Research has demonstrated that after intense training with frequent stress hormone releases, the body may down-regulate certain hormone receptors in the targeted tissues, resulting in reduced responsiveness to these hormones' effects (Lehmann et al., 1998).

Recommendations

Athletes must strive for maximum performance within a limited time and difficult conditions. Moreover, swimming in open water can cause stress on the body, leading to metabolic adaptation and protection. Additionally, saliva provides accurate results in examining biochemical changes due to stress and exercise.

Acknowledgements

The 320km swimming event, consisting of 7 Turkish, 3 Russian, and 2 Turkish-German swimmers, was led by the Istanbul Yıldızlar Swimming Club, and supported by Turkish Sports Ministry, Turkish Ministry of Foreign Affairs, Turkish Coastal Guard, Marmara University, Giresun University, Turkish Swimming Federation, Turkish Radio and Television, and Giresun City Municipality.

GENİŞLETİLMİŞ ÖZET

GİRİŞ

Açık suda yüzme, bireylerin doğal su ortamındaki bir aktivitedir. Yüzme sırasında çeşitli koşullarla karşılaşıldığından benzersiz ve zorlu bir deneyim sunar. Antrenman sırasında bu koşullara uyum sağlanabildiği halde bazı bireyler için stres kaygıya neden olabilir ve oksidatif stres düzeylerini artırabilir (Baldassarre et al., 2017). Vücut stres altındayken kortizol ve adrenalin gibi stres hormonları

üretir ve bu hormonlar vücutta reaktif oksijen metabolitlerinin (ROM) üretimini artırabilir. Oksidatif stres, vücutta oluşan aşırı mikatrdaki ROM ve diğer serbest radikallerin, antioksidan savunma tarafından nötralize edilemediği, fizyolojik bir dengesizliktir (Katerji et al., 2019). Süperoksit dismutaz (SOD), katalaz (KAT) gibi antioksidan enzimler, glutatyon (GSH) gibi antioksidan moleküller ve sialik asit (SA) gibi yapısal moleküller ROM'u azaltabilir veya nötralize edebilir ve hasarı önleyebilir (Torres et al., 2004; Varki & Varki, 2007).

Tükürük, kolay erişilebilmesi ve biyokimyasal parametrelerin izlenmesinde non-invaziv bir tanı materyali olması nedeniyle değerli bir vücut sıvısıdır. Ayrıca tükürük bezleri hem serumdan hem de hücrelerden gelen çeşitli bileşenler ürettiğinden, fiziksel egzersizin vücutta neden olduğu değişikliklerive parametreleri incelemek için tercih edilebilir (Papacosta & Nassis, 2011).

Bu çalışma, uzun mesafe açık deniz yüzücülerinin, yüzme öncesi ve sonrasında alınan tam tükürük örneklerinde oksidasyon oluşumu, antioksidanların ve kortizol düzeyleri üzerindeki etkisini değerlendirmektedir.

YÖNTEM

Çalışmada, 320 kilometrelik Soçi (Rusya)-Giresun (Türkiye) parkuruna katılan 12 uzun mesafe açık su yüzücüsü (30-50 yaş arası, 9 erkek ve 3 kadın) bulunmaktadır. Yüzücülerin yüzme turu öncesi ve sonrasında toplanan tükürükler ile lipid peroksidasyon (LPO) ürünü ve oksidan bir parametre olan malondialdehit (MDA) (Aebi, 1984), antioksidan sistem parametreleri olarak SOD (Walter & Schült, 1974), KAT (Beutler, 1975) ve GSH (Warren, 1959) ve bir inflamasyon belirteci olan SA (Sureda et al., 2005) tayini yapıldı. Ayrıca, bir stres parametresi olarak kortizol seviyeleri (Salimetrics LLC, 101 Innovation Boulevard, Suite 302, State College, PA 16803), her yüzmeden önce ve sonra, 10 turda toplanan tükürüklerde ölçüldü.

İstatistiksel analiz

İstatistiksel analizler Graph Pad 9.0 Prism programı kullanılarak yapıldı ve çalışmanın verileri ortalama±standart sapma olarak sunuldu. Grupları karşılaştırmak için tek yönlü ANOVA analizi kullanıldı ve ardından grupları karşılaştırmak için Tukey'in çoklu karşılaştırma testleri uygulandı. $p<0,05$ değeri anlamlı olarak belirlendi.

BULGULAR

Yüzücülerin LPO düzeyi, başlangıç düzeyine göre anlamlı düzeyde arttı ($p<0,05$). Ayrıca yüzme sonrası tükürük SOD ve KAT aktiviteleri öncesine göre olarak anlamlı derecede azaldı ($p<0,01$, $p<0,05$). Yüzücülerin GSH düzeyi de başlangıç düzeyine göre hafif azalma gösterdi ancak sonuç anlamlı değildi. Ayrıca, yüzme sonrası tükürük SA düzeyinde başlangıca göre anlamlı bir azalma tespit edildi ($p<0,05$).

Kortizol tayini için yüzücülerin tam tükürüğü, her yüzme öncesi ve sonrasında toplam 10 turda alınmıştır. Yüzmenin 1. 2. ve 3. turlarında kortizol seviyeleri başlangıç seviyelerine göre anlamlı

düzeyde arttı ($p<0,001$). Ayrıca yüzme sonrası toplam kortizol düzeyleri yüzme öncesine göre arttı ($p<0,001$).

TARTIŞMA VE SONUÇ

Aşırı egzersiz sırasında ve stres altındaki koşullarda, artan ksantin oksidaz ve katekolamin düzeyleri, laktik asit birikmesi ve iltihaplanma nedeniyle vücutta serbest radikaller oluşabilir. Bu durumda, ekstra performans için oksijen kullanımı 10 kata kadar artırılabilir. Bu artış, mitokondriyel artışla beraber süperoksit ve hidrojen peroksitin metabolik sızıntısıyla da bağlantılıdır (Pippenger et al., 1998). Antioksidan enzimler; SOD ve KAT, radikalleri başka moleküllere dönüştürerek hücre korumasında önemli rol oynar. SOD, süperoksit radikallerini hidrojen peroksite dönüştürürken, CAT, oluşan hidrojen peroksiti oksijen ve suya dönüştürür. GSH, hayati hücresel bileşenleri çeşitli radikal türlerin neden olduğu hasarlardan koruyabilmektedir (Ali et al., 2020). Kanda ve hücrelerde olduğu gibi tükürük de vücudun korunmasında ve bağışıklıkta rol oynayan çok sayıda antioksidan bileşik içerir. Farklı spor disiplinleri ile yapılan önceki çalışmaların sonuçlarına benzer şekilde (McIntosh & Sapolsky, 1996; Sahin & Gümüslü, 2004; Haleagrahara et al., 2009), bu çalışmada da yüzücülerin yüzme sonrası tükürüklerinde başlangıç düzeyine göre LPO düzeyinde artış ve SOD ve KAT aktivitesinde azalma gözlenmiştir. Artmış LPO seviyesi oksidatif stresin oluşumu ile uyumludur. Ayrıca hızla artan radikal üretimi antioksidan kapasitesini aşmış ve vücutta SOD ve KAT'ın tükenmesine neden olmuş olabilir. Dolayısıyla bu durum yüzme sonrası tükürük SOD ve KAT aktivitesinde göre azalmaya neden olabilir. Başlangıç değeriyle karşılaştırıldığında, performanstan sonra yüzücülerin tükürük SA seviyelerinde artış tespit edildi. Bu durum, hücrenin performans sırasında artan metabolik süreçler nedeniyle oluşabilecek hasardan korunma mekanizmasından kaynaklanıyor olabilir.

Açık suda yüzmek zor şartlar altında maksimum güç harcanan bir etkinlik olduğundan hem metabolik yolları etkiler hem de hormonal tepkileri tetikler. Daha önce yapılan spor dallarındaki yayınlarla uyumlu olarak, çalışmamızda yüzme öncesine göre kıyaslandığında yüzme sonrası kortizol seviyeleri artmıştır, ayrıca 1. 2. ve 3. turlarda da aynı artış gözlenmiştir. Yüzme ile artan kortizol düzeyi, vücudu daha fazla performansa zorlamak için olabilir (Lehmann et al., 1998). Çalışmamızda, yüzme öncesi değerlere göre 3. tur sonrasında kortizol seviyesinde anlamlı bir artış görülmediği tespit edildi. Bunun nedeni koşullara uyum sağlamak olabilir.

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Literatür Tarama *Literature Review*	Çalışma için gerekli literatürü taramak *Review the literature required for the study*	Nihal Şehkar ULUCAN
Veri Toplama ve İşleme *Data Collecting and Processing*	Verileri toplamak, düzenlemek ve raporlaştırmak *Collecting, organizing and reporting data*	Kubilay ÇİMEN Cengiz KARAGÖZOĞLU Korkut ULUCAN
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