Asian Journal of Multidisciplinary Studies
Volume 2, Issue 7, July 2014

ISSN: 2321-8819 (Online)
2348-7186 (Print)
Impact Factor: 0.923

Change in the Malondialdehyde status of heart following exhaustive exercise in Rats

Bijan Goodarzi[1] and Amir Khosravi[2]
[1]Physical Education Department, Islamic Azad University, Boroujerd Branch,Boroujerd, Iran
[2]University of Ayatollah Alozma Boroujerdi, Boroujerd, Lorestan, Iran

Abstract

The aim of this study was to investigate of eight weeks treadmill training attenuates exercise-induced oxidative stress in rat heart. 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.

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 heart damage and endurance training attenuated exercise-induced oxidative stress in heart after a single bout of exhaustive treadmill running, probably by reinforced of antioxidant defense system during exercise.

Keywords: acute exercise**,** oxidative stress, heart

INTRODUCTION

Exercise is known to promote reactive oxygen species (ROS) production in humans and animals, mainly through increased leakage of oxygen-centered free radicals from the mitochondrial electron transport chain, ischemia-reperfusion-induced activation of xanthine oxidase, NAD(P)H-dependent superoxide release from activated neutrophils (Deaton and Marlin 2003) . ROS-based chemical attacks can lead to molecular damages and to oxidative stress, thereby cells possess highly conserved defense systems fulfilled by multiple interactions of antioxidant compounds, antioxidant enzymes, damage-removal and repair enzymes (König, Wagner et al. 2000) . Despite the increased ROS production during exercise, growing evidence derived from epidemiological and prospective studies strongly indicates that habitual, moderate physical activity reduces the incidence of oxidative stress-based diseases and retard the aging process (Ji, GOMEZ-CABRERA et al. 2006; Laukkanen, Rauramaa et al. 2011). This apparent paradox can be explained taking into account that reactive oxygen species produced during repeated exercise bouts could serve as mild stimulating stressors able to trigger hormetic responses. Organic adaptations can involve changes in cardiorespiratory and muscular physiology, the activation of redox-sensitive transcription pathways and the induction of the major endogenous defense systems. Indeed, Knez et al. (2006) (Knez, Coombes et al. 2006) and Radak, Chung et al. 2008 (Radak, Chung et al. 2008) demonstrated that exercise training triggers adaptations in body's antioxidant defense systems.

Given that around 2–5% of oxygen consumed can result in RONS generation, an increased cardiac oxidative metabolic rate arising from physical exercise becomes a predisposing condition for increased mitochondrial production of RONS, leading to alterations in the cellular redox state and activating pathways of cell death (Halliwell and Gutteridge 1999) . However, if this situation is repeated over time, it may have a strong modulating effect on various defense systems in cardiac cells (Ji 1995; Atalay and Sen 1999; Powers and Demirel 2001; Powers, Lennon et al. 2002). Many sources of stress, such as heat, irradiation, hyperoxia, hypoxia, inflammation and any increases in metabolism including exercise, injury, and even repair processes lead to increased production of free radicals and associated RONS. However, and in clear contrast to the conventional idea that reactive species mostly serve as a trigger for oxidative damage of biological structures, it is known that low physiologically relevant concentrations of RONS can regulate a variety of key mechanisms due to its role as signalling molecules (Nemoto, Takeda et al. 2000; Finkel 2001) .It seems consensual that acute prolonged exercise induces loss of cardiac homeostasis seen for instance in changed levels of oxidative stress, damage and apoptosis (Ji 1995; Atalay and Sen 1999; Phaneuf and Leeuwenburgh 2001; Powers, Lennon et al. 2002) . Conversely, as regular exercise has been used as a therapeutic measure in patients with chronic heart disease (Shern-Brewer, Santanam et al. 2000; Thompson, Buchner et al. 2003) and may lead to increased tolerance against adverse stressors that greatly exacerbate cardiac

oxidative stress such as ischemia–reperfusion (I/R), diabetes, acute severe exercise or doxorubicin (DOX-adriamycin) treatment (Somani, Frank et al. 1995; Venditti and Di Meo 1996). Despite the scarcity of direct evidence of increased oxidant production after acute exercise (Ohkuwa, Sato et al. 1997; Bejma, Ramires et al. 2000), changes in antioxidant systems as well as in myocardial oxidative injury markers following acute exercise are strong indirect signs of several redox disturbances. In fact, regardless the data provided by some studies reporting unchanged levels of malondialdehyde and carbonyls in whole heart after exhaustive running (Khanna, Atalay et al. 1999; Bejma, Ramires et al. 2000), increases in lipid peroxidation (Venditti and Di Meo 1996; Venditti and Di Meo 1997; Aydin, Ince et al. 2007) as well as in protein oxidation (Aydin, Ince et al. 2007) in rat hearts after a period of exhausting swimming have been described. The significant decrease of whole heart antioxidant capacity after exhausting swimming in both male and female rats may also be indicative of an additional RONS production (Venditti and Di Meo 1996).

The oxidative stress related effects on heart remained to be studied. The purpose of this study, therefore, was to determine the, whether endurance training alters the oxidative damage caused by acute exhaustive exercise in the heart. This study examined the effects of acute exhaustive exercise and 8-week endurance training on heart 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 heart were quickly removed, washed and stored at -80°C until analysis. The other half of all rats (N=12) underwent anesthesia immediately before the acute exhaustive exercise, then heart tissue was obtained according to the same program. These samples were used for the measurement levels of total protein concentration and MDA rat heart.

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). Samples were homogenized in ice-cold trichloroacetic acid (1 g tissue in 10 ml 10% trichloroacetic acid) in a tissue homogenizer (Heideloph Diax 900, Germany). Following centrifugation of the homogenate at 3,000 rpm for 10 min (Hermle Z 323 K, Germany), 750 µl of supernatant was added to an equal

volume of 0.67% (m/v) thiobarbituric acid and heated at 100□C for 15 min. The absorbances of the samples were measured at 535 nm. Lipid peroxide levels are expressed in terms of MDA equivalents using an extinction coefficient of 1.56 □□105 mol/cm.

Protein Determination

The protein content was measured colorimetrically by the method of Bradford (Bradford 1976) using bovine serum albumin (1 mg/ml) as standard. The sample was preincubated in an alkaline solution containing EDTA, which denaturates the protein and eliminates interference from magnesium ions. Benzethonium chloride is then added, producing a turbidity that was read at 600 nm.

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 ± 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). heart 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 ($p<0.05$).

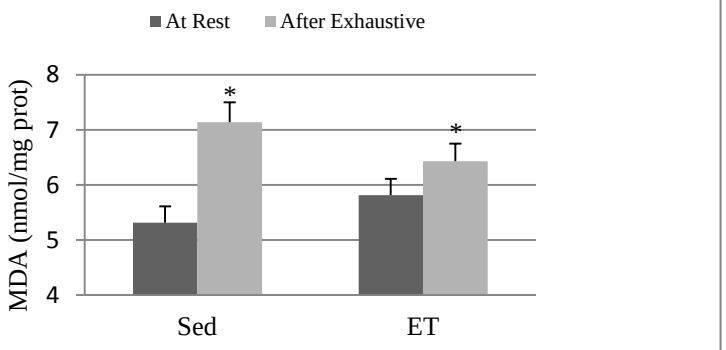

Figure 1. The level of MDA rat heart both befor and after an exhaustive protocol test.*P<0.05 significantly different between rest and after exhausted in two groups of Sed and ET.

Discussion

Regular physical exercise has many health benefits including a lowered threat of all-cause mortality along with a reduced risk of cardiovascular disease, cancer, and diabetes (Blair, Cheng et al. 2001).Paradoxically, it is also clear that contracting skeletal muscles generate free radicals and that prolonged and intense exercise can result in oxidative damage to cellular constituents (Alessio and Goldfarb 1988). The amount of oxidative damage caused by exercise can differ between various tissues and organs (Liu, Yeo et al. 2000).Previous research has established a number of different conditions that may precipitate various forms of cardiac muscle dysfunction (Ji 1995). As the central organ of the cardiovascular system with elevated aerobic metabolism, the heart is continuously working and has one of the highest oxygen consumption rates among all body tissues, which seems to favor reactive oxygen species (ROS) production. In fact, the weakness of the heart to oxidative damage may be in part explained by the fact that heart demonstrates a slow turnover and relatively lower levels of antioxidant enzyme activity when compared to most other tissues. Growing experimental evidence suggest that the increased production of ROS may play an important role in these dysfunctions (Lefer and Granger 2000) .Among other cell sources, heart mitochondria electron transport chain has been referred as one of the major sites of ROS production, through the so-called electron leakage. Whereas the activity of mammalian cytocrome C oxidase is O2-saturated at very low O2 tensions, the rate of electron leakage by mitochondria increases at high O2 concentrations during certain conditions such as exercise (Di Meo and Venditti 2001), favoring enhanced ROS production. Exhaustive exercise, such as run on a treadmill, affects the level of MDA in the heart of both trained and untrained rats. In the present study we found that heart MDA levels a significant increase in TBARS in the two groups of animals after exhaustive run on a treadmill. In agreement with the present findings, Venditti *et al* and Aydin *et al* have reported that the after exhaustive swimming exercise, increases in MDA levels in the heart tissue (Venditti and Di Meo 1997; Aydin, Ince et al. 2007). On the contrary, Gul *et al*. (Gul, Demircan et al. 2006) have reported that the MDA level in the heart tissue was not affected in rats that ran on the treadmill until exhaustion. Similarly, Liu *et al*. (Liu, Yeo et al. 2000) have observed that

level of MDA in the heart and skeletal muscle tissues are not different between the control and exhausted rats. An interesting finding in our research is that regular 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 regular run on a treadmill may provide protection to acute insult in the heart mitochondria herein measured by oxidative stress markers. It seems probable that endurance exercise training provides myocardial protection against many cardiac insults. Although the exact mechanisms responsible for this protection continue to be debated, it has been argued that they are in part, associated with the decreased free radical production and with increased response of antioxidant defense systems (Ji 1995). As well as regular exercise can also reduce acute exercise-induced oxidative stress (Alessio and Goldfarb 1988; Jenkins, Krause et al. 1993; Sen and Packer 2000). In conclusion, our results indicate that heart MDA level is affected by an acute exhaustive exercise. We demonstrate that acute exhaustive exercise increases lipid peroxidation in heart, especially in untrained rats. However, we find that, endurance training decreased the levels of MDA in the rat heart induce of acute exhaustive exercise.

References

Alessio, H. M. and A. H. Goldfarb (1988). "Lipid peroxidation and scavenger enzymes during exercise: adaptive response to training." Journal of Applied Physiology **64**(4): 1333-1336.

Atalay, M. and C. K. Sen (1999). "Physical Exercise and Antioxidant Defenses in the Hearta." Annals of the New York Academy of Sciences **874**(1): 169-177.

Aydin, C., E. Ince, et al. (2007). "Protective effects of long term dietary restriction on swimming exercise-induced oxidative stress in the liver, heart and kidney of rat." Cell biochemistry and function **25**(2): 129-137.

Bejma, J., P. Ramires, et al. (2000). "Free radical generation and oxidative stress with ageing and exercise: differential effects in the myocardium and liver." Acta physiologica scandinavica **169**(4): 343-351.

Blair, S. N., Y. Cheng, et al. (2001). "Is physical activity or physical fitness more important in defining health benefits?" Medicine and science in sports and exercise **33**(6; SUPP): S379-S399.

Bradford, M. M. (1976). "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding." Analytical biochemistry **72**(1): 248-254.

Brooks, G. A. and T. P. White (1978). "Determination of metabolic and heart rate responses of rats to treadmill exercise." Journal of Applied Physiology **45**(6): 1009-1015.

Deaton, C. M. and D. J. Marlin (2003). "Exercise-associated oxidative stress." Clinical Techniques in Equine Practice **2**(3): 278-291.

Di Meo, S. and P. Venditti (2001). "Mitochondria in exercise-induced oxidative stress." Neurosignals **10**(1-2): 125-140.

Esterbauer, H., R. J. Schaur, et al. (1991). "Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes." Free Radical Biology and Medicine **11**(1): 81-128.

Finkel, T. (2001). "Reactive oxygen species and signal transduction." IUBMB life **52**(1): 3-6.

Gul, M., B. Demircan, et al. (2006). "Effects of endurance training and acute exhaustive exercise on antioxidant defense mechanisms in rat heart." Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology **143**(2): 239-245.

Halliwell, B. and J. M. Gutteridge (1999). Free radicals in biology and medicine, Oxford university press Oxford.

Jenkins, R., K. Krause, et al. (1993). "Influence of exercise on clearance of oxidant stress products and loosely bound iron." Medicine and science in sports and exercise **25**(2): 213-217.

Ji, L. L. (1995). "Exercise and oxidative stress: role of the cellular antioxidant systems." Exercise and sport sciences reviews **23**(1): 135-166.

Ji, L. L., M. C. GOMEZ-CABRERA, et al. (2006). "Exercise and hormesis." Annals of the New York Academy of Sciences **1067**(1): 425-435.

Khanna, S., M. Atalay, et al. (1999). "α-lipoic acid supplementation: tissue glutathione homeostasis at rest and after exercise." Journal of Applied Physiology **86**(4): 1191-1196.

Knez, W. L., J. S. Coombes, et al. (2006). "Ultra-endurance exercise and oxidative damage." Sports Medicine **36**(5): 429-441.

König, D., K. Wagner, et al. (2000). "Exercise and oxidative stress: significance of antioxidants with reference to inflammatory, muscular, and systemic stress." Exercise immunology review **7**: 108-133.

Laukkanen, J. A., R. Rauramaa, et al. (2011). "Intensity of leisure-time physical activity and cancer mortality in men." British journal of sports medicine **45**(2): 125-129.

Lefer, D. J. and D. N. Granger (2000). "Oxidative stress and cardiac disease." The American journal of medicine **109**(4): 315-323.

Liu, J., H. C. Yeo, et al. (2000). "Chronically and acutely exercised rats: biomarkers of oxidative stress and endogenous antioxidants." Journal of Applied Physiology **89**(1): 21-28.

Navarro, A., C. Gomez, et al. (2004). "Beneficial effects of moderate exercise on mice aging: survival, behavior, oxidative stress, and mitochondrial electron transfer." American Journal of Physiology-Regulatory, Integrative and Comparative Physiology **286**(3): R505-R511.

Nemoto, S., K. Takeda, et al. (2000). "Role for mitochondrial oxidants as regulators of cellular metabolism." Molecular and cellular biology **20**(19): 7311-7318.

Ohkuwa, T., Y. Sato, et al. (1997). "Glutathione status and reactive oxygen generation in tissues of young and old exercised rats." Acta physiologica scandinavica **159**(3): 237-244.

Phaneuf, S. and C. Leeuwenburgh (2001). "Apoptosis and exercise." Medicine and science in sports and exercise **33**(3): 393-396.

Powers, S. K. and H. Demirel (2001). "Exercise, heat shock proteins, and myocardial protection from IR injury." Medicine and science in sports and exercise **33**(3): 386-392.

Powers, S. K., S. L. Lennon, et al. (2002). "Exercise and cardioprotection." Current opinion in cardiology **17**(5): 495-502.

Radak, Z., H. Y. Chung, et al. (2008). "Systemic adaptation to oxidative challenge induced by regular exercise." Free Radical Biology and Medicine **44**(2): 153-159.

Sen, C. K., E. Marin, et al. (1992). "Skeletal muscle and liver glutathione homeostasis in response to training, exercise, and immobilization." Journal of Applied Physiology **73**(4): 1265-1272.

Sen, C. K. and L. Packer (2000). "Thiol homeostasis and supplements in physical exercise." The American journal of clinical nutrition **72**(2): 653s-669s.

Shern-Brewer, R., N. Santanam, et al. (2000). "The paradoxical relationship of aerobic exercise and the oxidative theory of atherosclerosis." Sen CK, Packer L, Hanninen O: 1053-1067.

Somani, S., S. Frank, et al. (1995). "Responses of antioxidant system to acute and trained exercise in rat heart subcellular fractions." Pharmacology Biochemistry and Behavior **51**(4): 627-634.

Thompson, P. D., D. Buchner, et al. (2003). "Exercise and physical activity in the prevention and treatment of atherosclerotic cardiovascular disease a statement from the Council on Clinical Cardiology (Subcommittee on Exercise, Rehabilitation, and Prevention) and the Council on Nutrition, Physical Activity, and Metabolism (Subcommittee on Physical Activity)." Circulation **107**(24): 3109-3116.

Venditti, P. and S. Di Meo (1996). "Antioxidants, tissue damage, and endurance in trained and untrained young male rats." Archives of biochemistry and biophysics **331**(1): 63-68.

Venditti, P. and S. Di Meo (1997). "Effect of training on antioxidant capacity, tissue damage, and endurance of adult male rats." International journal of sports medicine **18**(7): 497-502.