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Effect of Exercise and Vitamin E on Cardiac Troponin Alterations in Myocardium and Serum of Rats after Stressful Intense Exercise

N.S. AL-Sowyan
Department of Biology, College of Science, AL-Qassim University,
P.O. Box 30230, Buraydah (51477), Saudi Arabia

Abstract: Increased concentrations of biomarkers reflecting myocardial stress such as cardiac troponin have been observed following strenuous exercise. The aim of this study was to determine whether the stress of forced exercise would result in injury to the myocardium. The effects of stress induced by short bout strenuous exercise and long term exercise on serum, cardiac and skeletal muscle troponin, also blood glucose and insulin were measured. Moreover, to determine whether vitamin E supplementation could modulate these effects or not. Five groups of rats were investigated, control, strenuous exercised rats, exercised and supplemented rats with vitamin E, long term exercise and long term exercised rats supplemented with vitamin E. Strenuous exercised rats and supplemented rats with vitamin E. produced significant increase in serum, cardiac and skeletal muscle troponin concentration. Long term exercise and long term exercised rats supplemented with vitamin E induced insignificant elevation of serum and muscle troponin concentration with significant increase in cardiac troponin level. In rats subjected to both strenuous and long term exercise and after supplementation of both group with vitamin E, there was a significant decrease in blood glucose and insulin level. These results suggest that stressful exercise induces alteration in myocardial troponin and that training before exercise and vitamin E attenuates the exercise induced heart damage. Accordingly, we can advise individuals who are subjected to strenuous exercise to supplement their diet with vitamin E to protect their heart from myocardial damage and sudden death which may be recorded in some athletes. Furthermore, these results demonstrate another support for the importance of exercise in diabetes mellitus.

Key words: Strenuous, stress, myocardium, heart damage, oxygen species

INTRODUCTION

Exercise increases the generation of oxygen free radicals and lipid peroxidation. Strenuous exercise in a person who is unconditioned or unaccustomed to exercise will induce oxidative damage and result in muscle injury (Evans, 2000).

Epidemiological studies have demonstrated that regular exercise is associated with a reduction in the risk of myocardial events (Dalleck *et al*., 2008). The risk of death from coronary heart disease decreased about twofold in individuals who were physically active compared with less active individuals (Roth *et al*., 2007).

On the other hand, heavy physical exercise has been recognized as a factor that triggers severe myocardial events such as impaired ventricular performance (Sirenko *et al*., 2006), cardiac ischemia (Di Napoli *et al*., 2007), cardiac arrest myocardial injury (Ortega *et al*., 2006).

Konig *et al*. (2007a) reported an increased concentrations of biomarkers following strenuous, long lasting endurance exercise. It appears a greater concentration of biomarkers may be as a reflection of myocardial stress.

On the other hand, exercise causes protein oxidation in skeletal muscle after a single bout of exercise, which appeared to be related to an exercise induced decrease in lipophilic antioxidants (Iborra *et al*., 2008).

It was suggested that supplementation of antioxidants including vitamin E will reduce oxidative stress and rates of lipid peroxidation and that vitamin E requirements may increase in exercise (Dillard *et al*., 1978).

The goal of this study was to determine whether the stress of forced exercise would result in injury to the myocardium.

MATERIALS AND METHODS

This study was designed to evaluate the effect of stressful exercise on cardiac, skeletal muscle and serum troponin, fasting blood glucose and serum insulin levels.

In addition, this work aimed to studying the possible modulation of such stressful exercise response after using vitamin E.

Forty adult male albino rats were used, their body weight ranged from 150-200 g. All animals were maintained under the prevailing atmospheric condition throughout the experimental period and maintained on commercial rat chow.

The rats were subjected to a reversed 12 h light dark cycle and exercised during their most active period.

- The experiment was carried out through February-April 2009
- The rats were divided into the following group's eight rats each
- **Group I:** Served as a control (not exercised)
- **Group II:** Short bout strenuous (short term) exercised group
- **Group III:** Short bout strenuous (short term) exercised group supplemented with vitamin E in a dose 75 mg kg^{-1} body weight orally for 4 weeks 5 days/week (Reznick *et al*., 1992)
- **Group IV:** Long term exercised group
- **Group V:** Long term exercised group supplemented with vitamin E in a dose 75 mg kg^{-1} body weight orally for 4 weeks 5 days $week^{-1}$

Exercise Protocol

All rats were accustomed to the treadmill exercise for variable periods. After a week of acclimatization, exercise were performed.

Animals in group II, III (short bout exercise) were made to exercise on the treadmill at 10 m min^{-1} for (90-120 min) until exhaustion, which is defined as loss of righting reflex when the rats were placed on a supine position (Bejma *et al*., 2000). Then rats were immediately anesthetized, the blood was collected from the orbital sinus according to Simmons and Brick (1987).

The animals in long term exercise group were exercised at the desired level at a rate 10 m min^{-1} for 10 min day^{-1}, then the animals were exercised at this level for 4 weeks, the animals were rested for two days, before scarification (Somani and Arroyo, 1995) and blood was collected from the orbital sinus.

The control group were kept in the used treadmill without exercise for a period similar to the period of exercise protocol according to Somani and Arroyo (1995).

Blood samples were centrifuged and the separated sera were analyzed for assessment of serum troponin T, glucose and insulin. Also frozen myocardial and gastrocnaemius muscle were homogenized and centrifuged. The supernatants were used for western blotting according to O'Brien *et al*. (1998).

All biochemical results were expressed as Mean±SE. Significant differences among the groups were determined by one way analysis of variance followed by Students t-test.

RESULTS AND DISCUSSION

Table 1 shows that short strenuous exercise and short term exercised rats supplemented with vitamin E produced significant increase in serum, cardiac and skeletal muscle troponin concentration compared to the control group. While long term exercise (15 min day^{-1}) and long term exercised supplemented with vit. E resulted in insignificant elevation of serum and muscle troponin concentration with significant increase in cardiac troponin in comparison to the control group.

In rats subjected to both short strenuous exercise and long term exercise and after supplementation of both group with vit. E., there was a significant decrease in blood glucose and insulin levels compared to the control rats (Table 2).

Serum and cardiac troponin (cTn 1) measurements are specific in the assessment of cardiac injury in the presence of skeletal muscle damage (Shave *et al*., 2007).

In the present study, strenuous exercise and long lasting endurance exercise showed very highly significant increase in serum, cardiac and skeletal muscle troponin, meanwhile after long moderate exercise an insignificant increase in both serum and skeletal muscle troponin level while cardiac troponin T showed a significant increase.

Supplementation of vitamin E after short strenuous exercise still produced significant increase in serum, cardiac and skeletal muscle troponin but the level of significance was decreased. However the same results were obtained after supplementation with vitamin E to long moderate exercised rats.

Table 1: The changes in serum, cardiac and skeletal muscle troponin concentration (ng nL^{-1}) after short term and long term exercise and after supplementation of vitamin E

	Serum		Cardiac		Muscle	
Group	Mean±SE	Level of significant	Mean±SE	Level of significant	Mean±SE	Level of significant
Control	0.19±0.05	-	0.14±0.04	-	0.11±0.04	-
Short term exercise	0.67±0.08	0.0005***	0.71±0.06	0.0005***	0.64±0.07	0.0005***
Short term exercise +vit. E	0.32±0.06	0.0125**	0.37±0.04	0.0025**	0.25±0.03	0.0125**
Long term exercise	0.18±0.03	0.45	0.23±0.02	0.025*	0.17±0.03	0.15
Long term exercise +vit. E	0.20±0.03	0.20	0.22±0.03	0.05*	0.12±0.03	0.45

Mean difference is significant at the 0.05 level. *Significant p<0.05, **Highly significant p<0.01, ***Very highly significant p<0.001

Table 2: The changes in fasting blood glucose level (mg %) and serum insulin level (μ mL^{-1}) after short term and long term exercise and after supplementation of vitamin E

	Blood glucose		Serum insulin	
Group	Mean±SE	Level of significant	Mean±SE	Level of significant
Control	93.00±1.68	-	4.26±0.62	-
Short term exercise	79.13±4.24	0.005**	0.25±0.057	0.005**
Short term exercise+vit. E	69.88±3.61	0.0005***	0.31±0.074	0.0005***
Long term exercise	57.00±5.40	0.0005***	0.51±0.11	0.0005***
Long term exercise+vit. E	55.88±2.13	0.0005***	1.00±0.33	0.0025**

Mean difference is significant at the 0.05 level. **Highly significant p<0.01, ***Very highly significant p<0.001

It is well known that serum and cardiac troponin T is highly sensitive and specific marker for myocardial injury in human and animals (Kukla *et al*., 2007; Bass *et al*., 2009).

Present results indicated that serum and cardiac troponin T were increased a highly indicative of myocardial injury and acute depression in cardiac function (Dawson *et al*., 2008).

However, present finding are in agreement with recent findings of (Konig *et al*., 2007a) who recorded that exercise cause myocardial damage as indicated by increased serum troponin T concentration. Michielsen *et al*. (2008) reported a transient cardiac dysfunction after prolonged strenuous running in healthy athletes.

Furthermore, several recent reports that used cTnT as a cardiospecific biomarkers showed the occurrence of subclinical and irreversible myocardial injury during long term endurance exercise in health people (Konig *et al*., 2007b; Lippi *et al*., 2008).

Present results revealed the protective effect of vitamin E supplementation in strenuous exercise induced myocardial and skeletal muscle damage.

However, in long moderate exercise, our findings revealed that troponin levels are less than in strenuous exercise clarifying that the degree of impairment is decreased in these groups. Also, the changes before and after supplementation with vitamin E as antioxidant the same results were obtained.

These results can be related to different metabolic processes between strenuous and moderate exercise including alteration in glucose and fatty acid utilization (Stiegler *et al*., 2008).

Present findings also can be explained by the protective effects of long moderate exercise induced myocardial and skeletal damage as a consequence of training (Leetmaa *et al*., 2008).

These results suggest that moderate exercise for long period could protect the heart and skeletal muscle from damage.

From other side, the results indicate that strenuous exercise induced highly significant decrease in fasting blood glucose and insulin level, while long term moderate exercise demonstrated very highly significant decrease in fasting blood glucose and insulin levels.

These results are in agreement with Kristiansen *et al*. (2000) and Hawley (2004) who attributed the decrease in blood glucose and insulin to the fact that the contracting muscle develops superior glucose utilization capacity and trained rats can utilize glucose at a high rate during exercise.

However, Colberg (2007) revealed that glucose transport into muscle cells can be activated by at least two distinct mechanisms: one stimulated by insulin and the other activated by contraction exercise. Phosphatidylinositol 1-3-kinase (PI3-kinase) is involved in insulin-stimulated Glut-4 translocation and glucose transport, whereas 5'-AMP-activated protein kinase (AMP-kinase) is likely to be involved in the contraction response (Goodyear, 2000; Higaki *et al*., 2008).

The effects of exercise are not dependent on the insulin-signaling pathways, but do have a beneficial effect on insulin action. Muscle contraction increases AMP: ATP ratio, resulting in the activation of AMP-kinase. In turn, AMPK activation leads to an increase in glucose transport possibly through plasma membrane (Dohm, 2002). AMPK can also phosphorylate and activate endothelial NO synthesis (eNOS) and NO production might contribute to exercise-stimulated glucose transport as well as improvement in endothelial function (Hardie, 2004). Also, as peroxisome proliferator-activated receptor coactivator-1-α(PGc-1-α) is increased after exercise, it may be involved in mediating the exercise-induced increase in muscle Glut-4 content and mitochondrial proliferation, thereby regulating the capacity of muscle to take-up and oxidize glucose (Wu *et al*., 1999; McCarthy, 2005). Also, Misra *et al*. (2008) reported that moderate exercise resulted in significant improvement in insulin sensitivity and glycerin.

In the present study supplementation of vitamin E to exercised rats produced the same changes in blood glucose level as regard to rats subjected to short and long exercise. These results can possibly due to that our animals reached maximum activation of Glut-4 or AMPK before supplementation of vit.E (Ryder *et al*., 2001).

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