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Effects of Ascorbic Acid Supplementation on Lipid Peroxidation and Glutathione Levels in Exhaustive Physical Exercise

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The purpose of this study was to examine the effects of exhaustive physical exercises on lipid peroxidation in blood, liver and skeletal muscle and also to assess the effect of ascorbic acid on the differentiation in antioxidant mechanism in rats. For this purpose rats were divided into four groups; control, exhaustive exercise, ascorbic acid supplementation + exhaustive exercise and ascorbic acid supplementation. Exercised rats were run on a rodent treadmill at a speed of 27 m/min at % 15 grades. Average exhaustion time was determined as 55 min. Ascorbic acid was administered 20 mg/kg/d i.p. for 45 d prior to exercise. Thiobarbituric acid reactive substance, glutathione and ascorbic acid amounts were measured by spectrophotometer. As a result of this study, statistical significance of changes were observed in thiobarbituric acid reactive substance amounts in liver and plasma; glutathione levels in muscle and plasma and ascorbic acid amounts in liver especially in the group in which ascorbic acid was supplemented. The fact that the ascorbic acid supplement was performed in exhaustive physical exercises comprised the different responses in the distinct tissues due to the antioxidant effects. On the other hand ascorbic acid supplementation without exercise played an oxidant role in different tissues.

Key Words: Thiobarbituric acid reactive substance, Exhaustive exercise, Vitamin C, Glutathione, Rat.

INTRODUCTION

During exercise the oxygen uptake of skeletal muscle can increase up to more than 100 times[1]. Strenuous physical exercise results in enhanced uptake of oxygen leading to increased metabolism, which in turn increases

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the production of free oxy radicals[2]. Under oxidative stress the increases of these toxic oxygen species can elicit widespread damage to constituents of the cell such as membrane lipids, mitochondria and DNA[3]. A direct relationship between exercise intensity and lipid peroxidation has been reported[4]. The major cause for the oxidative damage of acute physical exercise on liver, skeletal muscles, blood and other tissues[5,6] is the increase of the malondialdehyde (MDA), the final product of lipid peroxidation determined as thiobarbituric acid reactive substance (TBARS)[7]. Many scientists stated that exercise dependent muscle injuries and muscle fatigue are caused by the formation of free radicals[8,9] which increases the oxidative stress and thus the reactive oxygen species. Davies *et al.*[10] show that in gastrocnemius, soleus and plantaris muscles MDA content increased 80 % following an exhaustive exercise.

Organisms have evolved enzymatic and non-enzymatic systems for scavenging free radicals and destroying potentially harmful products before further damage can occur. The non-enzymatic system consists mainly of β-carotene, α-tocopherol, ascorbic acid and various sulphydryl compounds whereas the enzymatic system consists largely of superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase[11].

The potential of dietary antioxidants as an endogenous defense to detoxify lipid peroxides produced during exercise has received increasing attention in recent years[12]. Ascorbic acid provides in vivo antioxidant protection primarily as an aqueous phase peroxyl and oxygen radical scavenger; it is concentrated in tissues and fluids with a high potential for radical generation, such as the eye, brain, liver, lung, heart, semen and leukocytes[13]. Some studies have reported that supplementation with antioxidants such as ascorbic acid can reduce symptoms or indicators of oxidative stress as a result of exercise[14]. In experimental animals which are not able to synthesize ascorbic acid, the lack of ascorbic acid was shown to affect the running time[15].

Thus, the purpose of this study was to examine the responses of the antioxidant enzyme system and lipid peroxidation in rat skeletal muscle, liver and blood to acute physical exercise stresses and their interaction. Furthermore to determine of the adaptations of lipid peroxidation and antioxidant enzyme to exhaustive physical exercise under normal and ascorbic acid supplement conditions. The hypotheses were as follows: (I) Exhaustive exercise would result in oxidative stress as indicated by an increase in at least one of the biomarkers (TBARS and glutathione status) in the blood, liver and skeletal muscle; (II) antioxidant supplementation (ascorbic acid) would reduce the extent of oxidative stress as compared to the normal diet.

EXPERIMENTAL

Test subjects: Female wistar albino rats (The Military Medicine Academy of Gulhane Laboratories, Ankara, Turkey) were obtained at 7 weeks of age. 39 Rats (8 weeks old, 200 g) were randomly divided into four groups: Control (n = 10). The animals have been run till exhaustion without any supplementation (Ex.ex n = 9). The animals have been run till exhaustion after 45 d with ascorbic acid supplementation (20 mg/kg i.p per day) (AAS + Ex.ex n = 10). Control group that was supplemented ascorbic acid during 45 days (AAS n = 10).

Rats were maintained on a 12:12-h light-dark cycle and received food and water ad libitum. All procedures using these animals adhered to the guiding principles of the Ankara Gazi University Council on Animal Care. The study was conducted in the year of 2003.

Exercise protocol: All exercise groups were familiarized with running on a motor-driven treadmill. During the period of familiarization (1 week), rats exercised for 5-10 min/d at a speed of 20 m/min up a 10 % grade. Rats that are classified as acute exercise group ran at 27 m/min. 15% Grade until exhaustion level. Exhaustion was identified by the loose of righting reflex[16]. Average exhaustion time was determined as 55 min.

Taking blood, tissue samples and analysis: The animals in the acute exercise group just following the exhaustion were sacrificed. All rats were killed by decapitation and mixed blood was collected into vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA). Blood was centrifuged at 4 ºC at 3000 rpm for 20 min. Plasma was stored in a -80 ºC freezer until subsequent analyses of plasma TBARS, RSH and ascorbic acid. Plasma ascorbic acid measurements as described in a previous study[17] and for tissue ascorbic acid measurements as described by Berger *et al.*[18] in the same day. Portions of the soleus muscle and liver were excised immediately and placed in liquid nitrogen and stored at -80 ºC.

Lipid peroxidation was assessed by measurement of thiobarbituric acid-reactive substances (TBARS). The release of TBARS from muscle and liver was used to assess lipid peroxidation as previously described[19]. Plasma TBARS and determination of total sulfhydrly (RSH) groups measurements as described by Kurtel *et al.*[20]. The determination of liver and soleus muscle total glutathione (GSH) according to the details described earlier[21].

Statistical analysis: Significance of changes in means was tested with ANOVA and Kruskal-Wallis test. The results are expressed as mean ± standard deviation (SD). Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

As a result of this study, while the muscle GSH value of group AAS which ascorbic acid was supplied had the highest value, there were significant differences between control, Ex.ex, AAS + Ex.ex and AAS groups ($p < 0.05$) There was also no statistical difference between other parameters of both groups (Table-1).

TABLE-1
MUSCLE LEVELS OF TBARS, GSH AND AA (Mean ± SD)

Groups (n)		TBARS (nmol/g tissue)	GSH (μmol/g tissue)	AA (mg/g tissue)
I	Control (10)	60.73 ± 31.9	4.15 ± 1.6	6.46 ± 3.7
II	Ex ex. (9)	95.63 ± 54.2	4.79 ± 1.2	5.56 ± 2.2
III	AAS + Ex ex. (10)	70.01 ± 26.1	4.44 ± 1.8	4.69 ± 1.8
IV	AAS (10)	59.35 ± 45.3	9.27 ± 4.3	7.85 ± 3.2

(GSH I-IV, II-IV and III-IV) $p < 0.05$

Mean liver TBARS values were significantly high according to the controls after the exhaustive exercise and ascorbic acid supplementation ($p < 0.05$). In addition there was significant difference between groups Ex.ex and AAS + Ex.ex and AAS + Ex.ex and AAS ($p < 0.05$). In the liver ascorbic acid levels, while they had the highest values in AAS group which was supplied, there were statistically significant differences between control, Ex.ex, AAS + Ex.ex and AAS and also Ex.ex and AAS + Ex.ex groups ($p < 0.05$) (Table-2).

TABLE-2
LIVER LEVELS OF TBARS, GSH AND AA (Mean ± SD)

Groups (n)		TBARS (nmol/g tissue)	GSH (μmol/g tissue)	AA (mg/g tissue)
I	Control (10)	51.85 ± 11.9	5.46 ± 1.1	13.66 ± 5.7
II	Ex ex. (9)	83.22 ± 19.4	5.10 ± 0.7	9.17 ± 1.9
III	AAS + Ex ex. (10)	45.88 ± 16.1	5.85 ± 0.9	17.04 ± 1.3
IV	AAS (10)	96.21 ± 41.3	5.53 ± 1.0	26.75 ± 6.1

(TBARS I-II, I-IV, II-III and III-IV; AA I-IV, II-III, II-IV and III-IV) $p < 0.05$

When considering the results of plasma TBARS, there was only statistically significant difference between AAS + Ex.ex and AAS groups ($p < 0.05$) (Table-3). In plasma RSH values, while group AAS RSH level was also in the highest value, there were significant differences between control and group AAS ($p < 0.05$) (Table-3).

TABLE-3
PLASMA LEVELS OF TBARS, RSH AND AA (Mean ± SD)

	Groups (n)	TBARS (nmol/g tissue)	RSH (μmol/g tissue)	AA (mg/g tissue)
I	Control (10)	4.24 ± 1.3	138.54 ± 45.80	75.50 ± 18.4
II	Ex ex. (9)	4.35 ± 1.1	181.91 ± 61.50	78.33 ± 33.3
III	AAS + Ex ex. (10)	3.31 ± 0.9	187.56 ± 63.60	48.50 ± 15.8
IV	AAS (10)	4.71 ± 0.6	254.54 ± 86.99	67.50 ± 30.2

(TBARS III-IV; RSH I-IV) $p < 0.05$

Although there are benefits of physical exercise, there are so many studies claiming that strenuous and excessive exercise causes an imbalance between ROS and anti oxidant defenses as a result of oxidative stress in the body[22]. There are indications that the generation of oxygen free radicals may be the underlying mechanism for exercise-induced oxidative damage[23].

In present study, we carried out exhaustive exercise on rats which are able to synthesize ascorbic acid contrary to humans and instead of adding AA into the drinking water of animals, we examined the effect of 45 d intraperitoneally administratrated ascorbic acid on lipid peroxidation and GSH levels in blood, liver and muscle tissues.

The present study indicated that there was a marked increase in the amount of TBARS in liver as a result of acute exhaustive exercise. This had been documented by numerous investigations demonstrating increases in lipid peroxidation following strenuous exercise in various tissues[10,24-26]. In some other studies enhanced lipid peroxidation was also shown after exhaustive exercise in liver[27]. Decreased TBARS levels were observed in the group supplemented with ascorbic acid before exercise compared to the exercised group with no vitamin administration in liver. Several studies[28,29] have suggested that vitamin C is an important antioxidant *in vitro*. Although the antioxidant mechanism of vitamin C is well established, the importance in protecting against exercise-induced oxidative stress is not clear. Also we found that there was a significant increase in liver TBARS values as a result of daily intraperitoneal ascorbic acid injection alone. Ascorbic acid has not only antioxidant but also prooxidant effect by reducing the ferric ions into ferrous state by catalyzing the Fenton reaction which causes the generation of ascorbate hydroxyl radical ions and starts the lipid peroxidation. However it is not easy to predict the ascorbate effects. Although liver is equipped with abundant antioxidant enzymes and other scavenging systems, the high metabolic rate and central role in detoxification, makes it one of the main targets for free radical-mediated damage in the body. This made us to think the pro-oxidant effect of certain dose of ascorbic

acid on liver, the center of all metabolic processes, became effective. Based upon these findings we recommend using ascorbate on individuals prior to traumatic exercise. Also it seems that excessive intake of ascorbic acid seems to be harmful. The fact that the amount of ascorbic acid was the highest in liver tissue in ascorbic acid loaded group compared with other groups supporting the idea of the pro-oxidant effect.

On the other hand similar results were found in plasma TBARS levels as found in liver. So these results can be interpreted as done for liver before about the antioxidant and prooxidant effects of ascorbic acid. Although in our study we could not find a significant change in plasma TBARS level as a result of acute exercise, there are some studies showing that plasma lipid peroxidation increased in subjects doing intensive exercise[30] but TBARS levels of the subjects having exercises with same protocol but in a less intensive fashion were significantly higher compared with others[31]. This situation proves that the oxidative damage which takes place during exercise is related with the intensity and duration of the exercise[32]. Also Alessio *et al.*[4] reported a relationship between exercise intensity and serum MDA values.

GSH plays a multifunctional role in protecting tissues from oxidative damage during exercise[23]. In present study, we could not find a significant change in GSH levels after exhaustive exercise in all tissues. This may be related with the intensity and duration of the exercise. But in present study ascorbic acid supplementation resulted in an increase in GSH levels in muscle and plasma because of the prooxidant effect of ascorbic acid. Sastre *et al.*[33] also found an increase in GSH levels after one week antioxidant administration in rats. The merely administration of ascorbic acid may cause oxidation and to remove the H_2O_2 which is formed in media, formation of GSH may be increased.

The present study shows that ascorbic acid supplementation alone caused increase in ascorbic acid amount in liver. This result may be explained as follows: As the metabolizing organ for ascorbic acid is liver, this loaded ascorbic acid may accumulate in this organ and because of this increased levels of ascorbic acid might be found in liver. But in a study of Witt *et al.*[5] carried out on humans all plasma antioxidant concentrations increased after loading.

In summary, exhaustive exercise increases the lipid peroxidation in liver tissues. So increased levels of lipid peroxidation can be causes by the leakage of free oxy radicals from the other tissue sources. Merely administration of ascorbic acid increases TBARS level in liver tissue because of its oxidant effect. But ascorbic acid supplementation prior to exercise played an antioxidant role and resulted decreases TBARS level in liver. Also administration of ascorbic acid alone causes increased levels of GSH both in muscle and plasma because of its prooxidant effect. As a result moderate

intake of ascorbic acid may be beneficial before exhaustive exercise but in normal physiological conditions ascorbic acid should be used carefully. Also dose dependent studies of vitamin C should be carried out.

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