

## Oxidant/antioxidant Response of Swimmers During Ultra-long Swimming in Open Waters

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**Abstract: Background:** There is a controversy about the oxidant/antioxidant response in ultra long exercise. **Method:** This study focused on oxidant/antioxidant response in trained swimmers during 8 h of continuous swimming compared with subjects of control group with the same alimentation. Blood parameters were determined in capillary blood samples taken before, every hour during and after swimming. **Result:** During the first 3 h serum levels of TBARS decreased (48%,  $p < 0.01$ ) and total serum antioxidant capacity (AOC) increased (42%,  $p < 0.05$ ), followed by the recuperation of the initial levels of both parameters with high correlation ( $r = -0.95$ ,  $p < 0.05$ ). In women basal levels of TBARS was higher and of AOC lower ( $p < 0.05$ ). Also, in women, during the LDS these parameters reached their extremes and returned to initial levels 2 h before the same occurred in men. **Conclusion:** It seems that exercise induced the mobilization of non-enzymatic antioxidants from their reserves, then depleted the same. The possible mechanisms are discussed.

**Key words:** Oxidative stress, ultra-long exercise, gender differences, swimming, human

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### INTRODUCTION

The generation of Reactive Oxygen Species (ROS) is a normal process that occurs during the oxide-reductions of the respiratory chain as well as in other body functions. In case of prolonged exercise, the increased oxygen consumption generates an increase in ROS, representing a challenge for the limited capacity of antioxidant systems in different tissues (Sjodin *et al.*, 1990; Sastre *et al.*, 1992; Witt *et al.*, 1992; Banerjee *et al.*, 2003). Among the factors in the efficiency of such systems are age, sex, diet and the frequency and type of exercise (Leeuwenburgh *et al.*, 2004; Meydani *et al.*, 1993). Training favors antioxidant efficiency (Shimomura *et al.*, 1991; Senturk *et al.*, 2001).

There is little data about this process in relation to ultra-long (more 4 h) exercise, such as different ultra marathons (Kanter *et al.*, 1986; Rokitzki *et al.*, 1994; Sanchez-Quesada *et al.*, 1995; Mastaloudis *et al.*, 2001; Machefer *et al.*, 2004), ultra triathlon (Ginsburg *et al.*, 1996; Margaritis *et al.*, 1997), cycling (Aguilo *et al.*, 2005) and long-distance walking (Chevion *et al.*, 2003). Knez *et al.* (2006) emphasized the contradictory reports on the oxidant/antioxidant response measured before and after ultra-endurance exercise.

Whereas oxidative stress evaluated by the concentration of products of lipoperoxidation, oxidized glutathione, or carbonyls increased significantly in several studies (Kanter *et al.*, 1986; Aguilo *et al.*, 2005), it remained unchanged or decreased in others (Ginsburg *et al.*, 1996; Margaritis *et al.*, 1997;

Chevion *et al.*, 2003). A significant decrease was observed in non-enzymatic antioxidants as carotenoids (Rokitzki *et al.*, 1994; Machefer *et al.*, 2004; Cases *et al.*, 2006) and retinol (Ginsburg *et al.*, 1996). Data about vitamins C and E are controversial, showing a tendency to a decrease in some studies (Machefer *et al.*, 2004) or an increase in another study (Aguilo *et al.*, 2005). There is little information available on the response of antioxidant enzymes. However, one study reported a decrease in SOD, but no changes in GPx activity (Machefer *et al.*, 2004).

We observed significant changes in metabolic and immunological parameters of trained swimmers in the first 4 h of 8 h ultra-long swim in open waters (Kormanovski *et al.*, 2010). We suppose that this metabolic change during the LDS can affect the oxidant/antioxidant state measured after exercise. It was our objective to determine the changes of the oxidant/antioxidant state every hour during 8 h of an LDS carried out by these trained swimmers.

### MATERIALS AND METHODS

**Swimmers and training data:** The subjects of the present study were eight swimmers (experimental group) in training for Long Distance Swims (LDS) in open waters. These swimmers were in training for the marathon around Manhattan Island as well as the crossing of the English Channel, the San Pedro Channel and other events in open waters. The control group included 8 healthy people with

Table 1: Experimental and control group data

Data	Sex	N	Age (years)	Weight (kg)	Lactate threshold (m sec <sup>-1</sup> )	Velocity (m sec <sup>-1</sup> )
Swimmers	Male	4	31.5±4.4	75.5±3.4	1.15±0.2	0.90±0.08
Swimmers	Female	4	30.1±2.0	65.3±3.3	1.25±0.1	1.04±0.03
Control	Male	4	35.3±4.5	80.5±4.0		
Control	Female	4	28.3±2.1	63.0±4.2		

moderate physical activity. The research was approved by the Institutional Ethics Committee (Superior Medical School, National Polytechnic Institute). Written informed consent was obtained from the participants and throughout the study daily communication was maintained with them.

During 6 months the swimmers were under constant medical supervision by a complete capillary blood analysis and physical examination every 2-3 weeks. All participants were born and live in Mexico City (altitude, 2200 m). The data on the participants is presented in Table 1.

The lactate threshold (4 mmol L<sup>-1</sup> lactate) that reflected the performance of swimmers in open waters was determined before this study by the 5×400 m trial, with gradually increased velocity. All swimmers trained during the 10-15 years prior to this study. The performance level of these swimmers was high, but does not reach the level that determines an elite open water swimmer (lactate threshold 1.5-1.6 m sec<sup>-1</sup>).

The controlled training program for all swimmers consisted of up to 160 km month<sup>-1</sup>, 25% of which was intense swimming (>4 mmol L<sup>-1</sup> of lactate) and 75% of which was less intense. During the 6 months of training, five LDS were carried out by swimmers of the experimental group: one 4 h LDS carried out in a pool (month 1) and 3 h LDS in a salt water lake (water temperature 18-21 °C, 1800 m altitude), one for 6 h (month 1) and two for 8 h (months 3 and 6). Experimental evaluation was carried out in the final LDS, at which time swimmers had adapted to this type of exercise. Additionally, in the fourth and fifth month these athletes swam 20-30 min in very cold open waters (8-10 °C at an altitude of 4000 m) in order to adapt to this water temperature.

The average velocity of swimming during the LDS, measured by the frequency of strokes and distance swum, was steady in the first 4-5 h and after slowly decreased, a change that was not greater than 20% at the end of 8 h.

**Nutrition protocol during the LDS:** During of all LDS every swimmer drank approximately 300-400 mL h<sup>-1</sup> alternating each hour between two different beverages. One was a combined commercial beverage (50 g of proteins and carbohydrates in a proportion of 2:1, respectively in 500 mL) and the other a commercial

electrolyte beverage diluted 1:1 with water (10 g of carbohydrates in 500 mL). On the average 15±3 g h<sup>-1</sup> of carbohydrates, 18±4 g h<sup>-1</sup> of proteins and 400±100 mL h<sup>-1</sup> of liquids were consumed. The intake was moderately low in carbohydrates but sufficient for carrying out LDS without any problems. This addition of proteins did not attenuate the systemic indexes of muscle damage or inflammation that took place when carbohydrates were ingested alone (Betts *et al.*, 2009). Body weight change during the LDS varied from swimmer to swimmer between no change and a moderate increase of approximately 0.5 kg.

In order to evaluate the effects of the LDS nutritional regimen on changes in blood parameters, the persons of the control group followed the same nutritional protocol during 8 h of resting.

**Sampling:** Samples of capillary blood (from a finger) were taken before, during (every hour) and after the last 8 h LDS in the experimental group and every 2 h in subjects of control group during resting with the same nutrition protocol. For nutritional consumption and blood sampling, the swimmers got out of the water for approximately 3-4 min. At this time the principal problem is to avoid hemolysis in samples of capillary blood, above all when the skin becomes dehydrated. A specialist who had taken blood samples in athletes for 20 years solved this problem 0.3-0.5 mL of blood was deposited in an Eppendorf tube that was kept on ice. The serum was separated within 30 min, kept on ice and processed on the same day. The hematological parameters were immediately determined in 0.1 mL of blood with a QBC analyzer (Becton Dickinson).

The blood chemistry parameters (glucose, triglycerides, urea, uric acid, lactate, total proteins, albumin and cholesterol) as well as the Creatine Kinase (CK) and lactate deshydrogenase (LDH) activity (25 °C) were determined using routine reagents (RANDOX). The Coefficient of Variation (CV) for measurement was between 1 and 3%, depending on the parameter. Thiobarbituric acid reactive substances (TBARS) were determined in 0.02 mL of serum and presented in nmol mL<sup>-1</sup>. TBARS production velocity (a parameter contrary to the antioxidant capacity) was determined in 0.010 mL of serum and presented in nmol/mL/min. Total serum antioxidant capacity (AOC) was calculated [100-(TB-PV)/0.5] in relative units (Hicks and Medina-Navarro, 1995).

**Statistical analysis:** The results are presented in absolute values as the Mean±SD. Statistical comparisons of the initial parameter level or gender differences were

performed by a Student paired t-test. Correlation between changes of different parameters during the LDS was analyzed by a Pearson's test using the SPSS statistics program.

**RESULTS**

The changes in the average concentration of total proteins (within 7% of the initial level) and in hematocrit (HCT) (within 2%) confirm that the possible effect of a change in plasmatic volume on the blood parameters is not significant during this type of exercise (data not shown), which coincided with reports by other researchers (Rokitzki *et al.*, 1994; Aguilo *et al.*, 2005). The changes in TBARS and total serum AOC level in swimmers (during the 8 h of swimming) and in control group subjects (during 8 h of resting with the same alimentation of swimmers) are shown in Fig. 1. There was a drastic decrease in TBARS concentration (Fig. 1a) during the first 3 h of the LDS ( $10.0 \pm 3.4$  vs.  $5.2 \pm 3.3$   $\text{nmol mL}^{-1}$ ), a decrease that was significant from the second to the 5th h of swimming.

In the control group there were no significant changes in respect to the initial level. During the entire LDS the TBARS levels in swimmers was significantly

lower than in the control group ( $p = 0.05$ ). The behavior of the calculated AOC (Fig. 1b) had the opposite tendency, reaching its maximum level at the same time that the TBARS concentration was at its minimum level. Throughout the LDS the level of AOC remained higher in the experimental than control group ( $p < 0.05$ ). A negative correlation was observed between the behavior of the TBARS and AOC during the LDS ( $r = 0.95$ ,  $p < 0.05$ ).

The behavior of the TBARS and AOC was different in women than men (Fig. 2a, b). Unexpectedly, the TBARS levels were greater ( $p > 0.05$ ) and AOC lower ( $p = 0.015$ ) in women compared to men. The maximum decrease in TBARS (49%) and the maximum increase in AOC (89%) in women occurred between 2 and 3 h of the LDS, whereas in men this happened between the 4th and 5th h (49 and 24%, respectively). That is, the percentage of decrease in TBARS was similar in men and women, but the increase in AOC was 3.7 times greater in women.

By the end of the swim the TBARS and AOC of women returned to initial levels, whereas in the men this did not occur. The TBARS levels were higher and the AOC levels lower during the entire LDS in women (change that was significant starting in the 4 h).

In women the initial levels of TBARS and AOC in the control group did not differ significantly compared to the

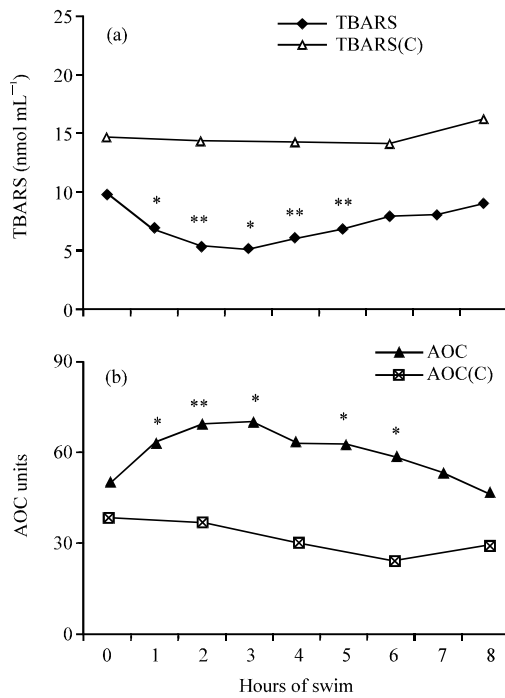


Fig. 1: (a) Serum TBARS and (b) AOC levels during LDS in experimental and control (C) group. \* $p < 0.05$ , \*\* $p < 0.01$  in relation to initial level

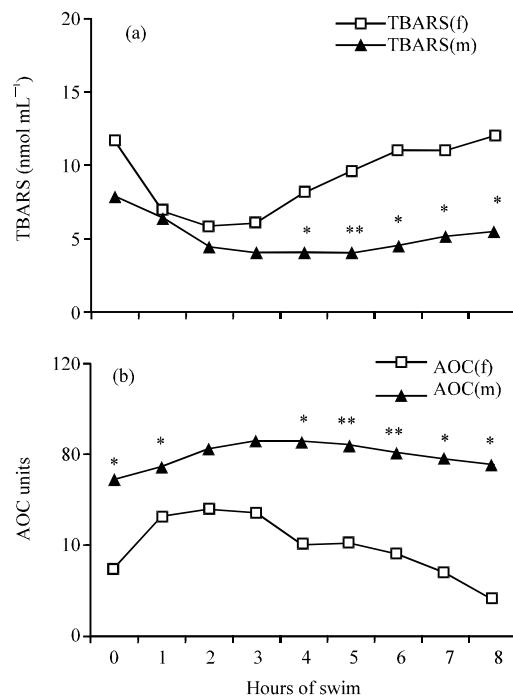


Fig. 2: (a) Gender differences of serum TBARS and (b) AOC levels during swimming. \* $p < 0.05$ , \*\* $p < 0.01$

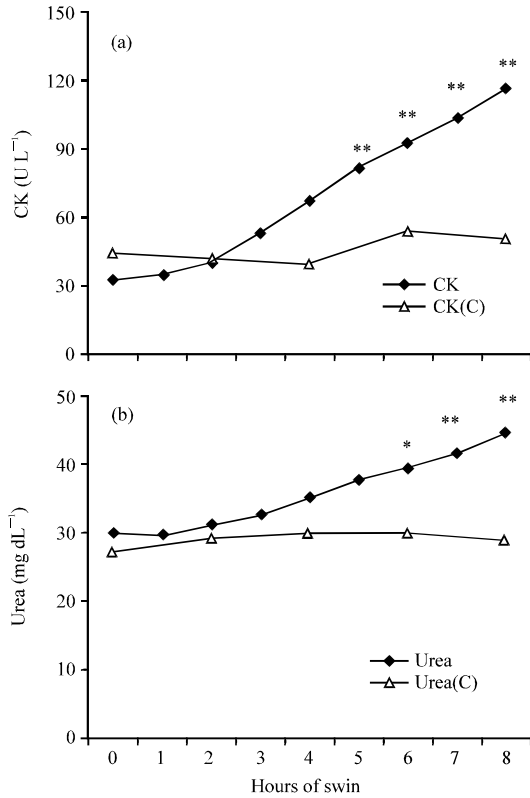


Fig. 3: (a) CK average activity and (b) urea level during LDS in swimmers and control (C) group. \* $p < 0.05$ ; \*\* $p < 0.01$  in relation to initial level

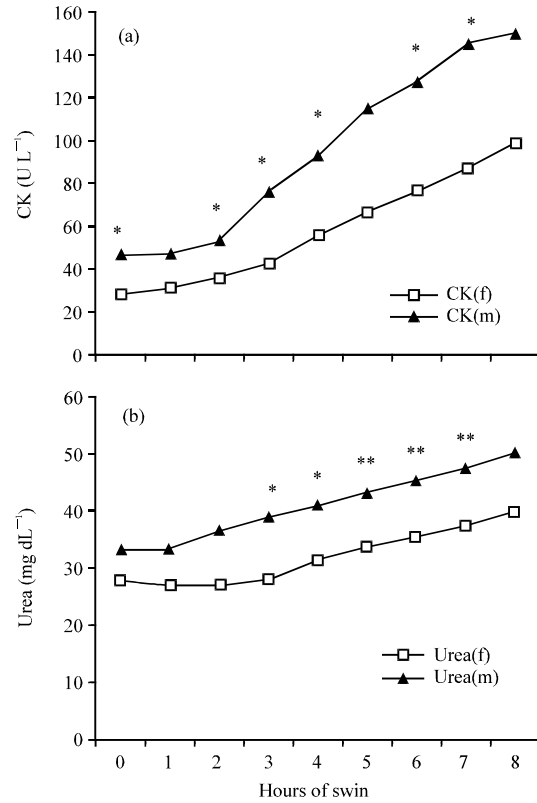


Fig. 4: (a) Gender differences of CK activity and (b) urea levels during swimming. \* $p < 0.05$ ; \*\* $p < 0.01$

experimental group, but in men the TBARS levels were significantly greater ( $17.6$  vs.  $7.9$   $\text{nmol mL}^{-1}$ ,  $p < 0.05$ ) and AOC levels significantly lower ( $42$  vs.  $69$  units,  $p < 0.05$ ) in the control group compared to the experimental group (data not presented). During the moderate swimming exercise in the control group there was no significant change in either parameter in either gender.

Changes in uric acid and other blood components with high antioxidant potential, such as platelets, erythrocytes and albumin were not significant. Albumin remained 5% above red blood cells and below the initial level during the entire LDS (data not presented).

Significant changes were found in the CK activity and urea concentration (Fig. 3a, b). The changes in this parameters are similar ( $r = 0.96$ ;  $p < 0.01$ ), showing a gradual increase after the first 2 h of swimming and reaching  $116$   $\text{uL}^{-1}$  and  $44.6$   $\text{mg dL}^{-1}$ , respectively.

The basal level of CK was lower in women (Fig. 4a) than men ( $p = 0.04$ ), remaining like this during the entire swim ( $p < 0.05$ ). The significantly lower levels of urea ( $p < 0.05$ ) in women were observed starting in the 3rd h of swimming (Fig. 4b).

LDH activity increased moderately ( $p < 0.05$ ) only in the 4th h and remained at this level up to the end of the swim (data not presented). This marker of muscle damage is not specific to muscle tissue, as its most important part comes from erythrocytes.

The average quantity of granulocytes over time had an S-form, reaching its maximum level ( $14000$   $\mu\text{L}^{-1}$ , or 280% of the initial level) in the 7th h (Fig. 5a). The agranulocytes (lymphocytes+monocytes) had their peak (Fig. 5b) in the 5th h ( $2990$   $\mu\text{L}^{-1}$ , or 126% of the initial level).

Only the agranulocyte response showed a gender difference (Fig. 5c): men reached the first peak in the first 2 h of swimming ( $p > 0.05$ ), whereas women had their minimum level during this same time period. The gender difference in relation to this parameter is significant for the first and 2nd h of the LDS. No significant changes or gender differences were found in relation to WBC in the control group.

The average concentration of glucose, triglycerides and lactate decreased approximately 20% ( $p = 0.082$ ,  $0.093$  and  $0.054$ , respectively) by the end of the LDS, without

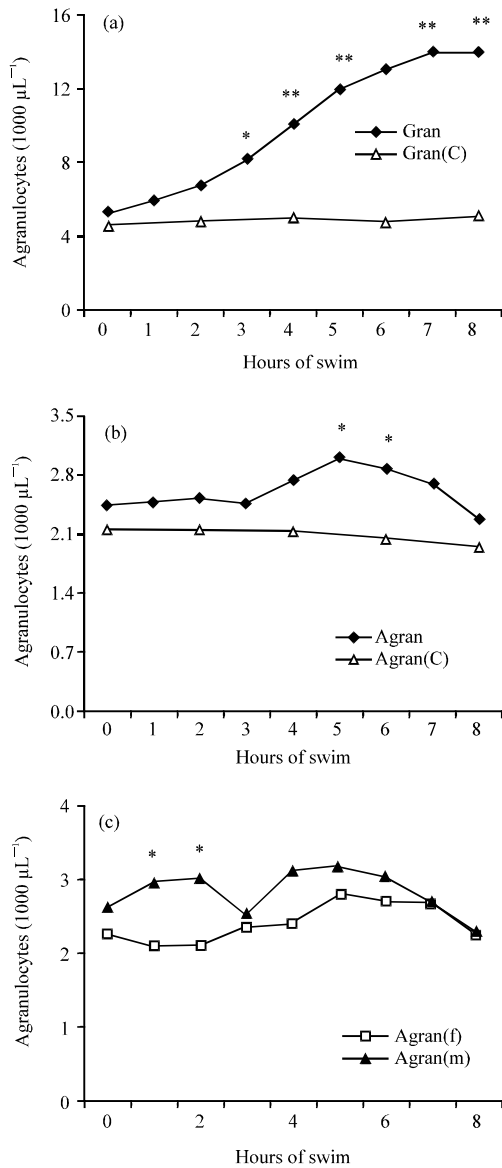


Fig. 5: (a) Granulocyte and (b) agranulocyte response and (c) gender difference in the agranulocyte response during swimming. \* $p < 0.05$ ; \*\* $p < 0.01$  in relation to (a, b) initial levels and (c) gender difference

any significant difference in respect to the control group or gender (not presented). Changes in cholesterol were not significant in either group.

### DISCUSSION

The majority of specialists attribute the changes in TBARS concentration in blood to their total antioxidant capacity (Ji, 1999), which includes the enzymatic part

(principally in erythrocytes and platelets) and the non-enzymatic part (vitamins, minerals, amino acids, albumin, thiols, etc.). Accordingly, in this study we found a high negative correlation between TBARS and AOC serum levels during exercise. AOC was measured in serum and determined principally by its non-enzymatic antioxidant capacity.

The increase of total serum non-enzymatic AOC during the first 3 h of the LDS may have been induced by the mobilization of non-enzymatic antioxidants from other tissues or from consumption during LDS. The latter reason is unlikely, as no significant difference was observed in the control group during resting with the same diet.

During the 1st h of continuous exercise, there is a decrease in muscular glycogen and an increase in the participation of lipids as a source of energy (Phillips *et al.*, 1996; Hawley *et al.*, 1998). This implies an increase in the capacity to mobilize lipids from their reserves and transport them to the muscle tissue, enabling the transport of antioxidant non-enzymatic liposoluble molecules from other tissues. The increase in total serum AOC in the first 4 h of swimming in the present study coincide with data from other studies where an increase in vitamin C and E was observed after 4.5 h of cycling (Aguilo *et al.*, 2005) and after 3-4 h of a marathon (Rokitzki *et al.*, 1994). On the other hand there was significant decrease in carotenoids reported by the majority of studies after 4-5 h of exercise (Rokitzki *et al.*, 1994; Machefer *et al.*, 2004; Cases *et al.*, 2006), as occurred with retinol (Ginsburg *et al.*, 1996) and GSH (Sastre *et al.*, 1992; Aguilo *et al.*, 2005).

The increase in the serum concentration of TBARS after the 3rd h and decrease in total serum AOC may be determined by the depletion of non-enzymatic antioxidants during the second part of swimming and/or the increase in the rate of ROS production. The high negative correlation between TBARS and AOC found in this study confirms the first supposition.

The intensity of exercise and consequently of  $\text{VO}_2$  in this study remained the same or even decreased in the second half of the LDS. Therefore, it should not have influenced the higher production of ROS during this period of the LDS. Such increase can possibly be explained by muscular inflammation and/or the neutrophils activated by exercise.

Muscle inflammation could have increased the production of free radicals in the blood (Slade *et al.*, 1993; Bonsignore *et al.*, 2003; Neubauer *et al.*, 2008) and the WBC, CK and LDH response during the LDS found in this study, a possibility that is consistent with the evidence after the 4th h of swimming.

Another possible factor in the increase in ROS production in the second half of the LDS is the elevated granulocyte levels found during the same period. This parameter indicates increased levels of neutrophils, which are known to generate more ROS (Babior, 1978) and to be activated during long duration exercise (Smith, 1996). In another study, an increase in ROS production was found after an ultra-marathon (Sato *et al.*, 1996) and marathon (Hessel *et al.*, 2000). Pearson's statistical analysis shows that the concentration of total WBC and granulocytes from the 3rd h on correlates positively ( $r = 0.82$  and  $0.82$ ,  $p < 0.05$ , respectively) with TBARS levels and negatively ( $r = -0.88$ ;  $-0.89$ ;  $p < 0.01$ , respectively) with AOC levels, in accordance with the supposition. However, in a recent study of the oxidant/antioxidant state in leukocytes of cyclists after 4.5 h of cycling, there were no significant changes observed (Cases *et al.*, 2006), which contradicts this supposition.

**Gender differences:** In spite of the low quantity of men and women in the current contribution (4 persons), statistical analysis gives certain value to the gender differences observed. The majority of Guinness records in long duration swimming or in cold waters (24 h in river or pool, triple crossings of the English Channel, ultra-long swims in the sea, crossing to the Bering Straits, etc.) were done by women, indicating a greater physical as well as mental capacity of the organism to handle extremely long-duration exercise.

Women oxidize proportionally more lipids than CHO and proteins compared to men, in both training periods and continuous exercise of moderate intensity (Friedlander *et al.*, 1998; Carter *et al.*, 2001). Muscular damage after eccentric exercise is less in women (Kerksick *et al.*, 2008). It is supposed that the mechanism of this difference is in the hormonal response and particularly of catecholamines (Horton *et al.*, 1998), which is lower in women. The possible antioxidant effect of estrogens in women is also worth mentioning. A recent study showed that during exercise a lower level of the product of peroxidation (8-isoprostan) and a higher level of SOD was found in women after eccentric exercise (Kerksick *et al.*, 2008). Gender differences in the metabolic response in the present study coincide with these data. During the entire LDS, women showed a lower index of muscular damage (CK), a lower level of urea and a lower lymphocyte response, with a tendency to a decrease in glucose, lactate and triglycerides.

Surprisingly, in the women swimmers of the present study the level of oxidative stress measured by the TBARS levels was greater and total serum AOC (principally non-enzymatic) was lower than in men during

the entire LDS. We suppose that the greater metabolic activity of lipids in women is accompanied by greater and earlier mobilization of unsaturated fatty acids (the substrate for lipoperoxidation) and consequently a greater decrease of non-enzymatic AOC in the blood. The initial levels of both these parameters in control group women did not differ from the same in experimental group women. In each case, there were no significant changes between the two groups in relation to these parameters. On the other hand, the initial TBARS levels was significantly greater and those of AOC significantly lower in experimental group men than control group men, confirming that training affects the oxidant/antioxidant state in men to a greater extent than in women.

## CONCLUSIONS

The decrease in the TBARS levels in the first half of the LDS correlates with an increase in total serum AOC, which most likely reflects the mobilization of non-enzymatic antioxidants from other tissues during this period.

Contrary changes in the second part of the LDS probably were determined by the decrease of AOC, as well as muscular inflammation during this period.

There is a significant gender difference in the oxidant/antioxidant response during swimming, probably related to peculiarities in energetic metabolism and its regulation during exercise in men and women.

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