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Intense and exhaustive exercise induce oxidative stress in skeletal muscle

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ABSTRACT

Objective: To assess the oxidative stress and antioxidant defense system in the skeletal muscle of male albino rats subjected to strenuous exercise programme. **Methods:** Wistar strain albino rats were subjected to exhaustive swimming exercise programme daily for a period of five days. The thiobarbituric acid reactive substances (TBARS), conjugated dienes, superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase were measured in the gastrocnemius muscle of the exercised animals. **Results:** The elevated levels of TBARS and conjugated dienes indicated the oxidative stress in the gastrocemius muscle of the exercised animals. The depleted activity levels of superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase in the exercise animals indicated the increased oxidative stress and decreased antioxidative defense system in the muscle. **Conclusions:** The study suggests that prolonged strenuous exercise programme can induce oxidative stress and therefore an optimal level of exercise schedule should be advocated to obtain the maximum benefit of exercise programme.

1. Introduction

Cells continuously produce free radicals and reactive oxygen species (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. Exercise can cause an imbalance between ROS and antioxidants, which is referred to as oxidative stress[1]. While regular exercise training is associated with numerous health benefits, it can lead to an intense physical stress or increased oxidative cellular damage, likely due to enhanced production of ROS[2]. The beneficial effects of regular physical exercise have long been known. The effectiveness of regular physical activity in the prevention of chronic diseases such as diabetes, cancer, hypertension, obesity, depression, and osteoporosis and premature death, is well established. However, the beneficial effects of exercise are lost with exhaustion. It is well known that exhaustive exercise, especially when sporadic, causes structural damage to or inflammatory reactions within the muscles. This damage is due to, at least in part, the production of ROS[3]. Strenuous exercise increases oxygen consumption and causes disturbance of intracellular prooxidant homeostasis. The mitochondrial electron transport chain, polymorphoneutrophil, and xanthine oxidase have been identified as major sources of intracellular free radical generation during exercise. ROS pose a serious threat to the cellular antioxidant defense system, such as diminished reserve of antioxidant vitamins and glutathione, and increased tissue susceptibility to oxidative damage.

However, enzymatic antioxidants have demonstrated great adaptation to acute and chronic exercise. The delicate balance between prooxidants and antioxidants suggests that supplementation of antioxidants may be desirable for physically active individuals under certain physiological conditions by providing a larger protective margin[4]. Antioxidant enzymes may be activated selectively during an acute bout of strenuous exercise depending on the oxidative stress imposed on the specific tissues as well as the intrinsic antioxidant defense capacity. Skeletal muscle may be subjected to greater level of oxidative stress during exercise than liver, heart due to increased ROS production. Therefore, the muscle needs greater antioxidant protection against potential oxidative damage occurring during

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and/or after exercise. SOD, CAT, and GPx provide the primary defense against ROS generated during exercise, and activities of these enzymes are known to increase in response to exercise in both animal and human studies[5].

However, studies on free radicals generation during exercise schedule are scanty. The previous reports have assumed that skeletal muscle provide the major source of free radicals and ROS generation[6]. Therefore the study has been undertaken to assess the oxidative stress and antioxidant defense system in the skeletal muscle of male albino rats subjected to strenuous exercise programme.

2. Materials and methods

2.1. Animals

Male albino rats of wistar strain weighing around 170-190 g were purchased from Tamilnadu Veterinary and Animal Science University, Chennai. The Animals were acclimatized to the laboratory conditions, fed with commercial pelleted rat chow (Hindustan Lever Ltd, Bangalore, India) and had free access to water. The experiments were designed and conducted in accordance with the guidelines of institutional animal ethics committee.

2.2. Swimming as an exercise

Male albino rats of wistar strain were employed throughout the study. The rats were subjected to swimming in groups of 5 to 6 with 2% of their body weight in lead weight attached to their tails, until exhausted as described earlier[7]. The swimming was continued for five days.

2.3. Experimental protocol

The rats were randomly divided into two groups. Group-1: Normal rats. Group-2: Rats that were subjected to exhaustive swimming exercise programme.

2.4. Biochemical estimations

2.4.1. Estimation of thiobarbituric acid reacting substances (TBARS)

The level of TBARS in gastronomies muscle were estimated by measuring malondialdehyde and TBARS reactivity with thiobarbituric acid (TBA) to generate a pink colour chromophere, which was read at 535 nm[8]. The transmissions were measured by calorimeter and expressed in terms of mM/ 100 g wet tissue.

2.4.2. Estimation of conjugated diene

Conjugated diene was measured in all the muscle groups of animals by the method of Klein[9]. The optical density of lipid was measured at 230-235 nm. Levels of conjugated dienes content in tissue expressed as micro moles of conjugated dienes (CD)/ mg protein.

2.4.3. Estimation of superoxide dismutase

Superoxide dismutase (SOD) was assayed utilizing the method of Kakkar *et al.*[10]. A single unit of enzyme was expressed as 50% inhibition of NBT (Nitroblue tetrazolium) reduction/ min/ mg protein.

2.4.4. Estimation of catalase

Catalase was assayed colorimetrically at 620 nm and expressed as μ moles of H_2O_2 consumed/ min/ mg protein as described by Sinha[11]. The reaction mixture 1.5 mL contained 1.0 mL of 0.01M pH 7.0 phosphate buffer, 0.1 mL of tissue homogenate and 0.4 mL of 2M H_2O_2. The reaction was stopped by the addition of 2.0 mL of dichromate-acetic acid reagent (5% potassium dichromate and glacial acid were mixed in 1:3 ratio).

2.4.5. Estimation of glutathione peroxidase

Glutathione peroxidase was measured by the method described by Rotruck *et al*[12]. To 0.2 mL Tris buffer, 0.2 mL of ethylene diamine tetraacetic acid (EDTA), 0.1 mL of sodium azide and 0.5 mL of tissue homogenate (Tris buffer 0.4 M, pH 7.0) were added. To the mixture, 0.2 mL of glutathione (GSH) followed by 0.1 mL of H_2O_2 was added. The contents were mixed well and incubated at 37 ℃ for 10 minutes, along with a control containing all reagents except tissue homogenate. After 10 minutes, the reaction was arrested by the addition of 0.5 mL of 10% trichloroacetic acid (TCA) and centrifuged. The activity was expressed as mg of GSH consumed/ min/ mg protein.

2.4.6. Estimation glutathione-S-transferase

Glutathione-S-transferase (GST) activity was assayed spectrophotometrically at 340 nm by the method of Habig *et al*[13]. The reaction mixture contained an aliquot of 0.1 M potassium phosphate buffer pH 7.4, 100 mM GSH and100 mM CDNB, which was used a substrate. The enzymatic activity was expressed as nmol CDNB conjugated/ min/ mg protein.

2.5. Statistical analysis

The results were expressed in ±standard deviation. Statistical analysis was carried out by using one way ANOVA as in standard statistical software package of social science (SPSS).

3. Results

The oxidative stress and the antioxidant defence system were studied in the gastrocnemius muscle of normal rat and rats subjected to swimming exercise.

The results were tabulated (Table 1, 2). The levels of

Table 1
Levels of thiobarbituric acid reactive substance (TBARS) and conjugated dienes (CD) in the gastrocnemius muscle of normal rats (Group-I) and rats subjected to swimming exercise (Group-II).

Parameters	Group-I	Group-II	% of changes (Group-I *vs* II)
TBARS (μ moles of mg protein)	0.29 ± 0.04	0.670± 0.040	+131.03*
Conjugated dienes (μ moles of mg protein)	1.39 ± 0.12	2.412± 0.130	+73.52*

Values are mean of 6 individual observations in each group ±S.D. '+' and '-' indicate % of changes over the normal group. * P<0.001.
TBARS= Content in tissue, expressed as μ moles Malondialdehyde (MDA)/mg protein, Conjugated dienes (CD) = Expressed as μ moles/ mg rotein.

Table 2
Activity levels of antioxidant enzymes *viz.*, superoxide dismutase, catalase, glutathione peroxide and glutathione-S-transferase in normal rats (Group-I) and rats subjected to swimming exercise (Group-II).

Parameters	Group-I	Group-II	% of changes (Group-I *vs* II)
SOD (Unit1/ mg protein)	0.560 ± 0.010	0.270 ± 0.020	- 51.78*
CAT (Unit2/ mg protein)	1.300 ± 0.012	0.810 ± 0.014	- 37.69*
GPX (Unit3/ mg protein)	6.100 ± 0.140	3.810 ± 0.080	- 37.54*
GST (Unit4/ mg protein)	0.151 ± 0.010	0.062 ± 0.010	- 58.94*

Values are mean of 6 individual observations in each group±S.D. '+' and '-' indicate % of changes over the norm al group. * P<0.001.
SOD -U1- One unit of activity was taken as the enzymes reaction which gives 50% inhibition of NBT reduction in one minute. CAT -U2- μ moles of hydrogen peroxide consumed per minute. GPX - U3- μ g of glutathione consumed per minute. GST - U4- μ moles of CDNB -GSH conjugate formed per minute.

thiobarbituric acid reactive substances (TBARS) and conjugated diens (CD) in the animals subjected to exhaustive exercise programme were significantly elevated to 131% and 73.52% respectively when compared to that of normal group.

The activity levels of antioxidant enzymes *viz.*, superoxide dismutase (SOD), catalase (CAT), glutathione peroxide (GPx) and glutathione-s-transferase (GST) were significantly depleted to 51.78%, 37.69%, 37.54% and 58.94% respectively in the gastrocnemius muscle of rats subjected to exhaustive swimming schedule.

4. Discussion

The oxidative stress leading to increased lipid peroxidation in the gastrocnemius muscle of animals subjected to exhaustive swimming exercise programme was indicated by elevated levels of TBARS and conjugated dienes. 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[14,15]. When a hydroxyl radical reacts with an unsaturated fatty acid, a lipid peroxyl 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[16,17]. Small amounts of malondialdehyde were produced during peroxidation and can react in the thiobarbituric acid test to generate a coloured product for photometric measurement. The conjugation dienes were formed during the oxidation of unsaturated fatty acids side chains. The measurements of levels of TBARS and conjugated dienes, which are the markers of oxidative stress induced lipid peroxidation, reveal significant elevated levels in the gastrocnemius muscle of exercised animal group.

Similar observation, where the free radicals generated lipid peroxidation were recorded in previous studies[4].The increased levels of TBARS and CD in the gastrocnemius muscle of exercised animals might be due to increased oxidative stress and decreased levels of antioxidant enzyme defense mechanism in the muscle tissue.

Further probe into the antioxidant defense mechanism of the gastrocnemius muscle during strenuous exercise programme was carried out. The activity level of cytosolic enzyme SOD recorded a significant decrease, indicating decreased inhibition in the formation of hydroxyl ion (.OH) from hydrogen peroxide, thereby increasing the free radical concentration leading to oxidative stress. It is well known that SOD is regarded as the first line of defense by the antioxidant enzyme system against ROS generated during exhaustive exercise[18,19]. The CAT and glutathione peroxidase activity levels recorded depletion in the present study, prevent the formation of hydroxyl radicals that can initiate lipid peroxidation by converting the hydrogen peroxide into water and diatomic oxygen. Glutathione peroxidase reduces H_2O_2 by oxidizing glutathione (GSH)[20].

The enzymatic antioxidant SOD, which catalyses the conversion of the oxygen radical (O_2.) to H_2O_2 and H_2O[15]; the enzyme catalase which then converts H_2O_2 to H_2O and O_2; glutathione peroxidase reduces H_2O_2 to H_2O by oxidizing glutathione (GSH). Further CAT is involved in detoxification of high concentration of H_2O_2 and glutathione peroxidase is

sensitive to lower concentration H_2O_2.

Glutathione-S-transferase activity level decrease in the present study along with glutathione peroxidase play significant role in protecting the cells against oxidative damage by scavenging reactive oxygen species[22-26]. In addition GST and Gpx are bio-transformative enzymes involved in the detoxification of free radicals and peroxides by conjugating these toxic substances with glutathione (GSH), ultimately protecting cells and organs from oxidative damage.

The present study reveals that exhaustive swimming exercise programme induced oxidative stress in the gastrocnemius muscle of albino rats. This suggests that in spite of the various physiological benefits due to physical exercise programme, a control and close monitored exercise schedule is necessary to avoid oxidative damage due to exhaustive physical exercise.

Conflict of interest statement

We declare that we have no conflict of interest.

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