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The Physiological Responses to 5000 m Open Water Swimming Exercise in Children

Çocuklarda 5000 m Açık Su Yüzme Egzersizine Fizyolojik Cevaplar

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ABSTRACT Objective: Open water swimming (OWS) is an endurance sport. Nitric oxide (NO) is a vasodilator gas, therefore it is related to endurance. Physiological stress levels of OWS on children are unknown. The purposes of this study were to investigate the physiological responses to 5000 m OWS and its relation with serum NOx (nitrite plus nitrate) levels in children. **Material and Methods:** Trained OWS athletes (9 males and 5 females; age=13.8±1.12 years) participated in the study. Serum NOx, oxidative stress index (OSI): total antioxidant status (TAS)/total oxidant stress status (TOS) ratio, muscle damage markers (MDM): serum creatine kinase (CK), aspartate aminotransferase (AST) activity, leucocyte counts and serum glucose (GLU) levels were determined before and after the OWS. Lactate (La), whole blood glucose (WGLU) analyses were done from the fingertip blood samples and heart rate (HR) was measured after every 1000 m. **Results:** After 5000 m OWS, HR and La levels were recorded as 166 beat/min and 2.95 mM. The results indicated that MDM significantly increased ($p<0.01$), and OSI significantly decreased however there were no significantly changes in TAS, NOx and serum glucose (GLU) values. Basal NOx levels was only related to CK ($r=-0.529$, $p=0.052$), but not other parameters. **Conclusion:** 5000 m OWS exercise induced a moderate physiological stress without any oxidative stress, hypoglycemia and health risk for the investigated parameters risk. Although this exercise did not increase blood NOx levels, the negative relationship between basal NOx and CK parameters may show the anti-inflammatory role of NO during OWS exercises in the children.

Keywords: Swimming; nitric oxide; oxidative stress; stres, physiological

ÖZET Amaç: Açık su yüzme (ASY) bir dayanıklılık sporudur. Nitrik Oksit (NO) damar genişletici özelliği nedeniyle, dayanıklılıkla ilişkili bir gazdır. ASY'nin çocuklar üzerindeki fizyolojik etkileri bilinmemektedir. Bu çalışmanın amacı; çocukların 5000 m ASY egzersizine verdikleri fizyolojik cevapları ve bu cevapların serum NOx (Nitrit + nitrat) düzeyleri ile ilişkisini araştırmaktır. **Gereç ve Yöntemler:** Çalışmaya aktif ASY sporcuları (9 erkek, 5 kız; yaş=13,8±1,12 yıl) dâhil edilmiştir. Yüzmeden önce ve sonra serum NOx, oksidatif stres indeksi (OSI): toplam antioksidant statüsü (TAS)/toplam oksidatif stres statüsü (TOS), kas hasarı göstergeleri (KHG): serum kreatinkinaz (KK), aspartat aminotransferaz (AAT) aktiviteleri ve lökosit) ölçümü yapılmıştır. Her 1000 m yüzmenin ardından kalp atımı, el parmak ucu kanından laktat ve tam kan glukoz (WGLU) seviyesi ölçülmüştür. **Bulgular:** 5000 m ASY sonunda ortalama kalp atımı (HR); 166 atım/dk, laktat düzeyi; 2,95 mM olarak bulunmuştur. KHG'nin yükseldiği ($p<0,01$), TAS, NOx ve serum glikoz (GLU) değerlerinin anlamlı olarak değişmediği fakat OSI'nin düştüğü ($p<0,01$) tespit edilmiştir. Bazal NOx ve KK aktivitesi arasında korelasyon bulunmuştur ($r=-0,529$, $p=0,052$). **Sonuç:** 5000 m ASY egzersizi çocuklarda, orta seviyede bir fizyolojik strese neden olmuştur, fakat oksidatif stres, hipoglisemi ve araştırılan parametreler için herhangi bir sağlık riski yaratmamıştır. ASY her ne kadar NOx'da bir artış yaratmamış olsa da, NOx ve KK arasındaki ilişki NO'nun ASY esnasında anti-inflamatuar bir rolü olabileceğini gösterir.

Anahtar Kelimeler: Yüzme; nitrik oksid; oksidatif stres; stres, fizyolojik

Open water swimming (OWS) competitions were taken place during the 2008 Beijing Olympic Games Aquatic Events for the first time, due to the rapidly increasing popularity of the sport. This swimming event is similar to marathons because of the 5, 10 and 25 km swimming distances and the performance times involved.[1] However, it includes longer swimming distances than both Olympic and Ironman triathlon contests (1.5-3.9 km). Therefore, its physiological requirements differ from these events.[2] Knowledge of the physical and physiological properties of a sport is crucial for planning an efficient training and good performance as well as health.

Training may positively or negatively affect oxidative stress (OS) levels depending on training load, type, and specificity.[3] The high levels of oxygen consumption may lead to increase OS during long-duration of OWS exercises; OS can also negatively affect performance and cellular integrity.[4] In a study of well-trained adult male swimmers, no OS was found after ultra-marathon swimming.[5] Although it was not observed any serious OS in the studies carried out on adults, the physiological stress and OS levels of OWS in child swimmers are unknown.[5-7] The responses of children may differ from those of adults, because there are several metabolic and physiologic differences between children and adults.[8] It has been reported that children can be more sensitive to OS during chronic exercise and exhibit disturbances in redox balance at rest.[9] It has been shown that trained male and female swimmers had higher OS levels and lower antioxidant capacity in comparison with their sedentary counter parts.[9,10] However, cold stress can cause alterations in both metabolic and physiological aspects due to increased free radical production as a result of shivering and muscle movement to maintain body temperature.[11] Therefore, antioxidant capacity and immune function may also be impaired during long-duration swimming in cold water.[12] In addition, excess production of oxidant substances may impair muscle and diaphragm function.[13] These factors may lead to the deterioration of swimming performance and also some health problems as well. Furthermore, overtraining may also lead to OS and is associated with a number of health risks.[14] Humans respond to acute cold exposure with two major physiological adjustments: Vasoconstriction and thermogenesis. These responses are affected by factors such as age, subcutaneous body fat, body face area and fitness of the subject.[15]

Exercise that has hypoxic properties such as swimming causes increment in the production of nitric oxide (NO) which has both vasodilatory and antioxidant properties.[16,17] NO also increases glucose (GLU) uptake and reversibly inhibits glycolysis and phosphocreatine breakdown.[17] Therefore, the increased blood flow through NO to important organs such as the liver and heart as well as muscle, which is an important lactate consumer, may decrease lactate concentrations.[18] In addition, it has been found that there is a significant relationship between aerobic fitness level and NO.[17,19] Therefore, increasing NO levels may have a positive effect on physiological stress and OS levels during OWS. NO is synthesized from L-arginine, which is catalyzed by nitric oxide synthase (NOS). NOS has three isoforms: Type-I (neuronal NOS, nNOS), Type-II (inducible NOS, iNOS), and Type-III (endothelial NOS, eNOS).[20] NO is generated during muscle contraction as a result of nNO, eNO, and iNOactivation.[17,21] The best-known stimulus of NO production is shear stress, which is produced during increased blood flow and can increase NOS activity.[17,22] The effects of 8 weeks of training were shown to increase NO production in young men.[23] In addition, it has been shown that three months of severe swimming exercise induces a significant increase in NOx (as nitrate plus nitrite) levels.[16] Thus, long-duration swimming activities such as OWS may increase blood NO levels. In addition, NO is essential for maintaining a salutary cardiovascular status.[17] Furthermore, NO is an independent risk factor and a strong prognostic marker of long-term cardiovascular morbidity and mortality.[16,17] Therefore, this possible increase in NO during OWS may contribute to both antioxidant capacity and endurance capacity of athletes as well as their health status. These factors may contribute to a tolerable stress for chil-

dren performing long-duration OWS. But NO has both oxidant and antioxidant features depending on physiological conditions.[17,24]

Moderate-intense aerobic exercises improve endothelial function; while high intensity exercises increase OS levels and affect endothelial function negatively, decreasing the bioavailability of NO.[25] Increased superoxide levels may also lead to the conversion of NO to a stronger radical (such as peroxynitrite),[2]which impairs vasomotor function by decreasing the bioavailability of endothelium-derived NO and by inhibiting the synthesis of the vasodilator prostacyclin.[24] However, the physiological stress and OS levels of OWS on children and NOx's related to these parameters are unknown. Therefore, we hypothesized that 5000 m OWS will significantly increase physiological stress (PS), OS and blood NOx levels in children and that NOx can be associated with related to PS, OS and 5000 m swimming performance time. Turkish Open Water Junior National Team candidate swimmers swam 5000 m in the sea to test our hypothesis.

For this purpose, we investigated the effects of acute and long distance swimming exercise on physiological stress, inflammatory responses, oxidative stress and some endogenous non-enzymatic antioxidants. Physiological stress was evaluated by heart rate (HR), lactate (La), creatine kinase (CK), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) activities. The inflammatory responses were limited to leucocyte counts.[26] The oxidative stress was determined with total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI=TOS/TAS ratio) and NOx (as total nitrite), as well as the endogenus non-enzymatic antioxidants were limited to uric acid (UA), albumin (ALB) and ferritin (FRT).[17,27] In addition, the relationships between NOx and the above mentioned parameters were investigated.

MATERIAL AND METHODS

PARTICIPANTS

Nine male and five female Turkish Open Water Junior National Team candidate swimmers with 4-5 years of sports experience participated in the study (age= 13.78±1.21 years, height= 168.06±6.67 cm, body weight= 59.06±7.99 kg, body mass index [BMI]= 20.83±1.61 $kg \cdot m^{-2}$). No significant difference was detected between male and female subjects in terms of basic parameters pre- and post-swim, including TAS, TOS, and NOx (pre swim-TAS; p=0.125, post swim-TAS; p=0.061, pre swim-TOS; p=0.841, post swim-TOS; p=0.463, pre swim-NOx; p=0.289, post swim-NOx; p=0.416). Therefore, the athletes were evaluated as a single group (n=14). A briefing was held one week prior to the test for the athletes and their parents in order to provide information on the procedures followed during the study. Participants and their parents each signed a personalized document stating that they were participating in this project voluntarily. This study was also approved by the Ege University Faculty of Medicine Clinical Research Ethics Board (Approval No: 09-9.1/10).

EXPERIMENTAL DESIGN

The tests were conducted 20 days after the competition session. Initially, health checks were performed by taking an anamneses and carrying out physical examinations for each athlete. Subjects were told that they should eat their breakfast 3 hours before the test and arrive at the test center 1 hour before the test start. On day 1, an anaerobic threshold test was held in the olympic indoor pool (Test 1). Anthropometric measurements were performed prior to the test and three days later from 5000 m the open water test (Test 2). Air, sea, and pool temperatures at the time of the anaerobic threshold test and the 5000 m OWS test were measured as 21 C°, 24 C°, and 27 C° respectively.

ANAEROBIC THRESHOLD TEST

Anaerobic threshold levels (ANT; the work load at 4 mM lactate concentration), which are used as an indicator of mid- and long-term aerobic endurance capacity, were tested 3 hours after breakfast at 11:00 a.m. in a 50m indoor swimming pool.[28] A protocol was adapted and applied from the study of Wakayashi which consisted of 4x400-m accelerating freestyle swimming to individual maximum with 1-minute passive resting intervals.[28] Loading intensity was identified as a percentage of each

swimmer's individual 400m maximum time (T400max). According to these values, the load was gradually increased to T400 (55%), T400 (75%), T400 (90%), and T400max. Each athlete was helped in regulating their rhythm by a signal sound every 50m. Before testing, a 600 m standardized warm-up protocol was used.[29] The HR of each athlete at each level was measured telemetrically via a Polar S810i (Polar Electro, OY, Finland) HR recorder. Moreover, blood samples were taken from the fingertip into heparinized capillary hematocrit tubes after every 400 m of swimming. Twenty μL of samples which were put into the special tubes of the analyzer (BIOSEN C-line, EKF Diagnostics, Barleben/ Magdeburg, Germany) were used to measure La and Whole blood glucose (WGLU) levels using the electro enzymatic chip method. Completed measurements were put on metric graphic paper in a La performance curve as specified by Coen to identify ANT levels.[30] The corresponding speed and HR for 4 mM La concentration were calculated using mathematical interpolation.[20]

ESTABLISHING 5000 M OF OPEN WATER SWIMMING PERFORMANCE

A 5000 m freestyle swimming test was held in September following a 3-hour after breakfast (at 06:00 a.m.) in the Aegean Sea (Foça), İzmir/Turkey. During the open water swimming test, participants were continuously observed by the national team coach and doctor. During the 5000 m test, participants were allowed 2-minute passive rest periods and isotonic fluid intake (250 mL) every 1000 m HR levels, La and WGLU measurements after each 1000 m were conducted in alignment with procedures followed in the ANT test.

BLOOD DRAWAL AND MEASUREMENT METHODS

Blood Sampling and Storage

Three hours after breakfast (at 06:00 a.m.) before the 5000 m open water swim, participants were rested for 10 minutes in a sitting position and blood samples totaling 12 mL were taken and placed in two separate tubes (EDTA and anticoagulant-free serum vacutainer). The same procedure was followed 5-10 minutes after the 5000 m swim. Blood hemogram analysis from tubes with EDTA was applied after 4-5 hours using an automatic blood count device (BC-3000 Plus, Mindray, China). The hemogram analysis included erythrocytes (RBC), leucocytes (WBC), hematocrit (HCT), hemoglobin (HGB), mean erythrocyte volume (MCV), and thrombocyte levels (PLT). The tubes containing the blood drawn for serum were left at room temperature for 30 minutes and then centrifuged for 15 minutes at 1500 g. The serum samples were then stored at -20 °C until analysis (within 10 days).

BIOCHEMICAL MEASUREMENTS

Glucose (GLU), urea (UREA), UA, creatinine (CR), total protein (TP), ALB, and liver function tests AST, ALT, gamma glutamyl transaminase (GGT), CK, lactate dehydrogenase (LDH), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), triglyceride (TG), iron (FE), and FRT were analyzed from serum samples via standard kits (Dialab Gmbh, Austria) and auto analyzer (Beckman, CX7, Brea, CA, USA). Low density lipoprotein (LDL) cholesterol was calculated according to the LDL-C = TC- (HDL-C +TG/5) formula.[31]

TOS and TAS Measurements

Serum TOS and TAS levels were determined by auto analyzer (Beckman, CX7, USA) using commercial kits (Real Assay Diagnostics kit; Gaziantep, Turkey) with chromogenic methods. The TOS method is based on the principle that the oxidant molecules in the serum create a new color with the chromogenic substance.[32] The absorbance of this compound is proportional to the amount of oxidant substance in the serum and reflects the amount of oxidant molecules present. The sample to be analyzed was calibrated with hydrogen peroxide and the results were expressed as μM hyd rogen peroxide per liter (μM H_2O_2 Eq/L). The intra and inter coefficient of variation (CV) was smaller than 3.0%. The TAS method is based on bleaching of the characteristic color of a more s 2,2'-azino-bis [3-ethylbenz-thiazoline-6-sulfonic acid (ABTS)] radical cation caused by antioxidants in serum.[33] Results were stated as μM trolox per liter (μM Trolox Eq/L). The intra and inter CV was smaller 3.0%. The ratio of TOS level to TAS level was used as OSI.

Nitric Oxide (NOx; Nitrate+ nitrite) Measurement

Serum NOx levels were measured spectrophotometrically via a microplate reader (Dialab Gmbh, Dia reader ELx800G, Austria) with a commercial kit (Oxis International Inc., USA). The method is based on the fact that nitrate (NO_3), which is the metabolite of NO, is broken down into nitrite (NO_2) by cadmium (CD^{+2}). The absorbance of the pink-colored azo dye this forms through the "Griess reaction" is measured spectrophotometrically. Through this method, the total NO_2 level in the sample was determined. The CV for intra and inter assay of NO kit were 7.1% and <9.2% respectively.

Calculation of Plasma Volume Changes (ΔPV %)

The plasma volume (**ΔPV %**) change in the blood samples collected before and after the 5000 m swim was calculated using HGB and HCT parameters by using the formula of Dill.[34] **ΔPV %** corrections were made for all biochemical parameters and these were presented in tables.

STATISTICAL ANALYSES

Shapiro-Wilk test was done to test the normality assumption of the data and skewness, kurtosis and CV % values were also checked. As central tendency measures, means and standard deviation were used for the data fit the normal distribution and median for those not fit the normal distribution. Repeated Measures of ANOVA was used for the analysis of the significance of difference in the repeated measures of the data in conformity with normal distribution (5x1000 m swimming; HR, La). In addition, among post-hoc tests, LSD was used to determine the repeated measure causing the significant difference. Friedman test was used for the analysis of the significance of difference in the repeated measures of the data which were not in conformity with normal distribution (5x1000 m swimming; GLU, speed). To find the measure that led to the significant difference, Wilcoxon signed-rank test with a Bonferroni's correction was used for pairwise comparisons. Among the hematologic, biochemical and oxidant parameters measures taken pre and post 5000 m swimming, in order to determine the significance of the difference between two measures, paired-samples t-test was used in the data that complied with normal distribution (WBC, HGB, RBC, HCT, MCV, PLT, FRT, GLU, WGLU, URE, UA, GGT, TC, TG, HDL-C, TP, NOx) and Wilcoxon signed-rank test for the data that did not comply with normal distribution (FER, CR, AST, ALT, CK, ALB, LDL-C, TOS, TAS, OSI). Moreover, numerical differences and variation ratio of the numerical differences (Δ %) between two measures were also included.

In order to determine the relationship between the data (hematological and biochemical parameters, and oxidative stress indices), Pearson correlation coefficient analysis was used for normally distributed data, whereas Spearman rank-order correlation coefficient analysis was used for the data that did not comply with normal distribution. SPSS for Windows release 15.00 analysis program was used for statistical evaluation and level of significance was accepted as $p<0.05$ and $p<0.005$ acceptable significance level for the Bonferroni's correction.

RESULTS

HR, WGLU, LA, AND SPEED VALUES OF EVERY 1000 M OF OWS

The defining statistics for OWS performance and comparisons of HR, WGLU, La, and speed parameters for 1000 m swims are presented in Table 1. The swimming speed of the athletes, corresponding to their anaerobic threshold values, was 1.23±0.08 $m \cdot sec^{-1}$, and their HR was 169.64±6.7 $beats \cdot min^{-1}$. After the last 1000 m, WGLU and La levels as well as swimming speed displayed a significant decrease from those of the first 1000 m (WGLU 1-5, La 1-5, Speed 1-5; Table 1).

HEMATOLOGICAL PARAMETERS

A significant increment was detected in the athletes' WBC and FRT levels (98.37%, p=0.01; 11.09%, p=0.05 respectively). Other parameters did not exhibit any significant changes. ΔPV % (ΔPV=- 0.61%) change corrections were made for all biochemical parameters and these were presented in Table 2.

TABLE 1: Comparison of heart rate, WGLU, lactate and swimming speed between 1000 m swims, [mean±SD (median)].

1000 m S	HR (beats•min-1)	WGLU (mM)	La (mM)	Speed (m•sec-1)
1 (Basal)	164.71±20.75	6.05±0.73[¥] (6.06)	3.92±0.85[&#β¥]	1.00±0.04[&] (1.01)
2.	169.57±15.43[&]	5.84±0.84[*α] (5.63)	3.3±1.36&[α∞*]	0.99±0.06[#] (1.02)
3.	167.92±10.8[¥]	5.32±0.97 (5.02)	2.81±1.32[#αμ]	0.99±0.07 (1.03)
4.	166.64±12.33	5.2±0.94* (4.81)	2.28±1.22[β∞μ]	0.98±0.06[¥] (1.00)
5.	161.57±12.01[&¥]	4.79±1.27[¥α] (4.79)	2.45±1.16[¥*]	0.96±0.05[&#¥] (0.98)
5000 m mean	166.08±14.26	5.44±0.95	2.95±0.99	0.99±0.06 (1.02)

1000 m S: 1000 m swimming; HR: Heart Rate; La: Lactate; WGLU: Whole Blood Glucose

Super scripts represents statistitically significant differencies between;

[&] HR 2-5 and La 1-2 ($p<0.05$), Speed 1-5 ($p<0.005$). [¥] HR 3-5 and La 1-5 ($p<0.05$), WGLU 1-5 and Speed 4-5 ($p<0.005$). * La 2-5 ($p<0.05$), WGLU 2-4 ($p<0.005$). [α] La 2-3 ($p<0.05$), WGLU 2-5 ($p<0.005$). [#] La 1-3 ($p<0.05$), Speed 2-5 ($p<0.005$). [β] La 1-4 ($p<0.05$). [∞] La 2-4 ($p<0.05$). [μ] La 3-4 ($p<0.05$).

TABLE 2: Pre- and post-swim comparisons of hematological parameters [mean±SD (median)].

Parameters	Pre-Swim (Basal)	Post-Swim	Difference	Δ%
WBC (x10^3/mm^3)	5.53±0.95	10.97±1.68	↑ 5.44**	↑ 98.37
HGB (g/dL)	13.51±0.58	13.4±0.45	↓ 0.11	↓ 0.81
RBC (x10^6/mm^3)	4.8±0.16	4.71±0.21	↓ 0.09	↓ 1.87
HCT (%)	40.22±1.69	39.64±1.25	↓ 0.58	↓ 1.44
MCV (fL)	83.88±4.32	83.77±4.02	↓ 0.11	↓ 0.13
PLT (x10^3/mm^3)	273.85±37.18	295.15±60.64	↑ 21.3	↑ 7.77
FRT (ng/mL)	25.69±9.74	28.54±10.76	↑ 2.85*	↑ 11.09
FE (μg/dL)	92.28±39.09 (77.50)	92.74±46.28 (80.36)	↑ 0.46 (2.86)	↑ 0.49 (3.69)

WBC: Leukocyte; HGB: Hemoglobin; RBC: Erythrocyte; HCT: Hematocrit; MCV: Mean RBC volume; PLT: Platelets; FE: Iron; FRT: Ferritin; Difference - Numerical difference between pre- and post-swim; Δ% - Variation ratio of numerical difference between pre- and post-swim. *$p<0.05$, **$p<0.01$.

BIOCHEMICAL PARAMETERS

Post-swimming, AST and CK activities increased significantly from basal values (32.53%, p=0.014; 94.97%, p=0.010 respectively) (Table 3). Other parameters did not display any significant changes.

OXIDATIVE STRESS INDICES

The results indicated that except TAS (5.79%, p=0.272) and NOx (3.46%, p=0.803), the values of TOS, and OSI levels significantly decreased (35.84%, p=0.01; 38.71%, p=0.010, respectively) (Table 4).

CORRELATIONS

In this study, a significant relationship was found between 5000 m OWS time and ANT (r=-0.843, p=0.001). The findings of the current study showed that there was a significant relationship between pre-swimming NO and CK (r=-0.529, p=0.052), but there was no correlation between NOx and any other parameter (including 5000-m OWS time). Pre- and post-swimming UA and WBC both showed negative correlations (r=-0.620, p=0.05; r=-0.541, p=0.05); AST and CK positive correlations (r=0.553, p=0.05; r=0.600, p=0.05), and ALT and TOS positive correla-

TABLE 3: Pre- and post-swim comparisons of biochemical parameters [mean±SD (median)].

Parameters	Pre-Swim	Post-Swim	Difference	Δ%
GLU (mg/dL)	77.71±5.51	74.63±10.09	↓ 3.08	↓ 3.96
UREA (mg/dL)	23.57±4.41	24.22±5.46	↑ 0.65	↑ 2.75
CR (mg/dL)	0.84±0.09	0.85±0.11	↑ 0.01	↑ 1.19
	(0.84)	(0.82)	(0.02)	(2.38)
UA (mg/dL)	3.17±0.86	3.18±0.85	↑ 0.01	↑ 0.31
AST (U/L)	20.87±3.66	27.66±7.15	↑ 6.79**	↑ 32.53
	(20.15)	(26.61)	(6.46)	(32.05)
ALT (U/L)	13.27±5.1	15.56±5.52	↑ 2.29	↑ 17.25
	(12)	(15.02)	(3.02)	(25.16)
GGT (U/L)	15.5±2.59	15.95±2.88	↑ 0.45	↑ 2.90
CK (U/L)	143.35±52.15	279.49±123.13	↑ 136.14**	↑ 94.97
	(146)	(273.72)	(127.72)	(86.98)
TC (mg/dL)	156.57±25.37	163.67±29.37	↑ 7.1	↑ 4.53
TG (mg/dL)	90.57±34.64	90.74±26.17	↑ 0.17	↑ 0.18
HDL-C (mg/dL)	58.4±6.49	59.44±7.32	↑ 1.04	↑ 1.78
TP (g/dL)	7.41±0.39	7.75±0.43	↑ 0.34	↑ 4.5
ALB (g/dL)	4.32±0.17	4.57±0.32	↑ 0.25	↑ 5.78
	(4.35)	(4.50)	(0.15)	(3.44)
LDL-C (mg/dL)	79.21±21.14	86.02±20.96	↑ 6.81	↑ 8.59
	(75.50)	(80.22)	(4.72)	(6.25)

GLU: Serum Glucose; CR: Creatinine; UA: Uricacid; AST: Aspartate amino transferase; ALT: Alanine amino transferase; TC: Total cholesterol; TG: Triglyceride; HDL-C: High density lipoprotein cholesterol; TP: Total protein; ALB: Albumin; LDL-C: Low density lipoprotein cholesterol; Difference: Numerical difference between pre- and post-swim; Δ%: Variation ratio of numerical difference between pre- and post-swim. *p<0.05; **p<0.01.

TABLE 4: Pre- and post-swim comparisons of oxidative stress indices [mean±SD (median)].

Parameters	Pre-swim mean (median)	Post-swim mean (median)	Difference	Δ %
TOS (μM H2O2 Equiv./L)	22.46±18,67	14.41± 13,19	↓ 8.05**	↓35.84
	(16.62)	(10.89)	(5.73)	(34.47)
TAS (mM Trolox Equiv./L)	2.76±0,93	2.6±0,41	↓ 0.16	↓ 5.79
	(2.51)	(2.50)	(0.01)	(0.39)
NOx (μM)	54.29±18.15	52.41±16.73	↓ 1.88	↓ 3.46
OSI	8.78±7.75	5.39±4.73	↓ 3.39**	↓ 38.61
	(6.58)	(4.38)	(2.2)	(33.43)

TOS: Total oxidant status; TAS: Total antioxidant status; NOx: Nitric oxide as total nitrite; OSI (TOS/TAS ratio): Oxidative stress index; Difference: Numerical difference between pre- and post-swim; Δ%: Variation ratio of numerical difference between pre- and post-swim. *p<0.05, **p<0.01.

tions (r=0.582, p=0.05; r=0.657, p=0.05). A significant relationship was found between post-swimming TAS and FRT levels (r=0.560, p=0.05), and between GGT and GLU levels (r=-0.798, p=0.001).

DISCUSSION

The main findings of this study demonstrated that 5000 m swimming caused a mid-level physiological stress in line with our hypothesis. In contrast to our hypothesis, OSI level was decreased significantly and blood NOx levels were not significantly changed. The expected significant relationships between blood NOx levels and other measured parameters including 5000 m OWS time and OS were not found. But the current study's findings showed that there was the negative relationship between basal serum NOx level and CK activity.

Post-exercise, the physiological stress marker;[26] HR (basal value; first 1000 m swimming, Table 1) as well as the inflammation and muscle damage indicators such as WBC, CK and AST were significant increased from basal values (Pre-Swim, Table 2, 3). The study carried out at similar exercise intensity and distance reported that hematological parameters remained within the reference ranges, expect the WBC, post swimming exercises in male adults.[35] The present study shows that this form of exercise as 5000 m swimming causes a mid-level physiological stress that was below or at ANT. In contrast to our study, serum UREA increased above the reference range following the 120-km open-water marathon swim in adult men.[6] Kormanovski et al. found that WBC levels and CK activity increased after ultra-long swimming in adult open water swimmers.[36] The fact that biochemical parameters including blood UREA levels, which are indicators of protein break-down, GGT levels, which are used as a more specific indicator than ALT for liver damage, CR levels, which are markers for kidney function, and all other biochemical parameters including blood lipids and lipoprotein remained within the normal ranges pre-and post-swimming in the present study, which confirms the mid-level physiological stress. The fact that post-swimming serum GLU values also remained within normal ranges showed that 5000 m swimming does not constitute a risk for hypoglycemia (Tables 1, 3) similar to the study of Haralambie and Senser conducted on young adult swimmers.[35] All these data also show that there is no significant health risk for the above-mentioned parameters in children.

Post-swimming serum TAS values, an indicator of the overall antioxidant capacity of serum, did not present any significant change from basal values, whereas OSI and TOS values decreased significantly. Except for blood FRT, other non-enzymatic endogenous antioxidant parameters such as UA and ALB were not exhibited any significant changes (Tables 2, 3) in the present study.[37] Kormanowski et al. demonstrated that serum thiobarbituric acid–reactive substance (TBARS) levels, similar to TOS in the present study, decreased significantly (48%, p = 0.01) after the first 3 hours in trained male long-distance swimmers during an 8-hour swim.[36] In contrast to the present study, TAS and UREA levels increased significantly (42%, p=0.05). In another study after a 15 km ultra-marathon that lasted 3.4 hours, similar findings of increased WBC levels were found, but TBARS and TAS did not change significantly.[5] In this study, only FRT levels displayed a significant rise post-swimming, and there was a significant relationship between TAS and FRT levels, which might indicate that FRT, which is both an acute phase protein and an antioxidant, may have an antioxidant effect during the exercise.[38] Therefore, this decrease in TOS and OSI post-swimming may have resulted from a rise in the mobilization of other non-enzymatic antioxidants from tissues including FRT. Another study, which has used the same swimming distance demonstrated that serum UA level, one of the non-enzymatic antioxidant marker, significantly increased in young adults.[24] In the present study, although UA didn't show any significant change after the swimming, the negative relationship between UA and WBC may support our opinion mentioned about the decrease in TOS. After the last 1000 m swim, WGLU and La levels significantly decreased from the start of the test (Table 1). These results showed the changes in energy metabolism during the last phase of the 5000 m swim. The decrease of WGLU level in the last phase of swimming may indicate a decrease in muscle glycogen supply and a higher level of lipid metabolism. The changes in energy metabolism may be one of explanation of the decrease in TOS level after the 5000 m swim. For example, during exercise, the increase in lipid metabolism in fat reserves might increase the transportation of non-enzymatic antioxidants that dissolve in lipids from other tissues.[39] We didn't analyzed the free fatty acids in the present study, but a similar study demonstrated that free fatty acids and free glycerol levels, as markers of lipid metabolism increased markedly after average 5200m after 90 minute swimming in young adults.[35]

In the current study, a positive correlation was found between AST and CK, additionally between ALT and TOS values, at pre-and post-swimming measurement time. The inflammation markers can

increase after an intense exercise.[40] Therefore these findings indicate a post-exercise inflammatory response also in the present study.[41] Therefore, it is expected that the rise of post-exercise WBC levels and other inflammation markers including CK will increase the production of free radicals in the blood because leukocytosis may lead to the production of reactive oxygen species after endurance exercise and may cause oxidative damage.[42] However NO is a gas that, depending on the physiological environment, can be both oxidant and antioxidant. In highly oxidative circumstances, it can combine with superoxide to form strong oxidants like peroxynitrite and then the strong oxidant peroxynitrite may reduce increasing of NOx. Such an increase in OS was not observed in the present study, unlike it reduced. This might have been as a resulted of the elevated antioxidant capacity in trained athletes, or the insufficient oxidants production by inflammatory leukocytosis which did not cause serious OS.[17,43] Although trained child swimmers exhibited the increased OS.[9] It has been shown that swimming training also increases the antioxidants in children.[5] However, in the present study no significant relationship between TAS and NOx was observed, also NOx concentration didn't change. The low intensity of exercise and unchanged oxidative stress level may explain the absence NOx. On the other hand, the negative correlation between basal NOx and CK values might indicate that NO have both anti-inflammatory and an antioxidant effect against muscle damage because inflammation and antioxidant events occur generally together. This correlation possibly may suggest an antioxidant capacity resulting from training adaptation, and it also may confirm indirectly the antioxidant properties of NO during swimming.[17]

The differences in OS and muscle damage created by exercises performed on land and in water might stem from the nature of the sport.[44,45] Swimming is considered as a non-muscle-damaging type of exercise (except for intensive swimming exercise such as competition condition) because it involves mainly non-weight-bearing activity and concentric contraction that may therefore cause no or minor muscle damage.[46] The fact that the exercise in this study was shorter in duration and causes mid-level physiological stress in comparison to the other examples may explain the serious level of OS commonly associated with land-based actives which were not conducted in the present study. This data supports the view that a 5000 m swimming distance and a mid-level exercise intensity is insufficient to cause a serious level of OS and/or that any OS created is neutralized by the antioxidant defense mechanism upregulated by swimming training, and possibly mediated by NO.

In this study, contrary to our hypothesis, NOx did not significantly increase after swimming. It has been reported that both acute and regular aerobic exercise in swimmers and sedentary group increase blood NOx levels.[23,47,48] The best-established stimulus of NO production is shear stress, which increases during increased blood flow and can increase NOS activity. Furthermore, it has been reported that all isoforms of NOS can be regulated by transcription with hypoxia.[17] Therefore, the hypoxic nature of swimming, heat, hydrostatic pressure, and physical as well as physiological movement forms may also increase NOx levels.[17] Training stimulates endothelial cells to synthesize NO.[17,22] It has been reported that all isoforms of NOS can be induced by muscle contractions. nNOS expression can increase with severe injury and muscular activity.[17,21] At the cellular level, injured fibers within muscle are thought to induce synthesis of NO by neuronal NOS beneath the sarcolemma and eNOS in muscle smooth.[22] It has been shown that the induction of mechanical damage to gastrocnemius muscle increases NO formation, which is considered to start a signaling process for damage repair.[49] In another study, it has been indicated that the concentration of NO increased throughout about 5 months of training in elite swimmers (mean aged: 21.5 years).[48] Similar variations were also observed in the salivary NO concentration and muscle injury markers such as CK. Salivary NO levels showed a proportional response to oscillations in training intensity and load. There was also a significant increase in WBC, AST, and CK levels post-swimming in the present study, but contrary to the aforementioned study, NOx level did not increase.

The negative correlation was found between basal NOx and CK values in the present study are considered as a result of training adaptations. It is well documented that OS can inhibit NOS activity and decrease blood NO levels.[17,24,25,50] In the current study, it is not possible to claim that the increase of NOx was inhibited by OS during swimming, because in fact it did not changes significantly. The endothelial NO system is up-regulated after short durations training and it may be the mechanism that causes longer term structural changes that ultimately normalize shear stress and it may be return basal NO levels of sedentary individuals. Therefore it may not also seem significant changes after long-term trainings as in this study, because the participants in the present study had 4-5 years of sports experience.[17] Additionaly, there was no significant correlation between NOx and 5000 m OWS performance and ANT. It has been reported that, the basal NOx levels and exercise performance (ANT) was significantly higher in group of football players than the control group.[51] In another study conducted by Jungersten, it was determined that the plasma NO_3 levels of the athletes were significantly greater than the sedentary, and also that there was a positive correlation between VO_2max and basal NOx levels.[19,52] Result of studies indicated that, even though there was no significant difference in terms of basal NO levels between sedentary and exercise groups, there was a significant relationship between aerobic capacities and blood NO levels. These findings may indicate the presence of a vasodilator and metabolic regulator potential that forms through training and can be triggered with exercise, which may have a role of NO in these results.[17]

Shear stress increases NO production during exercise. NO causes the vasodilatation of veins, and increase in blood flow. Thus the increases in blood flow of heart and skeletal muscle, rising oxygen levels, and substrate and regulatory hormone transport may increase aerobic endurance levels.[17] Besides that NO can increase glucose intake independently from insulin and therefore may limit glycolysis.[17] Therefore NO might have a role in the regulation of contractile function. For this reason, it is possible that even though there was no significant rise in NO post-exercise, and that there was no relation with 5000 m performance, NO has important functions in swimming performance. These findings demonstrate that child athletes can deal with 5000 m OWS with a mid-level physiological stress, without a severe oxidative stress and any significant health risk or hypoglycemia within the ranges of the examined biochemical markers. This study is the first study in the literature investigated the role of NO on physiological and oxidative stress induced by OWS as well as performance and health status.

There are a number of limitations in the present study. The low number of participants may have restricted the discussion. The fact of hypothermia that occurs during the long distance swimming and the lack of measurements of core temperature are other limitations of the current study' methodology. Furthermore, the important electrolytes such as sodium and potassium were not analyzed. Further investigations are recommended that are conducted in large number of athletes with absence of the above mentioned limitations.

CONCLUSION

5000 m low intensity OWS created a moderate physiological stress without any significant oxidative stress response, hypoglycemia and health risk for the investigated parameters. The role of anti-inflammatory of NO may be during OWS exercises in the children.

Conflict of Interest

Authors declared no conflict of interest.

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Authorship Contributions

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