Influence of Endurance Exercise on Lipid Peroxidase Enzyme

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ABSTRACT

Aim: *The purpose of this study was to determine the effects of endurance exercise on lipid peroxidase enzyme.* ***Methods:*** *Thirty rats (15males and 15 females), the mean age of all rats was 6 weeks old and the mean weight was 150(±10g), were participated in this study and they were chosen randomly. They are divided into two groups, control group (N= 10 rats), group (I) (N=10 rats) and group (II) (N=10 rats). Group (I) received treadmill exercises for 9 minutes duration at 25m/minute, 3 times per week for 4 weeks and group (II) received treadmill exercises for 9 minutes duration at 25m/minute, 6 times per week for 4 weeks.* ***Results:*** *The rats of all groups were scarified; their heart tissues were homogenized in phosphate buffer saline and then were centrifuged to obtain the fluid through which the malondialdehyde (MDA) was measured. One-way ANOVA showed a significant difference between the three groups of malondialdehyde.* ***Conclusion:*** *These results demonstrate that endurance training induced decline in lipid peroxidation such as malondialdehyde.*
Key words: *Endurance training, oxidative stress, lipid peroxidation.*

INTRODUCTION

There is growing evidence that oxidative stress is increased in myocardial failure and may contribute to the structural and functional changes that lead to disease progression; it has an obvious effect on glutathione peroxidase and lipid peroxidation. Glutathione is an important intracellular antioxidant that protects against a variety of different antioxidant species[13].

However, reactive oxygen species (ROS) are produced in the body through several pathways, all of which appear to be relevant to the metabolic changes occurring during or after strenuous exercise. These pathways include the mitochondrial respiratory chain, peroxisomal oxidative enzymes, xanthine oxidase, neutrophil activation, and catecholamine[9].

Therefore, metabolism of oxygen by cells generates potentially deleterious reactive oxygen species (ROS). These species may be toxic to almost everything found in a living cell including proteins, lipids, carbohydrates and DNA. This has lead to the development of endogenous physiological antioxidant defense systems that cooperate to scavenge and detoxify these species.

However, around 2-5% of consumed oxygen can result in ROS generation, an increased cardiac oxidative metabolic rate arising from physical exercise becomes a predisposing factor for increased ROS production at the mitichondrial level, leading to increase in the direct and indirect markers of tissue oxidative injury. However, if this situation is repeated over time it may have a strong modulating effect on various cardiac antioxidant systems[1,8].

So that, under normal physiological conditions the rate and magnitude of oxidant formation is balanced by the rate of oxidant elimination and an oxidative balance is achieved. An imbalance between prooxidants and antioxidants is called oxidative stress. This is the pathogenic outcome of the overproduction of oxidants that overwhelm the cellular antioxidant capacity[14].

There are several marked adaptations associated with the regular performance of endurance training. Aerobic endurance training produces increases in VO2 max, but has no hypertrophy effect on muscle. Muscle fiber size has actually been shown to decrease. Capillary supply to the muscles has been shown to change in response to endurance training through an increase in the capillary to muscle fiber ratio[6].

There is an increase in the number as well as the size of mitochondria, the latter of which is associated with an increase in certain enzymes. These increases are most apparent in the type I fibers as they have the highest content of mitochondria. Smaller increases in muscle and blood lactate levels are produced at the same relative exercise intensity after completing an endurance-training program[3].

This study may provide additional insights into the role of exercise training on myocardial oxidative stress, (lipid peroxidation) and may show the importance of increase needing for improved antioxidant defenses to cope with the stress imposed by the exercise sessions.

MATERIALS AND METHODS

The experiment of this study was carried out on 30 (male and female) rats. They were divided into 3 equal groups, the mean age of all rats was 6 weeks old and the mean weight was 150(±10g)[11].

The rats were housed in individual cages labeled according to the group, and fed a commercial standard diet, according to National Research Council (NRC) of laboratory animals, that contained 5.10% calcium, and 4.10% phosphorus. All rats were given food and tap water throughout the experiment and the lighting duration in the breeding room was fixed at 12h/day.

They were divided into three equal groups:

- Both second and third groups received endurance training.
- All groups had lab analysis for lipid peroxidaton (Malonhdialdehyde).

Evaluation equipments

1. The homogenizer used for measurement of both MDA.
2. Reagent for MDA.
3. Spectrophotometer or colorimeter for MDA absorbance reading.

Treatment Equipments

1. Electronic treadmill (Tunturi, modular treadmill 880, made in Finland).
2. A wooden box was opened from both sides up and down; this box surrounded the treadmill device from all directions, so that the rat won't fall during the exercise.

Procedure of the study

1. The first Group served as a control group.
2. The second Group received treadmill exercises for 9 minutes duration at 25m/minute, 3 times per week for 4 weeks[11].
3. The third Group received treadmill exercises for 9 minutes duration at 25m/minute, 6 times per week for 4 weeks[11].

Animal treatment

All rats were anesthetized by using ether and then will be decapitated to dissect their cardiac tissue.

Standard procedure

During the experiment it was proven that the interference from sialic acids on heart homogenate lipid peroxide determination was diminished by the addition of sodium sulfate in the modified Slater's method. Based on this observation, the standard procedure has been established as follows:

1. 0.5ml heart homogenate, 2.5ml of 20mg/dl trichloroacetic acid was added and the tube was left to stand for 10 min at room temperature.
2. Centrifugation at 3500 round per minute for 10min.
3. After centrifugation at 3500 round per minute for 10min, the supernatant was decanted and the precipitate was collected.
4. The precipitate was washed once with 0.05M sulfuric acid.
5. Then 2.5 ml of 0.05M sulfuric acid and 3.0ml of 0.2mg/dl the thiobarbituric acid (TBA) in 2M sodium sulfate were added to this precipitate.
6. The coupling of lipid peroxide with TBA was carried out by heating in a boiling water bath for 30 min.
7. After cooling in cold water, the resulting chromogen is extracted with 4.0ml of n-butyl alcohol by vigorous shaking.
8. Separation of the organic phase is facilitated by centrifugation at 3000rev./min for 10 min.
9. Its absorbance is determined at the wavelength of 350nm.
10. Finally concentration of malondialdehyde was calculated from standard curve.

Statistical analysis

Mean, standard deviation, independent t test and ANOVA will be used for treatment of collective data.

RESULTS

Malondialdehyde

Measurement of malondialdehyde in group (I) vs. the control group has no significant difference as the mean of control group was (8.223±0.97) while the mean of group (I) was (7.67±1.63) and SE of mean was 0.31 and 0.51 consequently and P value was 0.368. However, measurement of malondialdehyde in group (II) vs. the control group has a significant difference as the mean of control group was (8.223±0.97) while the mean of group (II) was (5.31±1.27) and SE of mean was 0.31 and 0.40 consequently and P value was 0.0001.

Table (1): Malondialdehyde differences among all groups.

	N	Mean	S.D.	S.E. mean
C. Group	10	8.223	0.970	0.31
Group I	10	7.67	1.63	0.51
Group II	10	5.31	1.27	0.40

Measurement of malondialdehyde in group (I) vs. group (II) has a significant difference as the mean of group (I) was (7.67±1.63) while the mean of group (II) was (5.31±1.27) and SE of mean was 0.51 and 0.40 consequently and P value was 0.0001.

Table (2): Malondialdehyde difference between group (I) and group (II).

	N	Mean	S.D.	S.E. mean
Group I	10	7.67	1.63	0.51
Group II	10	5.31	1.27	0.40

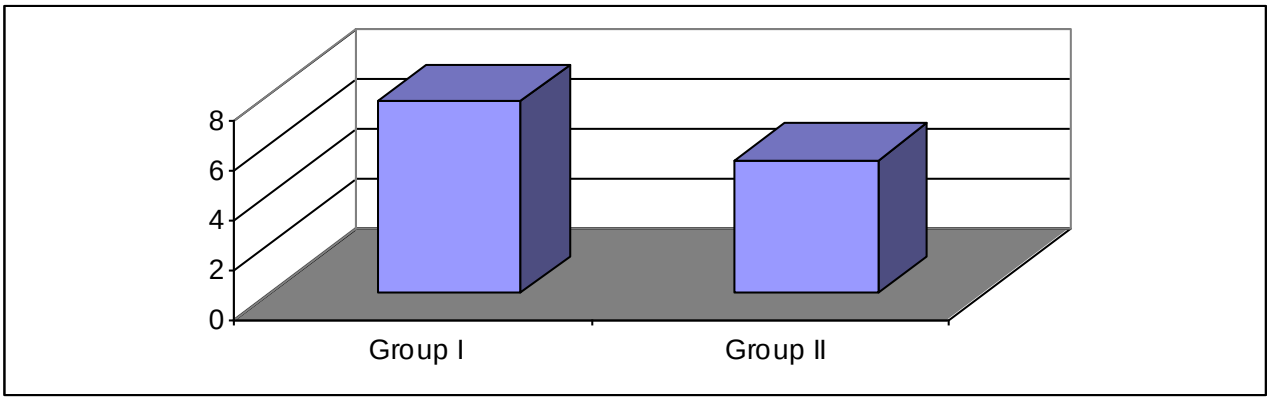

Fig. (1): Malondialdehyde difference in group (I) and group (II).

One-way analysis of variance (ANOVA) among the three groups showed a significance difference between the three groups (control group, group I and group II) where the mean value of the control group was (8.223±0.97) and group (I) was (7.666±1.627) and group (II) was (5.311±1.266) and the P value was 0.0001.

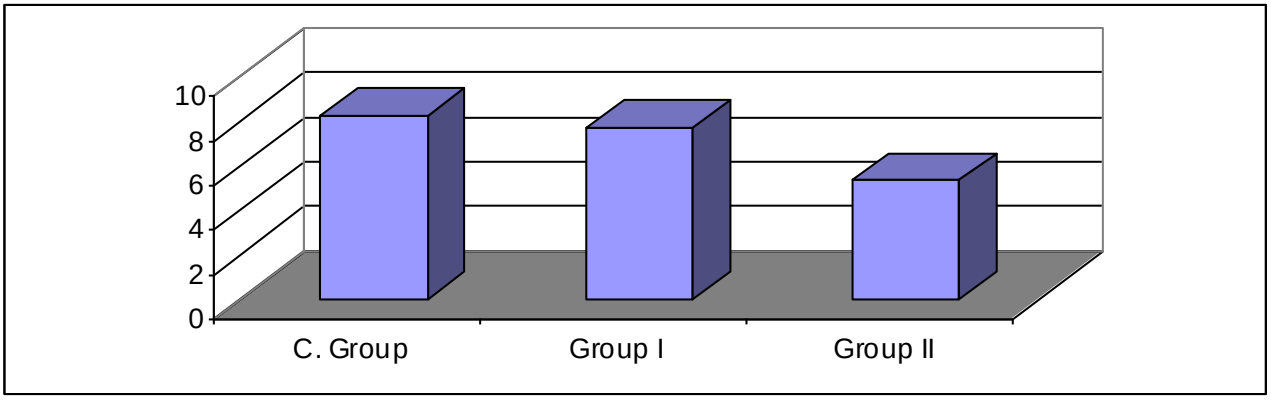

Fig. (2): Malondialdehyde difference in the control group, group (I) and group (II).

DISCUSSION

It seems clearly that cardiac muscle tissue, when simulated by acute exercise, presents changes in markers of cell damage due to oxidative stress notably lipid peroxidation and protein and DNA oxidation, on the other hand, the potential adaptability of the myocardium faced with systematic aggression presents a paradox, which appears to be O2- dependent, and therefore leads us to carefully analyze the effect of training,

particularly endurance training, on oxidative stress in major organ[16]. It is essential to recognize the fact that physical exercise of an inappropriate nature in terms of duration and intensity may itself act as a powerful source of ROS.

Some authors have suggested that endurance training may lead to increase tolerance to adverse situations that greatly exacerbate cardiac oxidative stress[5,7] and there is evidence that the intensity of endurance training and the duration of sessions influence the antioxidant system's ability to adapt[12].

However, endurance training could be considered an important stimulus for the antioxidant enzymes in myocardial protection such as glutathione peroxidase[2]. Endurance training seems to induce up-regulation in some antioxidant defenses, protecting cardiac muscle in potentially harmful situations that induce additional oxidative stress[3].

There is growing evidence that oxidative stress is increased in myocardial failure and may contribute to the structural and functional changes that lead to a disease progression; it has an obvious effect on glutathione peroxidase and lipid peroxidation[13].

There is an agreement that acute exercise promotes an increase in markers of cell damage from cardiac oxidative stress, as well as chronic alterations in the antioxidant defense system when practiced regularly[1,9].

Lipid peroxidation is a process normally occurring at low levels in all cells and tissues. It occurs when free radicals are generated adjacent to polyunsaturated fatty acids (PUFA) such as arachidonic and linolenic acids in membrane lipids[15].

In this study comparing the results of malondialdehyde in the three groups using one way analysis of variance showed a significant difference between the three groups as the P value was 0.0001.

It was suggested that the significant difference was due to the endurance training by using treadmill exercise for both group (I) and group (II). By comparing the results of lipid peroxidation between group (I) and group (II), it showed an obvious decrease of (LP) in group (II) more than group (I).

The findings were in disagreement with Vendetti and Di Meo (1996) who found an increase in cardiac markers of lipid peroxidation in rats after a period of exhausting swimming with a load equal to 2 % of the animal weight[15].

Further more, Gore, M. et al., (1998) have demonstrated that endurance training can cause deregulation of certain antioxidant systems or maintain them unchanged and they observed an unexpected increase in lipid peroxidation[4].

In addition, Somani, S.M. et al., (1995) noted an increasing in lipid peroxidation by products after exhaustive exercise[13].

The findings of this study were in agreement with Hunt, D. (1990) who trained rats at low to moderate intensity of 20 and 40 min/day of swimming showed a decline in lipid peroxidation in terms of malondialdehyde[6].

Conclusion

Within the limitation of this study, Endurance training produced significant changes of lipid peroxidation between the three groups. Further studies are recommended:to study the effect of strengthening exercise on lipid peroxidation, and to study the effect of short bouts exercise Vs long duration endurance exercise on cardiac stress.

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الملخص العربي

تأثير تمرينات التحمل على أنزيم الأكسدة الذاتية للدهون

تهدف هذه الدراسة إلى تحديد أثر تمرينات التحمل على أنزيم الأكسدة الذاتية للدهون ، ثلاثون فأراً من فئران التجارب يتراوح متوسط أعمارهم ستة أسابيع ومتوسط أوزانهم 150 ± 10 جرامات ، قسمت الفئران إلى ثلاث مجموعات متساوية احتوت كل مجموعة على عشرة فئران ، المجموعة الأولى استخدمت كمجموعة ضابطة ، المجموعة الثانية تلقت تمرينات التحمل على جهاز إحداث الحركة الدائرية لمدة تسعة دقائق بسرعة 25 م/ق ولمدة ثلاثة أيام في الأسبوع لمدة أربع أسابيع ، المجموعة الثالثة تلقت تمرينات التحمل على جهاز إحداث الحركة الدائرية لمدة تسعة دقائق بسرعة 25 م/ق ولمدة ستة أيام في الأسبوع لمدة أربع أسابيع ، ذبحت الفئران بعد نهاية فترة التدريب حيث تم نزع نسيج القلب ووضعه في محلول متجانس من ملح الفوسفات المحايد ، وضع المحلول بعد ذلك في آلة الطرد المركزي للحصول على السائل المستخلص الذي من خلاله تم قياس مالوندهيد (الأنزيم الخاص بالأكسدة الذاتية للدهون) ، استخدم قياس تحليل المتغيرات (أنوفا) حيث تبين أن هناك فروق ذات دلالة إحصائية بين المجموعات الثلاث ، وقد أظهرت النتائج انخفاضاً في أنزيم مالوندهيد أدى إلى اتزان في الأكسدة حيث أن عدم الاتزان يؤدي إلى ضغط الأكسدة .

الكلمات الدالة : تدريبات التحمل – ضغط الأكسدة – الأكسدة الذاتية للدهون .