

The Effects of an Acute Bout of Exhaustive Treadmill Exercise on Malondialdehyde Level in the Rat Skeletal Muscle

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Abstract:

The aim of this study was to investigate whether 8-week treadmill training attenuates exercise-induced oxidative stress in the rat Skeletal Muscle. Materials and methods: The study was carried out with 12 week-old male rats (N =24) were divided into untrained and trained groups. Endurance training consisted of treadmill running at a speed of 25 m/min for 1 h/day and 10% uphill grade 5 days a week for 8 weeks. To see the effects of endurance training on acute exhaustive exercise induced oxidative stress, untrained and trained rats were further divided into two groups: animals killed at rest and those killed after acute exhaustive exercise, in which the rats run at 30 m/min (10% uphill) until exhaustion. Results: After a single bout of exhaustive treadmill running, malondialdehyde level in untrained and trained rats were significantly increased, but were significantly lower in the trained group. Conclusions: The results suggested that single bout of exhaustive treadmill exercise may induce in the Skeletal Muscle damage and endurance training attenuated exercise-induced oxidative stress in the Skeletal Muscle after a single bout of exhaustive treadmill running, probably by reinforced of antioxidant defense system during exercise.

Key words: acute exercise, oxidative stress, Skeletal Muscle

Introduction

Cells continuously produce free radicals and ROS as part of metabolic processes. These free radicals are neutralized by an elaborate antioxidant defense system consisting of enzymes such as catalase, superoxide dismutase, glutathione peroxidase, and numerous non-enzymatic antioxidants, including vitamins A, E and C, glutathione, ubiquinone, and flavonoids. While regular exercise training is associated with numerous health benefits, it can be viewed as an intense physical stressor leading to increased oxidative cellular damage, likely due to enhanced production of ROS (BLOOMER, GOLDFARB et al. 2005). A single bout of exercise can result in activation of several distinct systems of radical generation and may be separated into both primary (e.g., electron leakage through the mitochondria during aerobic respiration, prostanoid metabolism, catecholamines, and the enzymes xanthine oxidase and NADPH oxidase), as well as secondary sources (e.g., phagocytic cells, disruption of iron containing proteins, and excessive calcium accumulation) (BLOOMER, GOLDFARB et al. 2005). Many studies have reported that acute aerobic exercise contributes to oxidative stress, especially when performed at high intensity levels. Two mechanisms linking acute aerobic exercise and oxidative stress are 1) increased pro-oxidant activity via a mass action effect when VO₂ is elevated 10- to 15-fold above rest, and 2) inadequate antioxidant activity relative to pro-oxidants (Alessio, Hagerman et al. 2000). Evidences indicate that acute aerobic physical exercise generated reactive oxygen species such as superoxide anion and hydrogen peroxide are able to cause muscular damage and inflammation (Alessio and Goldfarb 1988; Liu, Yeo et al. 2000). In spite of this, elaborate oxidative defense systems could be induced, counteracting oxidative damage, as a result of a

regular physical exercise program (Oh-Ishi, Kizaki et al. 1997; Liu, Yeo et al. 2000) . Physical exercise associated with oxidative damage depends on the type and intensity of exercise. However, studies have demonstrated that endurance training improves the antioxidant defense as well as oxidative capacity in skeletal muscle (Oh-Ishi, Kizaki et al. 1997; Terblanche 1999). Despite the great efforts expended, the causes of oxidante antioxidant imbalance in the Skeletal Muscle during exercise are remained to be studied. Thus, the aim of the present study was to investigate the, whether endurance training alters the oxidative damage caused by acute exhaustive exercise in the rat Skeletal Muscle. This study examined the effects of acute exhaustive exercise and 8-week endurance training on Skeletal Muscle MDA level in rats.

Materials and Methods

Animal care

Male Wistar rats weighing 245–270 g (n = 24, 12 weeks old) were purchased from Shahid Beheshti University of Medical Sciences and Health Services and were used in this study. All rats were housed in conventional wire-mesh cages, four rats per cage, in a room with the temperature regulated at 23 ±2°C, humidity 50-45% and in daily light / dark cycle (12h) (0700-1900 h dark; 1900-0700 h light), given standard rat chow and tap water ad libitum. All procedures were approved by the Tehran University Animal Care and Usage Committee and followed the guidelines established by American Physiological Society.

Experimental design

The animals were housed for two weeks prior to any special treatment. In the third-week all the animals were randomly divided mainly into two groups, group1, sedentary (Sed N=12), group2, exercise trained (ET n=12). Two groups were further

divided equally into two groups where the rats were studied at rest and immediately after exhaustive exercise. During the training period, the animals in the group2, was run on the treadmill 5 days a week for 8 weeks. Experiments were conducted between 10:00 and 12:00 h.

Training and Acute Exhaustive Exercise

After divided, the animals in the group (ET) were performed aerobic exercise on a treadmill for a period of eight weeks before the training, the group (ET) rats were introduced to treadmill running through the use of one 5-25 minute running session on a rodent treadmill at a speed of 16/6m/min and a 0-2% uphill grade (1 session a day, 5 times/wk, 1 wk). (Sen, Marin et al. 1992).The treadmill was equipped with an electric shock grid on the rear barrier to provide exercise motivation to the animals. The exercise protocol was performed in inclined treadmill one session a day during five days a week for 8 weeks. The exercise protocol was arranged as follows: in the first two weeks animals run with a speed of 16/67-18/33 m/min for 35-40 minutes and 3-4% uphill grade, in the following 3 weeks running speed was increased to 16/67-20 m/min and 3-5% grade uphill for 35-40 minutes and in the last 3 weeks, treadmill speed was adjusted to 25 m/min for one hour and 8-10% uphill grade. During the eighth week of the training program, the groups (Sed) were also introduced to treadmill running at speed of 16/67-20 m/min, for 15 min day, for 5 days before sample collection. This regimen was used to ensure that untrained rats could also tolerate the acute exhaustive exercise without having a significant training effect (Sen, Marin et al. 1992). At the end of the training period and after 2 days at rest, half of all rats were randomly selected into the acute exhaustive exercise group (each group N=6 , totality N=12). In acute exhaustive exercise, running speed was 25 m/min (10% uphill gradient) for the first 10 min; after that the speed was increased gradually to 30 m/min, and kept constant until the rats were exhausted. The loss of the righting

reflex when the rats were turned on their backs was the criterion of exhaustion. To eliminate diurnal effects, the experiments were performed at the same time (08.30–12.30 hours) (Brooks and White 1978). Immediately after exhaustion exercise, animals were sacrificed with Chloroform then their gastrocnemius muscle were carefully isolated and stored at -80°C until analysis. The other half of all rats (N=12) underwent anesthesia immediately before the acute exhaustive exercise, then gastrocnemius muscle tissue was obtained according to the same program. These samples were used for the measurement levels of total protein concentration and MDA rat gastrocnemius muscle.

Thiobarbituric Acid Reactive Substances (TBARS) Levels

Lipid peroxidation was estimated by measuring TBARS according to the method of esterbauer et al. (Esterbauer, Schaur et al. 1991).

Protein Determination

The protein content was measured colorimetrically by the method of Bradford (Bradford 1976) using bovine serum albumin (1 mg/ml) as standard.

Statistical Analysis

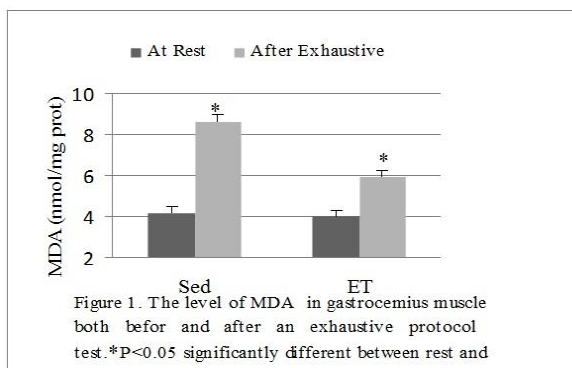
The Statistical Package for Social Sciences (SPSS, Ins, Chigaco, IL) version 17 was used for all analyses. Statistical significance was set at a level of $P < 0.05$, and data were expressed as the mean \pm SEM. One-way ANOVAs with Tukey's post-hoc tests were used to compare group means.

Results

Malondialdehyde level

Malondialdehyde results are presented in (Fig. 1). gastrocnemius muscle MDA level was significantly increased

after exhaustion in the Sed and ET groups but was significantly lower in the trained group compared with Sed group ($p < 0.05$).



Discussion

Evidence has accumulated, especially during the past two decades that strenuous aerobic exercise is associated with oxidative stress and tissue damage in the living organism. There are indications that the generation of oxygen free radicals and other ROS may be the underlying mechanism for exercise-induced oxidative damage (Davies, Quintanilha et al. 1982; Sen, Atalay et al. 1997). Exercise is associated with a large increase in O_2 consumption (Higuchi, Cartier et al. 1985) which probably results in an increased production of H_2O_2 . H_2O_2 as a potent oxidizing agent can result in extensive cellular damage in different tissues (Davies, Quintanilha et al. 1982). H_2O_2 resulting from two electron reactions can cross cell membranes rapidly whereas O_2 usually cannot. H_2O_2 can probably react with Fe^{2+} , and possibly Cu^+ , ions to form the hydroxyl radical (OH^\bullet) (Fenton reaction) and this may be the origin of its toxic effects (Halliwell and Gutteridge 1999). Davies et al. (Davies, Quintanilha et al. 1982) showed that exhaustive exercise decreases mitochondrial respiratory control, causing an increase in free radicals and thiobarbituric

acid reactive substances and suggesting that endurance training induces free radical damage. The rate of free radical or oxidant generation in biological tissue is closely related to oxygen consumption: under physiological conditions, the majority of oxidants are produced in the mitochondria. Thus it seems likely that mitochondria, in addition to being the sources of oxidant production, also should be the targets of oxidants. Exercise, by increasing the oxygen consumption rate, may result in oxidative stress in mitochondria. This results in an increased production of oxidants, which could be detrimental to tissue (Jenkins and Goldfarb 1993; Ji 1999). The oxidants cause damage to mitochondrial membranes and cytoplasmic structures through the peroxidation of phospholipids, proteins, and nucleotides. In the present study we found that gastrocnemius muscle MDA levels a significant increase in TBARS in the two groups of animals after exhaustive run on a treadmill. During exercise there is an increase in the requirement of oxygen. The process of delivering the oxygen to the working muscles may actually result in damage to polyunsaturated fatty acids in membrane structures. This has been documented by numerous investigations demonstrating increases in the byproducts of lipid peroxidation following exercise in previous studies (Sen, Atalay et al. 1997; Bejma and Ji 1999; Ji 1999; Ajmani, Fleg et al. 2003). When a hydroxyl radical reacts with an unsaturated fatty acid, a lipid peroxy radical is formed. In the presence of oxygen this new free radical incites a chain of events referred to as lipid peroxidation. Lipid peroxidation of cell membranes results in decreased membrane fluidity, inability to maintain ionic gradients, cellular swelling, and tissue inflammation (Alessio 1993; Okafor, Erukainure et al. 2011). Small amounts of malondialdehyde were produced during peroxidation and can react in the thiobarbituric acid test to generate a coloured product for photometric measurement. On the contrary, Liu *et al.* (Liu, Yeo et al. 2000) have observed that level of MDA in

the skeletal muscle tissues, are not different between the control and exhausted rats. An interesting finding in our research is that run on a treadmill decreased TBARS increases after the exhaustive protocol test in comparison to the control group. It has been shown that increased TBARS in the mitochondria membranes impair membrane-bound enzyme activities leading to mitochondrial dysfunction (Navarro, Gomez et al. 2004). These data suggest that run on a treadmill may provide protection to acute insult in the in the skeletal muscle mitochondria herein measured by oxidative stress markers. Similar to our results in most of studies related to the effects of regular aerobic exercise on oxidative stress have reported that endurance training reduces exercise-induced oxidative stress damage caused by acute exhaustive exercise (Miyazaki, Oh-ishi et al. 2001; Oztasan, Taysi et al. 2004; Radak, Chung et al. 2008).

In conclusion, our results indicate that skeletal muscle MDA level is affected by an acute exhaustive exercise. We demonstrate that acute exhaustive exercise increases lipid peroxidation in skeletal muscle, especially in untrained rats. However, we find that, regular aerobic exercise decreased the levels of MDA in the rat skeletal muscle induces of acute exhaustive exercise.

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