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ORIGINAL ARTICLE

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Effects of sprint exercise on oxidative stress in skeletal muscle and liver

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Abstract Although numerous studies have tested the effects of continuous exercise regimens on antioxidant defences, information on the effect of sprint exercise on the antioxidant defence system and lipid peroxidation levels of tissues is scant. The present study was designed to determine the effects of sprint exercise on the lipid peroxidation and antioxidant enzyme system in liver and skeletal muscle during the post-exercise recovery period in untrained mice. Mice performed 15 bouts of exercise, each comprising running on a treadmill for 30 s at 35 m·min^{-1} and a 5° slope, with a 10-s rest interval between bouts. They were then killed by cervical dislocation either immediately (0 h), 0.5 h, 3 h or 24 h after completion of the exercise. Their gastrocnemius muscle and liver tissues were quickly removed. It was found that blood lactate levels increased immediately after the exercise, but had returned to control levels by 0.5 h post-exercise. This exercise regimen had no effect on the activity of superoxide dismutase and glutathione peroxidase in these tissues. Levels of muscle thiobarbituric acid reactive substances (TBARS) had increased at 0.5 and 3 h post-exercise, and then returned to control levels by 24 h post-exercise. In conclusion, acute sprint exercise in mice resulted in an increase in TBARS levels in skeletal muscle; no change was observed in the liver. Antioxidant enzyme activities remained unaffected by acute sprint exercise in these tissues.

Keywords Exercise · Lipid peroxidation · Superoxide dismutase · Glutathione peroxidase · Blood lactate

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Introduction

Many studies have reported that physical exercise increases the production of reactive oxygen species (ROS), thereby inducing oxidative stress (Alessio et al. 1988; Child et al. 1998; Sen 1995). The majority of these studies utilised aerobic exercise as the fundamental cause of elevated levels of ROS. It has been suggested that elevated oxygen consumption generates high concentrations of ROS. ROS cause lipid peroxidation of polyunsaturated fatty acids in biological membranes and blood, inducing alterations of cell function (Duthie et al. 1990).

In the resting state the body is equipped with both non-enzymatic and enzymatic antioxidant reserves to prevent the potentially harmful effects of ROS. The fine physiological balance between oxidative reactions and antioxidant capacity may be perturbed by physical activity. Antioxidant defence systems preserve homeostasis for normal cell function at rest, and perhaps during mild oxidative stress. The primary components of the physiological antioxidant defence system are superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx). SOD catalyses the dismutation of superoxide to O_2 and H_2O_2, which catalase converts to water and O_2. GPx can reduce H_2O_2 to form glutathione disulphide and water (Benzi 1993).

Whereas a large number of studies have tested the effect of continuous treadmill running on the antioxidant defence system and lipid peroxidation, information on the effects of intermittent sprint exercise on the antioxidant defence system and lipid peroxidation is scant. Marzatico et al. (1997) measured the activity of antioxidant enzymes (SOD and GPx) and lipid peroxidation levels after sprint exercise in blood samples from sprint-trained athletes. Alessio et al. (1988) measured lipid peroxidation levels in the skeletal muscle of rats after sprint exercise. However, whether or not sprint exercise brings about changes in the activity of antioxidant enzymes in other tissues has not yet been examined. The aim of the present study was to assess the effects of sprint exercise on levels of thiobarbituric acid

reactive substances (TBARS), which provide an index of lipid peroxidation and antioxidant enzymatic activities, immediately after exercise and during the recovery period in the skeletal muscle and liver tissues of mice.

Methods

Animals

Male Balb-c mice ($n = 55$), weighing [mean (SEM)] 38.59 (0.42) g, were used. Animals were housed in a temperature-controlled room (20 ±2°C) with a 12:12 h light:dark cycle, and were allowed free access to standard rat chow and water. American College of Sports Medicine animal care standards were followed in our research and this study was approved by the Institutional Animal Care and Use Committee of the Dokuz Eylül University, Faculty of Medicine.

Exercise program

Running exercises were performed on three small animal treadmills. All procedures were carried out between 9.00 and 12.00 a.m. The mice ($n = 20$) were randomly assigned to one of four groups. They were run on a treadmill for 5 min·session^{-1} at a speed of 15 m·min^{-1} and a slope of 5°, five times a day for 1 week to adapt them to running before the experiment. Electric shocks were used sparingly to motivate the animals to run. In preliminary studies, mice were run at various speeds. When the treadmill speed was higher than 35 m·min^{-1}, mice were unable to maintain the pace on the treadmill. Therefore, the speed of 35 m·min^{-1} speed was chosen for experiments.

In order to determine the most appropriate exercise prescription that would result in a long-lasting increase in blood lactate levels, preliminary experiments were performed using three different exercise protocols. For group 1 mice ($n = 5$), each experimental exercise bout comprised running on the treadmill for 30 s at a speed of 35 m·min^{-1} and a slope of 5°, with a 30-s rest interval between bouts. For group 2 mice ($n = 5$), each experimental exercise bout comprised running on the treadmill for 30 s at 35 m·min^{-1} and a slope of 5°, with a 60-s rest interval between bouts. For group 3 mice ($n = 5$) each experimental exercise bout comprised running on the treadmill for 30 s at 35 m·min^{-1} and a slope of 5°, with a 10-s rest interval between bouts. The animals performed 15 bouts in total. The blood lactate levels of all of these mice were determined immediately after completion of the exercise procedure. The blood lactate levels of a group of control animals ($n = 5$) were determined without a prior running exercise procedure. Blood lactate concentration significantly increased in all groups after exercise, the longest-lasting increase being observed in group 3. Therefore, for the actual experiment, the protocol performed by group 3 was chosen: 30 s of running at a speed of 35 m·min^{-1} and a slope of 5°, with 10 s rest between bouts.

Mice ($n = 40$) were randomly assigned to one of five experimental groups: (1) unexercised control ($n = 8$), (2) cervical dislocation immediately after exercise ($n = 8$), (3) cervical dislocation 0.5 h after exercise ($n = 8$), (4) cervical dislocation 3 h after exercise ($n = 8$) and (5) cervical dislocation 24 h after exercise ($n = 8$). Two days after the last familiarisation run, animals (except for the control group) were subjected to the prescribed exercise regimen (30 s, 35 m·min^{-1}, 5° slope, 10 s rest intervals between bouts). The animals performed 15 of these exercise bouts.

Tissue sampling

The animals were killed by dislocation immediately (0 h), 0.5 h, 3 h or 24 h after the exercise. Control animals were killed without running exercise. Their gastrocnemius muscle and liver tissues were quickly removed. Tissues were washed in cold homogenate medium and visible clots were removed to minimise blood contamination. The tissue homogenates were prepared as described by Carrillo et al. (1991). An aliquot of the homogenate and supernatant was stored at –70°C until the determination of TBARS levels and enzyme activities.

Determination of antioxidant enzyme activities and TBARS levels

SOD activity (expressed as U·mg^{-1} protein) was determined using a RANSOD kit (Randox Laboratories Crumlin, UK). GPx (expressed as U·mg^{-1} protein) was determined using a RANSEL kit (Randox Laboratories; Paglia and Valentine 1967). TBARS levels were measured on homogenates according to the method of Rehncrona et al. (1980) and are expressed as nmol·mg^{-1} protein.

Determination of protein concentration

The protein contents of supernatant and homogenate were determined using a total protein kit (Randox Laboratories).

Determination of blood lactate

Blood samples were collected from the heart using a syringe and analysed for lactate concentration with an automated lactate analyser (Yellow Springs Instruments Model 23L; Weil et al. 1986).

Chemicals

RANSOD and RANSEL kits were obtained from Randox. All the other chemicals were from Sigma (St. Louis, Mo., USA).

Statistics

All results are expressed as mean (SEM). Statistical analysis of the data was performed using one-way analysis of variance followed by a post hoc Student-Newman-Keuls test. The level of statistical significance was set at $P < 0.05$.

Results

Blood lactate levels of the control, 0 h, 0.5 h, 3 h and 24 h groups were 6.53 (0.81), 10.66 (1.36), 8.67 (0.79), 6.25 (0.92) and 4.15 (0.78) mmol·l^{-1}, respectively. Blood lactate levels increased significantly immediately after exercise (0 h, $P < 0.05$).

TBARS levels, SOD and GPx activities in skeletal muscle are shown in Table 1. The exercise regimen had no effect on GPx and SOD activities in skeletal muscle. Muscle TBARS level increased after exercise (0.5 h $P < 0.05$, 3 h $P < 0.05$), then returned to control levels at 24 h post-exercise.

Liver TBARS levels, SOD and GPx activities are given in Table 2. TBARS levels and antioxidant enzyme activities in this tissue were unaffected by the exercise regimen.

Discussion

In the present study, muscle TBARS levels increased after sprint exercise, then returned to control levels at 24 h post-exercise. Few studies have investigated the effects of acute sprint exercise on lipid peroxidation.

Table 1. Mean (SEM) superoxide dismutase (*SOD*), glutathione peroxidase (*GPx*) enzyme activities and thiobarbituric acid reactive substances (*TBARS*) levels in skeletal muscle. The animals ($n = 8$ animals per group) were killed by dislocation, immediately (*0 h*), 0.5 h, 3 h and 24 h after the exercise. Control animals were killed without running exercise

Groups	SOD ($U \cdot mg^{-1}$ protein)	GPx ($U \cdot mg^{-1}$ protein)	TBARS ($nmol \cdot mg^{-1}$ protein)
Control	8.11 (0.51)	0.07 (0.01)	0.39 (0.04)
0 h	6.99 (0.50)	0.07 (0.01)	0.27 (0.03)
0.5 h	7.36 (0.75)	0.05 (0.01)	0.74 (0.01)*
3 h	7.36 (0.50)	0.06 (0.01)	0.84 (0.04)*
24 h	8.77 (0.98)	0.06 (0.01)	0.46 (0.04)

*$P < 0.05$, in comparison with control mice (Student-Newman-Keuls test)

Table 2. Mean (SEM) SOD, GPx enzyme activities and TBARS levels in the liver. The animals ($n = 8$ animals per group) were killed by dislocation, immediately (0 h), 0.5 h, 3 h and 24 h after the exercise. Control animals were killed without running exercise

Groups	SOD ($U \cdot mg^{-1}$ protein)	GPx ($U \cdot mg^{-1}$ protein)	TBARS ($nmol \cdot mg^{-1}$ protein)
Control	86.38 (3.80)	7.48 (0.86)	0.36 (0.03)
0 h	79.78 (3.84)	6.70 (0.46)	0.33 (0.03)
0.5 h	97.79 (7.46)	7.78 (1.02)	0.38 (0.04)
3 h	88.30 (7.02)	7.39 (0.90)	0.39 (0.02)
24 h	80.97 (8.41)	5.96 (0.56)	0.36 (0.04)

Marzatico et al. (1997) determined that lipid peroxidation levels in blood samples from sprint-trained athletes increased after sprint exercise. Alessio et al. (1988) have shown that lipid peroxidation levels in the skeletal muscle of rats increase after sprint exercise at a speed of 45 $m \cdot min^{-1}$ for 1 min. Many studies report increased lipid peroxidation in skeletal muscle after long-term submaximal exercise (e.g. Child et al. 1998; Sen 1995), but it is possible that supramaximal exercise has a larger ROS-producing and damaging potential. This is supported by the observations of Alessio and Goldfarb (1988) and Salminen and Vihko (1983), who noted a larger increase in TBARS after high-intensity exercise, in which lactate production is substantial, compared with moderate-intensity exercise. Moreover, Lovlin et al. (1987) observed a significant relationship between plasma lactate concentration and lipid peroxidation during progressive incremental exercise. In addition, it has been shown that lactate increases hydroxyl radical generation by the Fenton reaction (Ali et al. 2000), and that hydroxyl radicals cause lipid peroxidation (Halliwell and Gutteridge 1999). In the present study, lactate concentration increased after the sprint exercise. Therefore, the increase in TBARS levels in skeletal muscle may result from increased lactate concentration after exercise.

Marzatico et al. (1997) have determined that SOD and GPx activities increased after sprint exercise in blood samples from sprint-trained athletes. However, no data are available on the effects of acute sprint exercise on antioxidant enzymes in skeletal muscle. The results from the present investigation demonstrate that muscle SOD and GPx activities are unaffected by the acute sprint exercise procedure. The few sprint-training studies on oxidative stress have produced discrepant results about the effects on pro-oxidant-antioxidant balance. Criswell et al. (1993) studied the effect of 12 weeks of interval training and observed favourable changes in the soleus muscle of rats. On the contrary, they determined that SOD and GPx activities were not affected by interval training. In another study on sprint training in rats, it was observed that the GPx activity of the gastrocnemius and quadriceps femoris muscles increased significantly, but SOD activity was not influenced (Atalay et al. 1996). It might be that these disparate results are related to differences in the exercise type, intensity and/or duration used. Moreover, in these studies, antioxidant enzymes activities were measured in different muscles, which may be a cause of these different results.

One of the major functions of the liver, which has one of the highest antioxidant enzyme activities in the body, is detoxification (Parkhouse et al. 1995). No study has investigated the effects of acute sprint exercise on antioxidant enzymes in the liver. In our study, GPx and SOD activities in the liver were not changed by the acute sprint exercise. Inconsistencies have been found as to the effects of acute or chronic exercise on hepatic antioxidant capacity (Alessio and Goldfarb 1988; Ji 1993; Ji et al. 1988, 1990). The differences are probably related to variations in the intensity and duration of the exercise programs. Radak et al. (1996) demonstrated that exhausting treadmill running caused a significant increase in the activities of GPx in hepatic tissue immediately after running. In the same study, there was a definite increase in the immunoreactive content of mitochondrial SOD 1 day after the running; cytosolic immunoreactive copper- and zinc-SOD levels remained unchange. However, Alessio and Goldfarb (1988) determined that total SOD activity in the liver was unaffected by acute exercise. Ji et al. (1990) showed that acute exercise had no effect on hepatic SOD and GPx activities.

Acute exercise has been shown to elevate hepatic lipid peroxidation levels, indicating a significant increase in oxidative stress in this organ (Alessio and Goldfarb 1988; Davies et al. 1982). In the present study, TBARS levels were not changed after acute sprint exercise in the liver. Part of the reason for this contradictory finding could be the use of a different type and intensity of exercise in our study.

In conclusion, acute sprint exercise resulted in an increase in TBARS levels in skeletal muscle; no change was observed in the liver. The elevated TBARS levels in skeletal muscle after exercise may be attributable to increased lactate concentration, which can induce hydroxyl radical generation.

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