Effect of aerobic and anaerobic metabolism on free radical generation swimmers

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ABSTRACT

INAL, M., F. AKYÜZ, A. TURGUT, and W. M. GETSFRID. Effect of aerobic and anaerobic metabolism on free radical generation swimmers. *Med. Sci. Sports Exerc.*, Vol. 33, No. 4, 2001, pp. 564–567. **Purpose:** In this study, changes in antioxidant systems due to free radicals were investigated in short distance (100-m) and long-distance (800-m) swimmers, within whom the anaerobic and aerobic metabolisms dominate, respectively. **Methods:** For this study, swimmers aged between 15and -21 yr swam 800 m ($N = 10$) and 100 m ($N = 9$). Venous blood samples were taken before swimming, and at 1-, 20-, and 40-min intervals after swimming. Lactate, catalase (CAT), glutathione peroxidase (GPx), and reduced glutathione (GSH) levels were determined in the blood samples. **Results:** The increase of lactate levels was statistically significant in the swimmers, both after the 100- and 800-m distances as compared with the preswimming levels ($P < 0.001$, $P < 0.001$). Catalase activity was increased in the first minute postswimming as compared with preswimming levels. Catalase activity then decreased at the 20- and 40-min intervals as compared with the 1-min postswimming interval, at both 100- and 800-m distances ($P < 0.01$, $P < 0.001$). GPx activity was also increased in the first minute after swimming as compared with preswimming levels. GPx activity then decreased at the 20- and 40-min intervals when compared with the 1-min postswimming level. This occurred in both 100- and 800-m swimmers ($P < 0.001$, $P < 0.001$). GSH activity was decreased in the first minute after swimming, compared with the preswimming levels. GSH activity then increased at the 20- and 40-min postswimming intervals, as compared with the first-minute level. Again, this occurred in both the 100- and 800-m swimmers ($P < 0.001$, $P < 0.01$). **Conclusion:** We concluded that both long-distance and particularly short-distance (100-m) swimming increased the activities of antioxidant defense enzymes. **Key Words:** SWIMMERS, SWIMMING EXERCISE, ANTIOXIDANT DEFENSE

Free radicals are essential for many normal biological processes. However, they can become highly destructive to cells and tissues if their production is not tightly controlled (1,10). Oxidative stress, depending on the free radicals, is associated with a disturbance in the pro-oxidant–antioxidant balance in favor of the pro-oxidants (24).

Oxygen utilization may increase 10-fold during endurance exercise in association with an increase in the mitochondrial generation, or the metabolic "leak," of superoxide and hydrogen peroxide (4,26). Therefore, acute and prolonged physical exercise may result in an oxidative stress (21), which could lead to damage caused by free-radical-mediated lipid peroxidation (6,7).

It is now widely accepted that free radical generation is enhanced during strenuous exercise (15). This undoubtedly can cause alterations in cellular antioxidant status, both acutely and chronically, in the form of chorine adaptation. In the last decade, evidence has been accumulated suggesting that antioxidant enzyme adaptation is one of the fundamental changes of skeletal muscle in response to exercise training, much the same as in mitochondrial oxidative enzyme adaptation (15,20).

Skeletal muscle contains several naturally occurring mechanisms for protection against the injury caused by reactive oxygen metabolites. These protective mechanisms include the enzymes superoxide dismutase (SOD), catalase, and glutathione peroxidase. SOD catalyzes the dismutation of superoxide to O_2 and H_2O_2, which CAT then converts to water and O_2. GPx and reduced glutathione can reduce H_2O_2 to form glutathione disulfide (GSSG) and water (17,19). Although these enzymes are activated, or help to reduce oxidative stress reactions like lipid peroxidation during exercise, it is clear that these enzymes are not always adequate in preventing exercise-induced lipid peroxidation. Antioxidants are substances that help to reduce the severity of the oxygen stress either by forming a lesser reactive radical or by quenching the reactive oxygen species. The most well known antioxidants are reduced glutathione, vitamin E, and vitamin C (11,12,25).

The purpose of this investigation is to determine the responses of some antioxidant enzymes (CAT, GPx) and antioxidant substances (GSH) after 100 and 800 m of swimming exercise.

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TABLE 1. 100- and 800-m swimming lactate data (mmol·dL^{-1}).

	100 meter	800 m
Preswimming ± SE	0.74 ± 0.06	0.76 ± 0.04
Postswimming ± SE	4.16 ± 0.28	2.42 ± 0.13
	$t = -12.17$, $P < 0.001$	$t = 5.68$, $P < 0.001$

TABLE 2. 100-m swimming catalase data (U·g^{-1}Hb^{-1}).

Time	Mean ± SE	Compare
Preswimming (*N*=9)	1244.89 ± 34.07	A
Postswimming, 1 min (*N*=9)	1726.00 ± 29.78	D
Postswimming, 20 min (*N*=9)	1493.67 ± 41.21	C
Postswimming, 40 min (*N*=9)	1319.67 ± 22.47	B

$F_{3,24}$ = 117.97, P<0.001.

TABLE 4. 100-m swimming GPx data (U·g^{-1}Hb).

Time	Mean ± SE	Compare
Preswimming (*N*=9)	53.57 ± 1.34	A
Postswimming, 1 min (*N*=9)	67.00 ± 1.61	C
Postswimming, 20 min (*N*=9)	59.42 ± 1.35	B
Postswimming, 40 min (*N*=9)	57.88 ± 1.5	B

$F_{3,24}$=32.72, P<0.01.

MATERIALS AND METHODS

This work was done at swimming dock using volunteer swimmer of the Anadolu University Swimming Club. Informed consent was obtained from all swimmers. Blood samples were collected immediately after 100- and 800-m swimming.

The 100-m swimming was done by five male and four female (total $N = 9$) performance swimmers aged 15–21 yr old. The 800-m swimming was done by six male and four female (total $N = 10$) performance swimmers aged 15–21 yr old. None of the swimmers had any health problems on record and they have been regularly swimming for 5 ± 1 yr.

All swimmers were restricted from using any drugs 15 d before the study. Eating and drinking were not permitted before swimming during the experimental periods. Blood samples were collected by venous puncture from the right arm. Blood samples were taken as follows: 1) blood before swimming, 2) blood just after swimming the respective distances, 3) blood 20 min after the end of swimming, and 4) blood after 40 min after the end of swimming.

Blood was collected into tubes containing oxalate for working with lactic acid and into tubes containing heparin for working with GSH, catalase, and GPx. Blood samples were centrifuged at 400 $g \times 10$ min for lactate measuring (Sigma kit procedure no. 735, St. Louis, MO). All data were calculated as mg·dL^{-1}. GSH levels were measured as described by and according to the method of Beutler (2). Erythrocyte hemolysates were prepared from the blood to measure catalase and GPx activities. Catalase activity was determined using the Beutler method (3), the activity of GPx was determined as with Paglia and Valentina (22). These data were calculated as U·g^{-1} hemoglobin.

The absorbance was determined by using a UV-1201 Shimadzu spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Statistical analyses were done using Student's *t*-test and two-way variance analysis and the Tukey-ω test.

RESULTS

Table 1: Comparison of lactate levels before and after swimming was significantly different in both 100- and 800-m swimmers ($P < 0.001$, $P < 0.001$).

TABLE 3. 800-m swimming catalase data (U·g^{-1} Hb).

Time	Mean ± SE	Compare
Preswimming (*N*=10)	1192.40 ± 26.07	A
Postswimming, 1 min (*N*=10)	1536.50 ± 19.88	C
Postswimming, 20 min (*N*=10)	1356.30 ± 16.78	B
Postswimming, 40 min (*N*=10)	1200.10 ± 18.48	A

$F_{3,27}$=75.35 P<0.001.

Table 2: Comparison of catalase activity in 100-m swimmers before and after swimming revealed postswimming catalase values significantly increased at the 1-, 20-, and 40-min intervals. Catalase activity decreased after the 1-min level but remained significantly high as compared with the preswimming levels ($F_{3.24} = 117{,}97$ $P < 0.001$).

Table 3: Catalase activity increased in the 800-m swimmers at the 1-, 20-, and 40-min intervals and, like the 100-m swimmers, decreased at the 20- and 40-min intervals as compared with the 1-min level($F_{3.27} = 75{,}35$ $P < 0.001$).

Table 4: Although GPx activity increased at the 1-min postswimming interval as compared with the preswimming level, it decreased at the 20- and 40-min intervals as compared with the 1-min level. There was no significant difference in GPx levels, but they remained high as compared with the preswimming levels ($F_{3,24} = 32.72$, $P < 0.01$).

Table 5: GPx activity increased in the 800-m swimmers when comparing pre swimming levels with the 1- and 20-min postswimming intervals. The 20- and 40-min levels progressively decreased compared with the 1-min level, with the 40-min level arriving back to the preswimming level ($F = 94.27$, $P < 0.001$).

Table 6: GSH levels decreased in the 100-m swimmers at the 1-, 20-, and 40-min intervals. The levels increased progressively at the 20- and 40-min intervals, as compared with the 1-min level. Postswimming GSH levels decreased with statistical significance at the 20-min intervals when compared with the preswimming levels ($F_{3,24} = 12.23$ $P < 0.001$).

Table 7: GSH levels also decreased in the 800-m swimmers compared with the preswimming levels. Though the postswimming GSH levels increased at the 20-min interval as compared with the 1-min level, there was no statistical significance. GSH levels at the 40-min interval returned to the preswimming level. The postswimming level at 40 min increased significantly when compared to the 1- and 20-min intervals ($F_{3,27} = 7.29$, $P < 0.01$).

DISCUSSION

It has recently been pointed out that the production of free radicals depends upon the increase of oxygen consumption in the human body, with a clear relationship to exercise.

TABLE 5. 800-m swimming GPx data (U·g^{-1}Hb).

Time	Mean ± SE	Compare
Pre-swimming (*N*=10)	55.88 ± 1.31	A
Postswimming, 1 min (*N* = 10)	65.73 ± 1.23	C
Postswimming, 20 min (*N*=10)	61.11 ± 1.54	B
Postswimming, 40 min (*N*=10)	56.90 ± 1.4	A

$F_{3.27}$ = 94.97, P<0.001.

TABLE 6. 100-m swimming GSH data ($mg \cdot dL^{-1}$).

Time	Mean ± SE	Compare
Preswimming (N=9)	90.60 ± 2.09	D
Postswimming, 1 min (N=9)	78.86 ± 1.56	A
Postswimming, 20 min (N=9)	80.97 ± 1.11	B
Postswimming, 40 min (N=9)	84.53 ± 0.96	C

F=12.23, P<0.001.

TABLE 7. At 800-m swimming GSH data ($mg \cdot dL^{-1}$).

Time	Mean ± SE	Compare
Preswimming (N=10)	90.29 ± 1.23	B
Postswimming, 1 min (N=10)	81.64 ± 2.50	A
Postswimming, 20 min (N=10)	85.98 ± 1.84	A
Postswimming, 40 min (N=10)	89.10 ± 2.69	B

$F_{3,27}$ = 7.29, P<0.01.

Exercise causes more free radical production and increases metabolic processes by increasing the oxygen consumption according to the strenuousness and duration of the exercise (8).

In our study, increases of lactic acid levels were determined in both the 100- and 800-m swimmers as compared with the control group ($P < 0.001$, $P < 0.001$). The level of lactate in the 100-m swimmers was measured at 4.12 $mmol \cdot L^{-1}$, showing that anaerobic conditions were present (Table 1). Levels of lactate higher than 4 $mmol \cdot L^{-1}$ have been accepted as evidence of anaerobic metabolism (27). The lactate levels of the 800-m swimmers (2.42 $mmol \cdot L^{-1}$) showed that aerobic metabolism was occurring.

Catalase and GPx activities in the postswimming data were higher, and they were statistically significant compared with the preswimming levels in the 100-m swimmers within the 1-min interval ($P < 0.001$, $P < 0.01$). It has been suggested by other studies that acute exercise causes increase of reactive oxygen products (12,20). One of these products is superoxide, which is converted to H_2O_2 by superoxide dismutase. H_2O_2 is also transformed into water and oxygen by catalase and GPx. In this investigation, the activities of catalase and glutathione peroxide were increased because of substrate activation dependent upon the increase of the H_2O_2 level.

Postswimming catalase and GPx activities increased significantly when compared with the preswimming levels in the 800-m swimmers, within the 1-min interval ($P < 0.001$, $P < 0.001$). The consumption of oxygen in the 800-m swimmers is provided essentially by an aerobic pathway. Thus, the muscle is under severe oxidative control because of the excessive increase of oxygen consumption during aerobic exercise (20).

As a result of the increase of reactive oxygen products, increases of the activities of catalase and GPx to remove H_2O_2 were observed. Robertson et al. (25) have reported that the activities of catalase and GPx increased after 1 wk of exercise. Erythrocyte GPx activity is dependent on the age of the cell. However, we were unable to demonstrate a relationship between erythrocyte creatine content, a sensitive indicator of cell age, and the extent of physical training (9). The activities catalase and GPx in aerobic cells can be related to the metabolic rate and the production of oxygen radicals (5).

We found that the GSH levels significantly decreased within the first minute after swimming in both the 100- and 800-m swimmers ($P < 0.001$, $P < 0.01$). Superoxide radical is produced and is connected to the conversion of hemoglobin to methemoglobin in the erythrocyte during exercise (24). Superoxide radical is converted into H_2O_2 by SOD. The H_2O_2 formed is transformed to the $HO^{\bullet}$ radical by Fe^{+2} and Cu^{+2} ions, which are transition elements (14). These reactive oxygen species and especially $HO^{\bullet}$ radical convert the polyunsaturated fatty acids into a lipid peroxidation metabolite. These lipid peroxidation metabolites are removed by GSH (13). The decrease of GSH probably confirms the removal of the lipid peroxidation metabolites.

Depletion of GPx and catalase activities but significant elevation of GSH were observed at the 20- and 40-min intervals as compared with 1-min levels after both 100 and 800 m of swimming. Subjects in our study are swimmers who exercise regularly.

It has recently been pointed out that mild and regular exercise can increase the antioxidant capacity (1,23). From this point of view, the cells are protected from the injury caused by free radical production because antioxidant levels increase in those who exercise regularly. This idea is supported by our findings that catalase, GPx, and GSH levels were brought to near preswimming levels within 40 min postswimming in the 800-m swimmers ($P > 0.05$). Catalase, GPx, and GSH levels did not return to the preswimming levels within 40 min in the 100-m swimmers, indicating that free radical production was higher than the antioxidant capacity during anaerobic metabolism in acute exercises. Acute exercise induce free radical production in mitochondria during basal metabolism of aerobic cells (18). The preponderance of available evidence suggests that antioxidant supplementation, particularly with the vitamin C and E (16). Exercise endurance capacity was greatly increased by training (28).

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