Tohoku J. Exp. Med., 2002, **198**, 47-53

The Effect of Vitamin E Treatment on Oxidative Stress Generated in Trained Rats

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Department of Physiology and [1]Department of Biochemistry, Cerrahpaşa Faculty of Medicine, University of Istanbul, [2]Department of Sports Medicine, Istanbul Faculty of Medicine, University of Istanbul, Istanbul, Turkey

METIN, G., ATUKEREN, P., GÜMÜŞTAŞ, M.K., BELCE, A. and KAYSERILIOGLU, A. *The Effect of Vitamin E Treatment on Oxidative Stress Generated in Trained Rats*. Tohoku J. Exp. Med., 2002., **198** (1), 47-53 —— The aim of this study was to investigate the effect of vitamin E treatment on increased oxidative stress in rats exposed to a swimming exercise protocol. In order to examine the effects of physical swimming training on the antioxidant defences of tissues and on their susceptibility to damage induced by exercise, the levels of glutathione (GSH) and thiobarbituric acid reacting substances (TBARS) levels, on indicator of lipid peroxidation in various tissues, have been determined. In this study, four groups of female rats were used while the rats were trained to swim for 30 minutes a day and five days a week which lasted eight weeks and vitamin E (vit. E) supplementation (30 mg/kg/day) has been carried out for five days a week. TBARS levels are significantly found lower in both trained and sedentary vit. E supplemented groups, since vit. E is the most important antioxidant in an earlier line of defence in lipid peroxidation. Also, in vit. E supplemented trained rats, the glutathione response is observed to be significantly higher, supporting with the TBARS levels and in accordance with the literature. But in the sedentary group without vit. E supplementation, the GSH levels of the liver and the heart tissues were significantly lower than both vit. E supplemented sedentary and trained groups. These results evaluate that vit. E confers protection to GSH levels in these tissues where the GSH levels were found significantly lower in the groups not supplemented with vit. E. ———— Vitamin E; glutathione; TBARS; lipid peroxidation; training
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An elevated metabolic rate as a result of exercise can dramatically increase oxygen consumption in the locomotive muscles and heart as well as other tissues (Ji 1999). During exercise, bodily O_2 consumption is greatly increased up to 10 to 15 fold greater than resting levels, it

Received December 11, 2001; revision accepted for publication September 27, 2002.
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is very likely that free radicals are produced to a greater extent compared with rest. Considering that thousands of radicals are produced in each resting cell every day, it is tempting to speculate on the number of free radicals that may be produced as a result of elevated metabolism. Furthermore, during exercise, damage to active tissues is likely to occur, and oxidative stress reactions are known to increase in damaged tissue (Jenkins and Goldfarb 1993; Van Lente 1993).

The reactive oxygen species (ROS) pose serious threat to the cellular antioxidant defence system, such as diminished reserve of antioxidants and incresed tissue susceptibility to lipid peroxidation (Ji 1999). Vitamin E (Vit. E), as a scavenger of peroxyl radicals, is probably the most important inhibitor of the free radical chain reaction of lipid peroxidation (Packer 1991). However, enzymatic and nonenzymatic antioxidants have demonstrated great adaptation to acute and chronic exercise (Allesio and Goldfarb 1988; Evelo et al. 1992; Meydani et al. 1993; Ji 1999). The important role of glutathione (GSH) which is the most abundant thiol source in the cell, in protecting against exercise induced oxidative stress has been reviewed in detail in several articles (Kretschmar and Muller 1993; Ji and Leeuwenburg 1996). The delicate balance between prooxidants and antioxidants suggest that supplementation of antioxidants may be desirable for physically active individuals under certain physiological conditions by providing a larger protective margin (Gerster 1991; Goldfarb 1993).

In this study, the aim we focused on was to determine the antioxidant defence adaptation against the training process and to evaluate the possible effects of vit. E supplementation in trained and sedentary rats, on the antioxidant defence system.

MATERIALS AND METHODS

Animals

Female Wistar Albino rats weighing 169 g to 180 g were used. The rats were randomly divided into four groups. Two of the groups were subjected to swimming training while the other two were the sedentary groups. The animals were kept two to four per cage and had free access to water and normal chow. In order for the exercise to take place during their most active period, the animals were subjected to a reversed 12-hours light cycle. The properties of the groups are given in Table 1.

Exercise

The exercise groups of rats were submitted to swim for 30 minutes a day and five days a week which lasted 8 weeks. Rats swam individually in a 2-liter glass beaker filled with water ~30 cm deep. The beakers were submerged in a thermostatic water bath set at 30°C. The fur of the rat was washed with liquid soap prior to swimming and air bubbles trapped in the fur were removed periodically to reduce buoyancy and ensure the imposed work load.

TABLE 1. *The properties of experimental groups*

Groups	*n*	Initial weight (g)	Finalweight (g)	Vit. E injection	Training
Group 1	7	169±8.3	171± 9	(+)	Trained
Group 2	8	172.5±10	183±7.7	(−)*	Trained
Group 3	7	174±10	184.4±3.2	(+)	Sedentary
Group 4	7	180±5.5	187.5±6	(−)*	Sedentary

*Saline was injected.

Supplementation

During the experimental procedure, one of the trained groups and one of the sedentary groups were treated with vit. E (d-α-tocopheril acetate, Ephynale, Roche, 30 mg/kg/day) injected intraperitonally for five days a week where the other two groups were injected the same amount of saline only. All injections were performed 3 hours before swimming.

Tissue preparation

Eight weeks later, all rats, anesthetized with ether, were sacrificed by decapitation 2 hours after the last exercise. The tissues were removed from all animals in the same order: liver, heart and vastus lateralis muscles from both hind legs. The tissues were immediately cover with ice cold buffer and kept on ice and then immediately transported to a cold room where they were processed to small preparations. All the tissue preparations were frozen on dry ice and then transferred to a −80°C freezer where they were kept until the measurement.

Before the measurement, tissue samples were homogenated with saline (20%, V/V) and sonicated at a medium level. Then homogenates were centrifugated at 2000 rpm for ten minutes and supernatants were used in the biochemical analysis.

Measurement of lipid peroxidation levels

The thiobarbituric acid reacting substances (TBARS) in the tissues was determined as a marker of lipid peroxidation by the spectrophotometric method of Yagi (Ohkawa et al. 1979).

Measurement of GSH levels

GSH levels were determined as nonprotein sulphydryl content by reaction with 5, 5[1]-dithiobis (nitrobenzoic acid; DTNB) by the spectrophotometric method of Ellman (1959). Before the assay the samples were precipitated with a solution containing glacial metaphosphoric acid, disodium ethylene-diamintetraacetic acid and NaCl.

Statistical analysis

All results are from 7-8 animals. Means and S.E. were calculated, and all of the comparisions were performed by using two-way ANOVA and $p<0.05$ was considered significant.

Results

Heart tissue TBARS levels of both the group 2 and the group 4 were higher than the group 1 and 3, (Table 2, two way ANOVA, $p<0.001$). Liver tissue TBARS level of the group 4 was highest followed by the group 2 (two way ANOVA, $p<0.001$). Muscle tissue TBARS levels of the group 2 was highest and the level of the group 4 was secured highest (two way ANOVA, $p<0.001$). As shown in Table 3, mean GSH levels of heart, muscle and liver of the group 1 was higher than the other groups 2, 3 and 4 (two way ANOVA, $p<0.001$).

It was also remarkable that the GSH level of heart and liver of the group 3 were much higher than the group 4.

Table 2. *The TBARS levels of rats (nmol/g wet tissue)*

Tissues	Group 1 Vit. E (+) Trained ($n=7$)	Group 2 Vit. E (−) Trained ($n=8$)	Group 3 Vit. E (+) Sedantary ($n=7$)	Group 4 Vit. E (−) Sedantary ($n=7$)
Heart	123.2±14.4	191.8±43.6	129.1±24.9	187.1±29.8
Liver	158.4±39.9	219.3±34.3	148.6±71.5	234.2±42.2
Muscle	38.5±22.2	105.3±10.8	33.5±14	67.4±14.3

DISCUSSION

The benefit of exercise in promoting good health and preventing various diseases is well known. However, there has been many reports showing that exercise causes increases in oxidative damage biomarkers (Sen et al. 1997), effects on mitochondrial function (Ravalec et al. 1996) and decreases in levels of antioxidants and antioxidant enzymes in the heart (Somani et al. 1995a), blood (Ji 1993), lung (Salminen et al. 1984), liver (Ji 1993), brain (Somani et al. 1995b) and muscle tissues (Powers et al. 1994).

Vit. E is a potent fat soluble antioxidant that protects biological membranes against the damaging effects of reactive oxygen species. Several studies have shown the antioxidant effects of vit. E in different types of exercise models in which oxygen metabolism and consequently free radical production are greatly accelerated. As Aikawa et al. (1984) confirmed, there is clear evidence of vit. E depletion in both liver and skeletal muscle of rats during endurance training. Certainly, when dietary vit. E is depleted, all tissues rapidly form lipid peroxides (Bieri and Anderson 1960). Chen et al. (1980) reported that the values of TBARS in liver of rats which were not supplemented with vit. E was significantly found higher than the vit. E treated group, during 12 months. Also, Tiidus et al. (1993) found that the TBARS levels which were obtained from liver, heart and white and red vastus muscle tissues were lower in rats with group of vit. E treated compared to non supplemented.

In our study, tissue TBARS levels of both the trained and sedentary groups supplemented with vit. E, were observed significantly lower than the TBARS levels of the groups not supplemented with vit. E, except the liver tissue values of the trained group. Also, the GSH levels of the same groups treated with vit. E, except the values of the muscle tissue, were found higher. According to these results, vit. E not only reduces lipid peroxidation, but also maintains the GSH levels in high amounts in these tissues and this may be explained as its protective role and support to the endogenous antioxidant system. Haramaki et al. (1995) showed significant elevation of reduced GSH in vit. E supplemented normoxic rat hearts.

In this study, the significant increase in GSH levels seen in all the tissues of the vit. E supplemented trained group when compared with vit. E supplemented sedentary group, may also suggest the benefit of chronic training process in addition to the protective role of vit. E. Nevertheless, Evelo et al. (1992) found increases of the order of 50% for GSH after 20 weeks of training. GSH consentrations in most tissues are the milimolar range. However, clear differences exist among various tissues depending on their metabolic rate (Ji and Leeuwenburg 1996). Liver synthesizes GSH from endogeneous or dietary aminoacid de novo and supplies most of the circulating GSH (Meister and Anderson 1983). It is known that, there

TABLE 3. *The GSH levels of rats*

Tissues	Group 1 Vit. E (+) Trained ($n=7$)	Group 2 Vit. E (−) Trained ($n=8$)	Group 3 Vit. E (+) Sedentary ($n=7$)	Group 4 Vit. E (−) Sedentary ($n=7$)
Heart (nmol/g wet tissue)	12.4±2.3	5.8±1.6	7.1±1.3	4.4±1.6
Muscle (nmol/g wet tissue)	6.3±1.2	3.5±1	2.9±1.2	2.1±0.7
Liver (μmol/g wet tissue)	22.5±3.3	11.4±3.2	11.5±1.7	8.2±1.3

becomes an increase in the concentration of reduced GSH due to training (Lu et al. 1990). Although a substantial amount of GSH oxidized to GSSG during exercise due to ROS production, GSH redox state (GSH/GSSG) is not altered signicifantly because GSSG can be reduced back to GSH by glutathione reductase using NADPH as the reducing power. In a study, it was shown that during prolonged exercise the glutathione peroxidase activity decreased while the glutathione reductase activity increased, so the GSH activity was enhanced (Inal et al. 2001). On the other hand, vit. E is essential for normal cell function during exercise. Dietary vit. E supplementation has been shown to enhance GSH/GSSG redox status (Anzueto et al. 1993; Rojas et al. 1996). It is obvious that vit. E supplementation reduces GSH oxidation as it quenches the ROS generated during exercise.

Therefore, there is an increase in the concentration of the reduced glutathione due to training and vit. E supplementation. GSH levels of the group 1 in which the rats are trained and vit. E supplemented, are significantly elevated when compared with the group 4 which the rats are sedentary without vit. E treatment.

But, in our study, when the sedentary and trained groups not supplemented with vit. E were compaired, there was no significant difference in GSH and TBARS levels except in muscle tissues, as the primary mechanic tissue, muscles were prone to more oxidative stress during exercise. Hara et al. (1996) examined that, swimming enhanced the levels of lipid peroxidation products and the concentration of GSH and the GSH/GSSG ratio were decreased after a bout of swim-exercise in muscle. Previous results have shown that vit. E deficient animals show reduced endurance and higher levels of lipid peroxidation, and endogenous free radicals in both liver and muscle when compared to control animals (Davies et al. 1981). In our study, the training process without vit. E treatment elevated lipid peroxidation levels in all the tissues when compared with the sedentary groups supplemented with vit. E and this seems to be because of the oxidative stress induced by exercise. Besides, why there is no significant difference in between the GSH levels of these tissues when compared in the same groups, may be because the probable protective effect of vit. E against antioxidants is not revealed.

The results of our study showed that rats, not supplemented with vit. E, demonstrated exacerbated free radical production and excessive lipid peroxidation levels and decreases in GSH levels. So, our results suggest that, vit. E is essential for normal cell function during chronic swimming exercise. We conclude that, the advantage in receiving vit. E supplementation during exercise is compromised by its protective effects on the probable tissue antioxidant storages.

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