Full Length Research Paper

Oxidant/antioxidant response during fasting and exhaustive swimming in the kidney of trained mice

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There are no reports on the oxidant/antioxidant response in the kidney of trained mice, during the process of short-term fast or exhaustive exercise. Trained mice (TR) and untrained (NO) but only adapted to swimming were submitted to fasting or exhaustive swimming for 2, 4 and 6 h, and observed at 4 and 24 h of recovery. Measurements were made of the concentration of Thiobarbituric acid reactive substance (TBARS), total antioxidant capacity (TAC) and enzyme activity (glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT)), and were compared between the two groups. During fasting there was a sharp increase in all enzymes in the NO group and stability of all parameters in the TR group. Contrarily, during recovery from the fast, an increase was found in all parameters only in the TR group, and this was at the fourth hour. During exhaustive swimming (subtracting the effect of fasting), there was stability in TBARS and enzymes in the TR group, and a sharp decrease in SOD and CAT in the NO group. In both groups, a similar increase in the TAC was observed, reaching its maximum level at 2 h of swimming, followed by a return to basal levels. There are indices of non-enzymatic antioxidant components mobilization during exercise in both groups. The significant change in the antioxidant state during fasting as well as exhaustive exercise in the NO group probably represents a response to greater ROS production.

Key words: Training, oxidative stress, antioxidant enzymes, exhaustive exercise.

INTRODUCTION

There is little data about of the oxidant/antioxidant state in the kidney of rodents during the process of exhaustive exercise. In the majority of existing studies, measurements have only been made before and after the exhaustive exercise. After this type of exercise, blood

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Abbreviations: **TBARS,** Thiobarbituric acid reactive substance; **TAC,** total antioxidant capacity; **GPx,** total glutathione peroxidase; **SOD,** total superoxide dismutase; **CAT,** catalase.

flow and glomerular filtration diminish in the kidney (Upston et al., 1999; Guaquil et al., 2004), a process in which oxidative stress could have an important role. In a review article about the effect of acute exercise on the oxidant/antioxidant state in the rodent kidney (Fu and Liu, 2006), it was stated that the majority of studies show development of oxidative stress and an increase in the activity of antioxidant enzymes such as SOD. In a recent study (Kocer et al., 2008), a significant increase was observed in the level of TBARS and carbonyls in the kidney of untrained rats subjected to an exhaustive race. The authors attribute this increase to an augmented production of ROS, evidenced by the elevated activity of NADPH oxidase found in the mitochondria. Similar

results were reported in other studies (Liu et al., 2000; Radák et al., 1996; Huang et al., 2009) with untrained rats subjected to the same exercise.

It has been found that exhaustive swimming causes the development of oxidative stress in rodents (Alessio, 1993; Venditti et al., 1996). Other studies have confirmed these findings: a significant increase in rat kidney of the level of TBARS after seven sessions of forced swimming (Leeuweburgh and Li, 1998); a significant increase in CAT after exhaustive swimming (Terblanche, 2000); an increase in the level of oxidative stress and a decrease in the levels of glutathione (GSH), GPx and GR in the response to exhaustive swimming that was slowed by caloric restriction (Aydin et al., 2007); and a drastic decrease in GSH and an increase in GPx and SOD activity in the kidney of mice after non-forced exhaustive swimming lasting 4 and 5 h (Leeuweburgh and Li, 1995; Nayanatara et al., 2005). On the other hand, training of rats increases the efficiency of the antioxidant defense and diminishes the production of ROS in the mitochondria (Kocer et al., 2008). In a recent study (Coelho et al., 2010), it was shown that in rats trained for racing during 8 weeks, physical training before induction of a renal lesion reduced oxidative damage parameters and oxidant production, without altering renal function and the antioxidant defense system.

The rodents subjected to exhaustive swimming do not eat during the process of exercise. Prolonged fasting (greater than 12 h) affects the antioxidant capacity in the liver and intestine, particularly by diminishing the non-enzymatic antioxidant activity, such as that of GSH (Di et al., 1997). We found no data on the response in mouse kidney to fasts lasting less than 12 h. In order to take the factor of fasting into account during prolonged exercise, the present study focuses on measuring the oxidant/antioxidant response in mouse kidney during the process of 6 h of fasting as well as during 6 h of swimming of trained and no trained mice.

MATERIALS AND METHODS

Experimental design

Male Balb/C mice of two month age were kept (two per cage) in a room with a light/dark cycle (12:12 h, 8.00 to 20.00) and a temperature of 20 to 22°C. They were given standard food and water *ad libitum*. After a week of conditioning, the animals were randomly placed in two groups: control and experimental.

The experimental group (TR) was trained by non-forced swimming during 12 weeks (1 h of continuous swimming, 3 times per week). The control group (NO) was not trained, but during the last two weeks of the study was adapted to swimming. On the first day of the adaptation period, the mice were left in the water for 10 min, a time that was increased every second day until reaching 1 h at the end of the second week. The tub of water (32 ± 2°C) was made of transparent plastic, with separate areas (25 × 25 × 25 cm) for each mouse. The mice were given this adaptation period in order to reduce stress of an unknown stimulus, and to be able to

reach the maximum duration of swimming (6 h), very close to the exhaustive level.

At the end of the study, two basal subgroups were formed from both the experimental and control groups. The rest of the animals of every group were divided into two subgroups according to the treatment: fasting or swimming. Each of these subgroups was further divided into 5 subgroups according to the hours of the respective treatment (2, 4 or 6 h) and recovery (4 and 24 h). For each of the twenty two subgroups n = 6. All mice were sacrificed immediately after their swim or fasting session (including basal subgroup) by ether anesthesia. This study was approved by the Ethical Committee of the High Medical School of National Polytechnic Institute.

Sampling and proceedings

Samples of kidney were frozen with dry ice immediately after being obtained. Tissues were homogenized in 10 mmol/L phosphate buffer with 0.1% of Triton (1:10). After centrifuging (2500 r/min for 30 min), the supernatant was placed in Eppendorf tubes at -70°C until further processing. From these tissue samples, a determination was made of the concentration of total proteins, thyobarbituric acid reactive substances (TBARS, nmol/mg of total protein) (Hicks and Medina-Navarro, 1995), the total antioxidant capacity (TAC, nmol/mg of total protein), and the total activity (sum of all isoforms) of two antioxidant enzymes glutathione peroxidase (GPx, U/mg of total protein, dilution of homogenate 20) and superoxide dismutase (SOD, U/mg of total protein, dilution of homogenate 30) with RANDOX procedure and catalase (CAT; U/mg of total protein, dilution of homogenate 400) with the CYMANCHEMISTRY procedure.

The TAC was determined as the antioxidant capacity of a synthetic antioxidant (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), and the dilution of the homogenate was 40. The production of stable radicals involved ABTS[R] (3-ethylbenzthiazoline sulphonate), peroxidase (metmyoglobin) and peroxide to produce the radical cation ABTS[R+]. This has a relatively stable blue-green color, which is measured at 600 nm (RANDOX).

Statistical analysis

Results were expressed as means ± SD. Differences between groups were analyzed with ANOVA one way, using the SPSS statistical program. A P value of <0.05 was taken as evidence of significance.

RESULTS

Our interpretation of the oxidant/antioxidant response is based on the idea that the total antioxidant capacity (TAC) is the sum of the enzymatic antioxidant capacity and the non-enzymatic antioxidant capacity, which are closely related. Although this method of interpretation is debatable, it has three foundations: 1) the hypothesis of the close correlation between the parameters of oxidative stress and the response of endogenous antioxidants mentioned in various studies (Liu et al., 2000; Ji, 1993; Ji, 1996); 2) the supposition that stress provoked by fasting or exhaustive swimming strengthens the relation between these two parameters; and 3) the fact that the kidney has

Table 1. Basal levels of the parameters of the oxidant/antioxidant state in the kidney of untrained (NO) and trained (TR) mice. Mean ± SD, * - p < 0.05 between groups.

Group	TBARS (nmol/mg prot.)	TAC (nmol/mg prot.)	GPx (U/mg prot.)	SOD (U/mg prot.)	CAT (U/mg prot.)
NO	3.0 ± 0.32	812 ± 53	1.66 ± 0.48	5.4 ± 2.11	697 ± 189
TR	2.45 ± 0.72	982 ± 112*	1.81 ± 0.32	2.8 ± 2.15	414 ± 177

TBARS, thiobarbituric acid reactive substances; TAC, total antioxidant capacity; GPx, total glutathione peroxidase; SOD, total superoxide dismutase; CAT, catalase.

low activity during exhaustive exercise, and has diminished blood flow (Upston et al., 1999; Guaquil et al., 2004).

The basal levels of the oxidant/antioxidant state in both groups in kidney of mice are shown in Table 1. Compared to the NO group, the TR group had a significantly greater basal level of TAC and a tendency to a lower basal level of SOD and CAT. Supposing the aforementioned, that TAC is the sum of enzymatic and non-enzymatic antioxidant capacity, it is possible that training chiefly increased the basal level of the non-enzymatic component of TAC.

To evaluate the effects of exhaustive swimming (6 h), the results of both groups were compared with results from fasted only groups, as fasting also provokes oxidative stress. Since in the literature we only found corresponding data about fasts that lasted longer than 12 h, we investigated the effects of 6 h of fasting.

No significant difference was observed in the TBARS of either fasted group (Figure 1A). At the fourth hour of recovery, a significant increase was found in this parameter only in the TR group. The level of TAC in the kidney showed no significant change during fasting in the TR group, but compared to the basal level this parameter showed a significant increase in the fourth hour of recovery with significant difference between groups in this period (Figure 1B). The principal antioxidant enzymes showed stability in the kidney during the fasting period of the TR group. The increase in the TAC in the fourth hour of recovery of this group coincides with the rise in the levels of GPx and SOD, as well as with the increase in oxidative stress evidenced by the TBARS.

Surprisingly, in the NO group, a sharp increase was found in all antioxidant enzymes during fasting, reaching a maximum level between the second and fourth hour (Figure 1C, D, E), followed by a return to the basal level. This increase coincided with the stability of the TAC in this period, which leads to the conclusion that at this same time the levels of non-enzymatic antioxidant enzymes must have been declining. On the other hand, during the recovery period, the stability in TAC in the NO group coincides with an equivalent stability in the levels of antioxidant enzymes, leading to the supposition of a corresponding stability in the non-enzymatic antioxidant response during 24 h of recuperation.

Response to exhaustive swimming

We subtracted the level of each parameter during fasting from the same values during exhaustive exercise in order to eliminate the influence of this variable. Since the basal group is the same, whether for the trained or untrained mice, for both the swimming and fasting groups, the graphs begin from "zero". The only significant difference in the levels of TBARS between groups (Figure 2A) was found at 24 h of recovery, at which time there was a greater level in the TR group compared both to the NO group and the basal value (p < 0.05). The response of TAC was similar in both the TR and NO groups during swimming, with the maximum level at 2 h (Figure 2B) followed by the return to the basal value. At the fourth hour of recovery, a significantly greater level of TAC was observed in the NO compared to the TR group.

During swimming the activity of SOD and CAT diminished sharply in the NO group (Figure 2 C, D, E), while remaining unchanged in the TR group. At the 4 h of recovery, all levels of enzymes in both groups were similar to the basal levels, except for the increase in GPx in the NO group (Figure 2C). At 24 h of recovery, there was a tendency to an increase in all the enzymes in the TR group, while no change existed in the same parameters of the NO group.

DISCUSSION

Prolonged fasting (>12 h) decreased GSH level in liver and intestine of rodents (Di et al., 1997; Strubelt et al., 1981; Martensson,1986; Cho et al., 1981). In the case of the liver, this diminishment is most likely related to the efflux of GSH from the liver towards other tissues (Lauterburg et al., 1984; Lew et al., 1985). GSH is considered the principal non-enzymatic mitochondrial antioxidant, and its depletion markedly enhances sensitivity to mitochondrial dysfunction caused by oxidative stress, inducing degeneration of the mitochondrial structure (Fernandez-Checa et al., 1998; Meredith and Reed, 1982). In one study, no difference was observed in the levels of GSH, GPx or SOD in the kidney of mice (Nayanatara et al., 2005) between the control group with *ad libitum* feeding and that with 24 h of
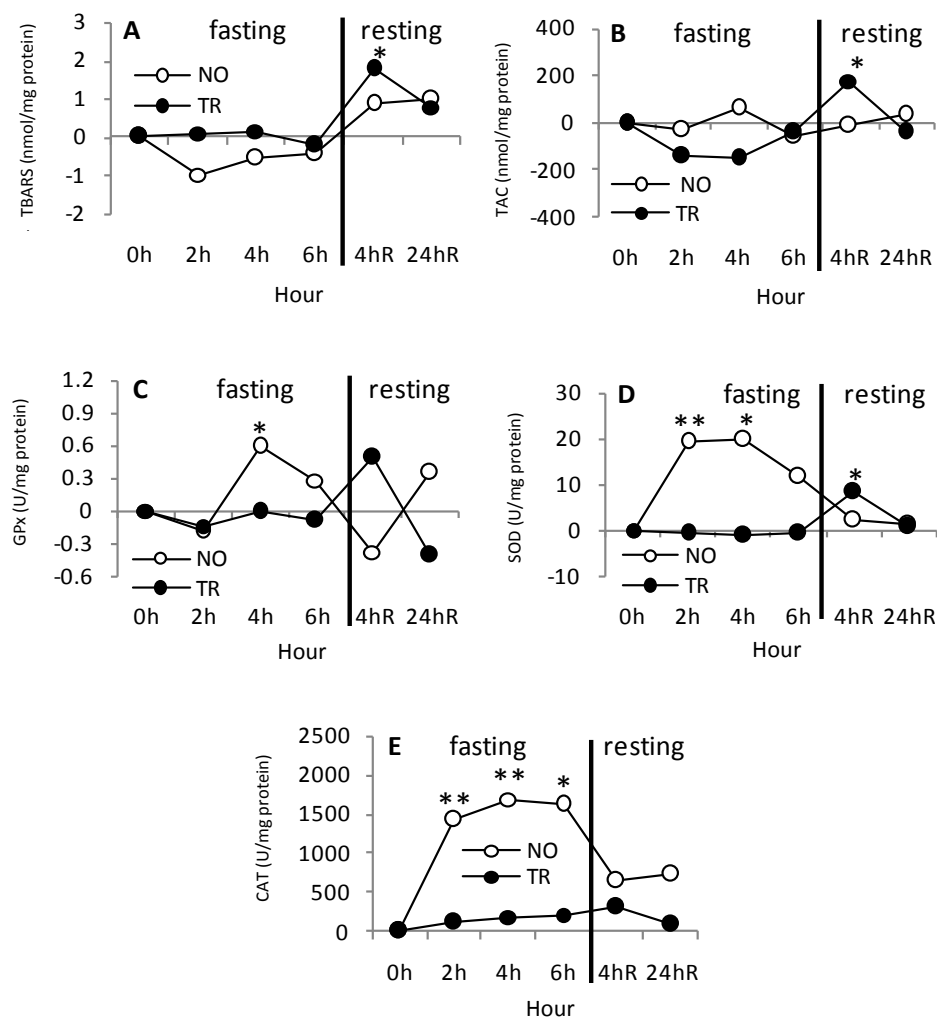
Figure 1. The response (subtracting the initial level) of the concentration of TBARS (A),TAC (B), total GPx (C), total SOD (D), and CAT (E) during fasting and recovery in the kidney of trained (TR) and untrained (NO) mice. * - p<0.05, ** - p < 0.01compared to the basal level.

fasting. We found no data on the response in mouse kidney to fasts lasting less than 12 h.

In the present study, we found a difference between the trained and untrained group in their response to 6 h of fasting. The untrained (but adapted to swimming) mice showed significant changes in the kidney in the majority of the parameters, being particularly notable the sharp increase in antioxidant enzymes. On the contrary, in the period of recovery, significant changes were mainly observed in the trained mice, which showed elevated levels of all parameters at the fourth hour, followed by a return to the basal level at 24 h.

Stability of all parameters, including those of oxidative stress, during fasting in the trained mice could mean that the antioxidant defense completely neutralizes the effects of stress caused by the fast, without any signs of

depletion in either the enzymatic or non-enzymatic elements of TAC. There are two principal factors that affect the oxidant/antioxidant state: the velocity of ROS production and the efficiency of the antioxidant system. If we suppose that the response to fasting of the NO group results in a greater production of ROS, then a greater activity of antioxidant enzymes would be necessary in order to maintain a low level of oxidative stress.

If our interpretation of the behavior of TAC is correct, there are two main effects during 6 h of fasting: 1) stability of all parameters in the trained group, and 2) a depletion of non-enzymatic antioxidant capacity in the untrained group, accompanied by a sharp increase in enzymatic antioxidant activity, followed by the decrease in the activity of these enzymes and an increase in non-enzymatic component of antioxidant defense as of the
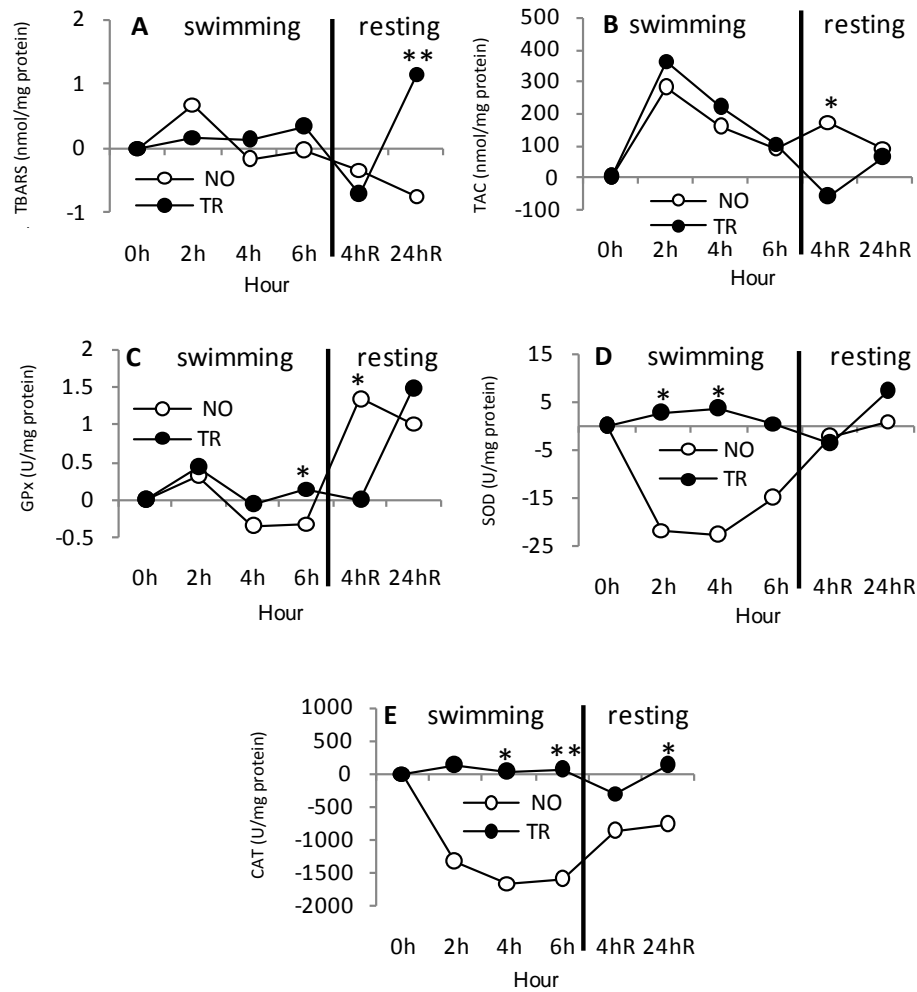
Figure 2. The response (subtracting the level of parameters during fasting) of the concentration of TBARS (A), TAC (B), total GPx (C), total SOD (D), and CAT (E) to swimming and recovery in the kidney trained (TR) and untrained (NO) mice. * - $p<0.05$, ** - $p<0.01$ for differences between groups.

sixth hour; 3) during the recuperation period, the response of all parameters in TR group is significant and quick.

In a recent study (Kocer et al., 2008), a significant increase was observed in the level of TBARS, carbonyls and proteinuria in the kidney of rats after an exhaustive race, which the authors attribute to the increased production of ROS, evidenced by the elevated activity of NADPH oxidase in mitochondria. In another study, training did not cause significant changes in the basal levels of malondialdehyde (MDA), carbonyls and glutamine synthetase activity in kidney. However, a significant decrease was found in the concentration of vitamin E and GSH in response to an exhaustive race in rats untrained but adapted to exercise (Liu et al., 2000). In the kidney of untrained rats, when derivatives of SOD

were administered before exercise, there was a decrease in oxidative stress (Radák et al., 1996), evidenced by the decrease in MDA level and the activity of xantin oxidase and myeloperoxidase. Contrarily, when arginine was administered before exercise, there was an increase in MDA and a decrease in the activity of both enzymes.

In two studies with a similar protocol of exhaustive swimming of untrained mice (Leeuweburgh and Li, 1995; Nayanatara et al., 2005), a significant decrease was found in oxidative stress in kidney, evidenced by a decrease in GSH and TGSH, as well as an increase in the activity of GPx and SOD. These effects depended on the duration of swimming. Nevertheless, we cannot use these studies for comparison, as in the current contribution: 1) untrained mice were adapted to swimming; 2) all animals swam for 6 h; 3) the response

was measured throughout the period of exhaustive swimming; and 4) a significant effect of fasting (for 6 h) was found to exist, and this effect was subtracted from the response to exhaustive swimming.

The stability of TBARS was observed in the present study in both trained and untrained mice during exhaustive swimming. However, a sharp increase in oxidative stress was found in the kidney of trained mice between the 4th and 24th hours of recovery, which requires an explanation. It was notable that the trained mice quickly set about looking for food and other activities after swimming, while the untrained mice were trembling more time and generally began their activity later. This is a probable explanation for the increase in oxidative stress in the trained mice during the recovery period.

According to our interpretation, the increase in TAC in both groups at the second hour of swimming, under conditions of either stability (TR group) or a sharp decrease (NO group) in enzymatic activity, shows that there must have been an increase in the non-enzymatic antioxidant capacity, followed by a depletion of the same. There are indices of mobilization of non-enzymatic components of antioxidant defense during exercise in both groups. This data coincided with reports that confirm that the liver can export non-enzymatic antioxidants, such as GSH, towards other tissues in response to the increase in glucagon or catecholamine (Lu et al., 1990; Ji et al., 1993). If our interpretation of TAC in the trained group is correct, the antioxidant defense is sufficient to not require the antioxidant enzymes depletion. On the other hand, in the untrained group, there was a notable depletion of both non-enzymatic and enzymatic antioxidant capacity.

If we suppose that the trained group produces a lower amount of ROS during exhaustive exercise, the explanation for the stability of oxidative stress and all the enzymes in this group is quite simple. Likewise, in the untrained group, it is easy to account for the sharp depletion found in the activity of antioxidant enzymes, if this is seen as a result of the greater velocity of ROS production. This conclusion coincides with data from other studies (Kocer et al., 2008; Liu et al., 2000).

As we have seen during fasting or exhaustive swimming, significant changes in the majority of parameters occur, and are later reversed and approach basal levels by the sixth hour. That is, when the response is measured at the end of exhaustive exercise, the lack of change masks the actual changes that take place during the entire process of exercise.

In conclusion, training of mice in swimming increased the basal level of the total antioxidant capacity and allowed the majority of parameters of the oxidant/antioxidant state to remain stable, both during fasting and exhaustive exercise. It seems that the difference between the response of the trained   and

untrained group is based on the lower ROS production in the former. There were indices of mobilization of non-enzymatic components of antioxidant defense during exercise in both groups.

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