Effects of vitamin E supplementation on renal non-enzymatic antioxidants in young rats submitted to exhaustive exercise stress

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Abstract

Background: Exercise stress was shown to increase oxidative stress in rats. It lacks reports of increased protection afforded by dietary antioxidant supplements against ROS production during exercise stress. We evaluated the effects of vitamin E supplementation on renal non-enzymatic antioxidants in young rats submitted to exhaustive exercise stress.

Methods: Wistar rats were divided into three groups: 1) control group; 2) exercise stress group and; 3) exercise stress + Vitamin E group. Rats from the group 3 were treated with gavage administration of 1 mL of Vitamin E (5 mg/kg) for seven consecutive days. Animals from groups 2 and 3 were submitted to a bout of swimming exhaustive exercise stress. Kidney samples were analyzed for Thiobarbituric Acid Reactive Substances to (TBARS) by malondialdehyde (MDA), reduced glutathione (GSH) and vitamin-E levels.

Results: The group treated with vitamin E and submitted to exercise stress presented the lowest levels of renal MDA (1: 0.16±0.02 mmol/mgprot vs. 2: 0.34±0.07 mmol/mgprot vs. 3: 0.1±0.01 mmol/mgprot; p < 0.0001), the highest levels of renal GSH (1: 23±4 μmol/gprot vs. 2: 23±2 μmol/gprot vs. 3: 58±9 μmol/gprot; p < 0.0001) and the highest levels of renal vitamin E (1: 24±6 μM/gtissue vs. 2: 28±2 μM/gtissue vs. 3: 43±4 μM/gtissue; p < 0.001).

Conclusion: Vitamin E supplementation improved non-enzymatic antioxidant activity in young rats submitted to exhaustive exercise stress.

Background

During exercise stress, free radicals may be produced in excess of the body’s natural defense. Strenuous exercise increases the whole body and tissue oxygen consumption up to 20 fold, which then elevates electron leakage from the mitochondrial transport system and disturbs the intracellular pro-oxidant and antioxidant homeostasis [1]. There have been many reports showing that exercise causes oxidative stress, e.g., the direct detection of free radical generation in rat skeletal muscle [2] and kidney [3] increases in oxidative damage biomarkers such as thiobarbituric acid reactive substances.

The protective capacity of the antioxidant defense system of sedentary individuals may therefore be more easily exceeded under conditions of acute physical exercise. Exogenous antioxidants, primarily obtained as nutrients or nutritional supplements, may help to counteract the oxidative stress of exercise in such subjects unaccustomed to physical activity. Supplementations are a non-pharmacological therapy that has been received attention in the literature [4-7]. More than 40% of people who practice physical activity use some type of dietary supplementation in order to keep a good health. Vitamin E (α-tocopherol) seems to be a very important agent in providing protection against oxidation of cellular lipids by free radicals that are potentially damaging byproducts of cellular metabolism [8]. Vitamin E supplementation was shown to present protective effects against deterioration of kidney function in rats with streptozotocin-induced Type 1 diabetes mellitus [9].

Exercise stress was already shown to increase oxidative stress in old [3] and just weaned rats [10]. However, reports of increased protection afforded by dietary antioxidant supplements against ROS production during...
exercise are conflicting. For instance, several authors observed little or no effect of vitamin C and E supplementation on plasma ascorbic acid [11] and lipid peroxidation (LP) [11-13] levels whereas others have demonstrated increases in the concentration of ascorbic acid [14] and unchanged LP level [15] in blood following supplementation. Thus, we aimed to evaluate the effects of vitamin E supplementation on renal non-enzymatic antioxidants in young rats submitted to exhaustive exercise stress.

**Methods**

**Animals**

Thirty-two just weaned male Wistar rats weighing an average of 95.5 g were used. The animals were bred at the Central Animal House of the Ribeirão Preto Campus of the University of São Paulo. The animals were kept in plastic cages (a maximum of 4 rats per cage) in a room with controlled temperature (24-28°C) and luminosity (a 12 h light:12 h dark cycle) with free access to water and balanced food rations. The animals were kept in accordance to the guidelines of the Committee on Care and Use of Laboratory Animals of the National Research Council of the National Institutes of Health. All procedures were approved by the Ethics Committee in Research of The Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo (protocol 003/08).

**Experimental Design**

Animals were randomized into three groups: 1) the control group (n = 8), in which rats were treated with gavage administration of 1 mL of water for seven consecutive days; 2) exercise stress group (n = 8), in which rats were treated with gavage administration of 1 mL of water for seven consecutive days and submitted to exhaustive exercise stress and; 3) exercise stress + Vitamin E group (n = 8), in which rats were treated with gavage administration of 1 mL of Vitamin E (5 mg/kg, gamma tocopherol) for seven consecutive days and submitted to exhaustive exercise stress. This period of treatment is able to achieve significant changes in antioxidant levels [10].

Groups submitted to exhaustive exercise stress were submitted to one bout of swimming exercise stress until exhaustion at the last day of the experiment (7th). They were submitted to exercise consisting of swimming in a glass tank (100 cm long, 50 cm wide, 80 cm deep) containing water and maintained at 32°C, when the animal stop swimming it was removed from the water, since it corresponds to a exhaustive situation [16]. The depth of the tank prevented the animals from resting their tails on the bottom of the tank while swimming. Swimming was selected because muscle trauma caused by prolonged running, exercise-stimulated electric shock, and plyometric contractions could be avoided. These factors alone could induce oxidative stress [17].

**Laboratory Methods**

After swimming, the animals were killed by ether inhalation and fragments of the kidney were removed for determination of malondialdehyde (MDA), reduced glutathione (GSH), and vitamin E concentrations. Concentration of MDA, an indirect product of lipid peroxidation, was determined by the thiobarbituric acid reactive substance (TBARS) test, a technique commonly used in lipoperoxidation studies [18]. Acid-soluble thiols were quantified to determine tissue GSH levels [19]. Vitamin E (α-tocopherol) was determined by high-performance liquid chromatography (HPLC) on a C-18 column (Shim-pack CLC-ODS 4.6 cm, 25 cm) and a 4 mm, 1 cm pre-column at a flow of 2.0 mL/min [20].

**Statistical Analysis**

Data are reported as mean ± standard error of the mean (SEM). Statistical significance was assessed by analysis of variance (ANOVA), followed by Dunn post hoc test. Differences were considered significant when the probability of a Type I error was lower than 5% (p < 0.05).

**Results**

We observed that GSH concentrations in the kidney were elevated in the group submitted to exercise stress treated with Vitamin E compared to control and exercise stress not treated groups (Figure 1). This finding indicates that Vitamin E treatment increased renal GHS levels in rats submitted to exercise stress.

Moreover, in the group submitted to exercise stress treated with Vitamin E renal Vitamin E concentration was also higher than the control and exercise stress groups (Figure 2). It supports the fact that Vitamin E supplementation increased non enzymatic antioxidants activity in the kidney of rats submitted to exercise stress.

In order to evaluate the renal oxidative stress, we evaluated the concentration of MDA, an indirect product of lipid peroxidation. Figure 3 indicates that MDA was higher in the group submitted to exhaustive exercise stress compared to the group submitted to exercise stress treated with Vitamin E and control group. This result indicates that Vitamin E treatment attenuated the increase in reactive oxygen species production caused by exhaustive exercise stress.

**Discussion**

In this investigation we endeavored to evaluate the effects of Vitamin E supplementation by gavage on MDA, glutathione and Vitamin E levels in the kidney of young rats submitted to exhaustive exercise stress. As a main finding, Vitamin E pretreatment attenuated the
responses caused by exhaustive exercise stress regarding oxidative stress, because it decreased the production of MDA in the kidney. In addition, Vitamin E pretreatment increased renal Vitamin E and GSH levels. We decided to use Vitamin E supplementation, because previous studies suggested that more than 40% of people from different ages who practice physical activity use some type of dietetic supplementation in order to keep a good

Figure 1 Concentrations of renal reduced glutathione (GSH) in control (Control), exercise stress (Stress) and exercise stress treated (Stress + Vitamin E) groups. *p < 0.0001: Different from Stress + Vitamin E group.

Figure 2 Concentrations of renal Vitamin E in control (Control), exercise stress (Stress) and exercise stress treated (Stress + Vitamin E) groups. *p < 0.001: Different from Stress + Vitamin E group. **p < 0.005: Different from Stress + Vitamin E group.
Major non-enzymatic antioxidants which detoxify peroxides/hydroperoxides. It has been demonstrated that intracellular redox status alterations are associated with depletion of reduced glutathione [25]. Based on our data, in association with reduced renal MDA levels, vitamin E supplementation increased renal reduced glutathione levels in rats submitted to exercise stress, which supports its antioxidant effect on rats submitted to exhaustive exercise stress. In addition, a previous study showed that vitamin E restored renal reduced glutathione levels and protected rat kidneys against oxidative stress induced by hyperthyroidism [26]. According to our results, the antioxidant effects of vitamin E supplementation on rat’s kidney are supported by the increased levels of renal vitamin E in the animals submitted to exercise stress and treated with vitamin E. Vitamin C and E treatment presented positive effects on the kidney of growing pigs [27]. Furthermore, vitamin E supplementation was already shown to present beneficial effects on renal non-enzymatic antioxidants, it maintained renal vitamin E and reduced glutathione levels in rats exposed to polychlorinated biphenyls [28].

In this study we focused the antioxidant effects of vitamin E on the kidney of rats submitted to exhaustive exercise stress. There are many studies which emphasize the effects of exercise stress on different organs. Aydin et al [29] suggested that long term dietary restriction protect against endurance and exhaustive swimming
exercise-induced oxidative stress in rats' brain by inhibiting oxidative stress. However, a study from the laboratory of Kayatekin [30] showed that a single bout of exhaustive exercise was not able to change antioxidant enzyme activities and lipid peroxidation levels in rat hippocampus, prefrontal cortex and striatum. Bachur et al [31] investigated the anti-oxidative systems in rat skeletal muscle after acute physical exercise and observed increased reactive oxygen species production in this tissue. We suggest futures studies to evaluate the effects of vitamin E treatment on the oxidative status in different organs.

The ability of vitamin E to provide non-enzymatic antioxidant protection makes it a potentially valuable tool in the treatment of dysfunctions resulting from increase reactive oxygen species production. As a consequence, the administration of vitamin E caused a significant increase in renal vitamin E and GSH and a significant reduction in renal MDA in experimental exhaustive stress.

Our results suggest the existence of a protective effect opposing the oxidative stress produced by swimming in animals treated with vitamin E. The decreased production of reactive oxygen species are reflected by the reduced levels of renal MDA. The increase in the levels of vitamin E and GSH in the kidney of the rats submitted to exhaustive exercise stress constitutes an adjustment response by activating the non-enzymatic antioxidant system.

Clinical aspects
Previous studies already investigated the impact of supplements on exhaustive exercise stress [32,33]. Vitamin E administration in children with immunoglobulin A (IgA) nephropathy, focal segmental glomerulosclerosis (FSGS) and type I diabetes demonstrated potential towards ameliorating progression. Oral vitamin E treatment was shown to reduce endothelial dysfunction, lipid peroxidation and oxidative stress in patients with chronic kidney failure (CKF) [34]. Considering that more than 40% of people who practice exercise make use of dietetic supplementation and that vitamin E is one of the most consumed supplementations [8], these issues regarding the efficacy and safety of vitamin E in the kidney are important to strength the relevance of this vitamin as a supplement to physical active people. Our results are important to indicate vitamin E as a good supplement in cases of subjects exposed to exhaustive exercise, such as marathon and also for those exposed to a high intensity stress which required high energy consumption. Due to its antioxidant property, vitamin E presents renal protective effects and could be used as nutrient supplements in kidney disorders caused by exhaustive exercise.

Limitations
Our investigation presents some points that should be addressed, we did not measure the concentration of hydrogen peroxide ($H_2O_2$), superoxide anions ($SO^2_2$) and NADPH oxidase subunits ($p22^{phox}$, $p40^{phox}$, $p47^{phox}$, $gp91^{phox}$ and $p67^{phox}$) in the kidney. It would significantly strengthen the impact of our results. Unfortunately, we did not measure those components due to the lack of such equipment in our laboratory. However, we clearly showed that vitamin E supplementation is able to reduce renal oxidative stress induced by exercise stress through renal MDA measurement. MDA is a rather insensitive index of lipid oxidation. Nevertheless, previous studies confirmed its significant relationship with oxidative stress [35-38].

Conclusions
Vitamin E supplementation improved renal non-enzymatic antioxidant activity in young rats submitted to exhaustive exercise stress. Our findings strength the importance of vitamin E supplementation in exercise stress situations.

Abbreviations
LP: Lipid peroxidation; MDA: Malondialdehyde; GSH: Reduced glutathione; TBARS: Thiobarbituric acid reactive substance; HPLC: High-performance liquid chromatography; ROS: Reactive oxygen species; VO2: Oxygen consumption; XO: xanthine oxidase; IgA: Immunoglobulin A; FSGS: focal segmental glomerulosclerosis; CKF: Chronic kidney failure; $H_2O_2$: Hydrogen peroxide; $SO^2_2$: Superoxide anions

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Authors’ contributions
All authors participated in the acquisition of data and revision of the manuscript. SAB, LCA, VEV and HV conceived of the study, determined the design, performed the statistical analysis, interpreted the data and drafted the manuscript. All authors conceived of the study, determined the design, interpreted the data and drafted the manuscript. All authors read and gave final approval for the version submitted for publication.

Competing interests
The authors declare that they have no competing interests.

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