

THE INFLUENCE OF VITAMIN E SUPPLEMENTATION ON THE OXIDATIVE STATUS OF RAT INTERSCAPULAR BROWN ADIPOSE TISSUE

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Abstract - We tested whether the additional intake of vitamin E in the form of α -tocopheryl-succinate would improve the interscapular brown adipose tissue (IBAT) antioxidative protection. Thus, we studied the tissue oxidative status in rats supplemented by two doses of vitamin E over a four-week period. Our results confirmed that vitamin E supplementation decreased the IBAT lipid peroxidation level, SOD and catalase activity levels, the hydrogen peroxide concentration and spared its vitamin C content.

Key words: Vitamin E, α -tocopheryl-succinate, vitamin C, interscapular brown adipose tissue, antioxidative protection, rats.

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INTRODUCTION

Brown adipose tissue is the most important site of cold-induced thermogenesis in rodents, which also contributes to diet-induced thermogenesis (Rothwell and Stock, 1979). In adult rodents it is found in discrete deposits scattered throughout the body, with interscapular brown adipose tissue (IBAT) being the major one. The control system of IBAT activity is located in the thermoregulatory centers of the hypothalamus. Acting through sympathetic nerves it directly innervates the brown adipocytes. The main fuel for stimulated thermogenesis is the oxidation of the IBAT free fatty acids (FFA) through noradrenalin-induced lipolysis.

During respiration a small portion of oxygen molecules is converted to superoxide radicals ($O_2^{\bullet-}$), mainly by mitochondrial complexes I and III (Turrens, 1997). Hydrogen peroxide, produced via $O_2^{\bullet-}$ dismutation, is the source of the more reactive oxygen species (ROS), such as the hydroxyl radical. Since heat generation in IBAT is accompanied with high oxygen consumption and FFA oxidation can also

represent the intracellular source of oxidative stress (Halliwell and Gutteridge, 1998), the significance of antioxidative protection in IBAT is emphasized.

Vitamin E is the major lipid-soluble antioxidant which interacts directly with a variety of oxygen radicals, including superoxide (Weiyang et al., 2009). Although the antioxidant properties of these molecules are similar (Packer et al., 2001), distinct biological effects can be explained at a molecular level. This specificity lies in the selective retention of α -tocopherol through the liver α -tocopherol transfer protein, as well as in the preferential interactions of some of the compounds with molecular components of the cells.

The membrane vitamin E is regenerated by vitamin C: one-electron oxidation of the α -tocopherol phenol group generates a phenoxyl radical on the head group; the latter then migrates to the cytoplasm leaflet of the lipid bilayer and reacts with ascorbate to become re-reduced (May et al., 1998). However, data describing the influence of vitamin E on vitamin C metabolism is still insufficient.

Our aim was to study the influence of two different doses of vitamin E in the form of α -tocopheryl-succinate on the antioxidative status of rat IBAT. The activities of the tissue copper zinc superoxide dismutase (CuZnSOD), manganese superoxide dismutase (MnSOD) and catalase, the hydrogen peroxide concentration, the level of lipid peroxidation and total vitamin C content were determined, as well as the serum ascorbate concentration.

METHODS AND MATERIALS

Design of experiment

Male rats of the Wistar strain (*Rattus norvegicus*) weighing 200 ± 45 g were used for the experiments. The animals were acclimated to $22 \pm 1^\circ\text{C}$ and maintained under conditions of 12 h periods of light and dark, with free access to tap water and commercial rat food.

The rats were divided into three groups, each consisting of six animals. The first group was the control. The second and the third group were formed by animals whose diet was enriched with a low or high dose of vitamin E in the form of α -tocopheryl-succinate.

Vitamin E supplementation

Vitamin E doses of 0.8 mg and 8 mg per kg of rat body weight daily (referred to as the low and high dose, respectively) were used. According to our previous experiment performed on a group of six animals during four weeks, the daily consumption of water in the rats was linear with respect to their body weight, with an average value of 240 ± 5 ml/kg. Therefore, both doses of α -tocopheryl-succinate were dissolved daily in an adequate volume of tap water and administered to the appropriate group of animals. This supplementation was permanent throughout the four weeks.

Sample preparation

The animals were killed by decapitation with a Harvard guillotine without anesthesia, as recom-

mended by the Local Ethical Committee. After decapitation, the IBAT was removed and blood collected.

The tissue was excised and divided into two equal portions. One portion was homogenized in 25mM phosphate buffered saline (PBS) pH 7.0, and centrifuged at 9,000xg in a semi-preparative Sorvall Super T21 centrifuge for 20 min at 4°C . The supernatants were used for determination of catalase, CuZnSOD and MnSOD activities, as well as for the measurement of H_2O_2 concentration and total lipid hydroperoxides (the level of lipid peroxidation). The other portion of the IBAT and blood serum were used for the determination of vitamin C content by using a similar procedure, except that 6% trichloroacetic acid (TCA) was used instead of PBS.

Methods

The total vitamin C content was determined in TCA samples by the method of Roe and Kuether (Roe and Kuether, 1943), against vitamin C standard curve absorption values.

Both lipid hydroperoxides and the H_2O_2 content were determined in the PBS samples by the ferrous ion oxidation (FOX) assay (Wolf, 1994). The concentration of hydrogen peroxide was measured by FOX-1 (Gay and Gebicki, 2000), and calculated against hydrogen peroxide standard curve absorption values. The concentration of lipid hydroperoxides was measured by FOX-2 (Jiang et al., 1991), with the level of lipid peroxidation in the samples expressed as the percent of lipid peroxidation level relative to the control animal group.

Total superoxide dismutase activity was determined in the PBS samples by the adrenaline method of Misra and Fridovich (Misra and Fridovich, 1972), with the use of potassium cyanide as a selective CuZnSOD inhibitor for the differential calculation of MnSOD activity (Weisiger and Fridovich, 1973).

Statistical analysis

The data were expressed as means \pm SEM. One-way ANOVA was used for the multiple range compa-

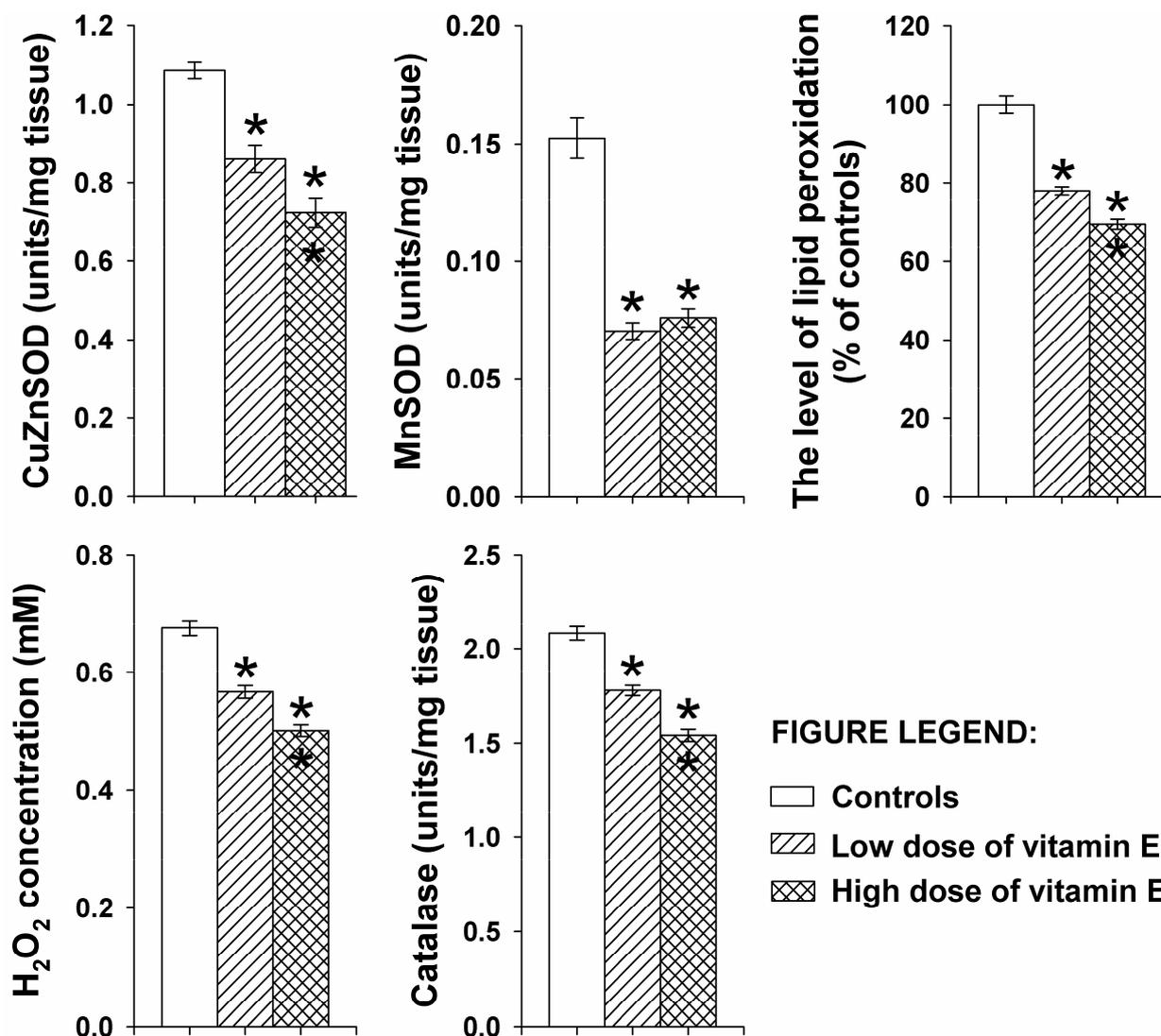


Fig. 1. Influence of low and high doses of vitamin E supplementation on the activity of CuZnSOD, MnSOD and catalase, the concentration of H₂O₂ and the level of lipid peroxidation in the interscapular brown adipose tissue of rats. Statistically significant differences ($P < 0.05$) in relation to the control are marked with an asterisk above the columns, while statistically significant differences between two doses of the vitamin E are marked with an asterisk inside the columns.

risson, with a posterior test for the determination of significant differences among the groups. The minimal level of probability of significance was set at $P < 0.05$.

RESULTS

As presented in Fig. 1, both doses of vitamin E decreased the activities of the IBAT CuZnSOD and

MnSOD, with the former in a dose-dependent manner. It could be expected that lowered SOD activities lead to the same changes in the H₂O₂ concentration and consequent catalase activity, which actually appears to have been the case (Fig. 1). In addition, the level of lipid peroxidation in the IBAT was also dose-dependently reduced under the influence of the antioxidant (Fig. 1).

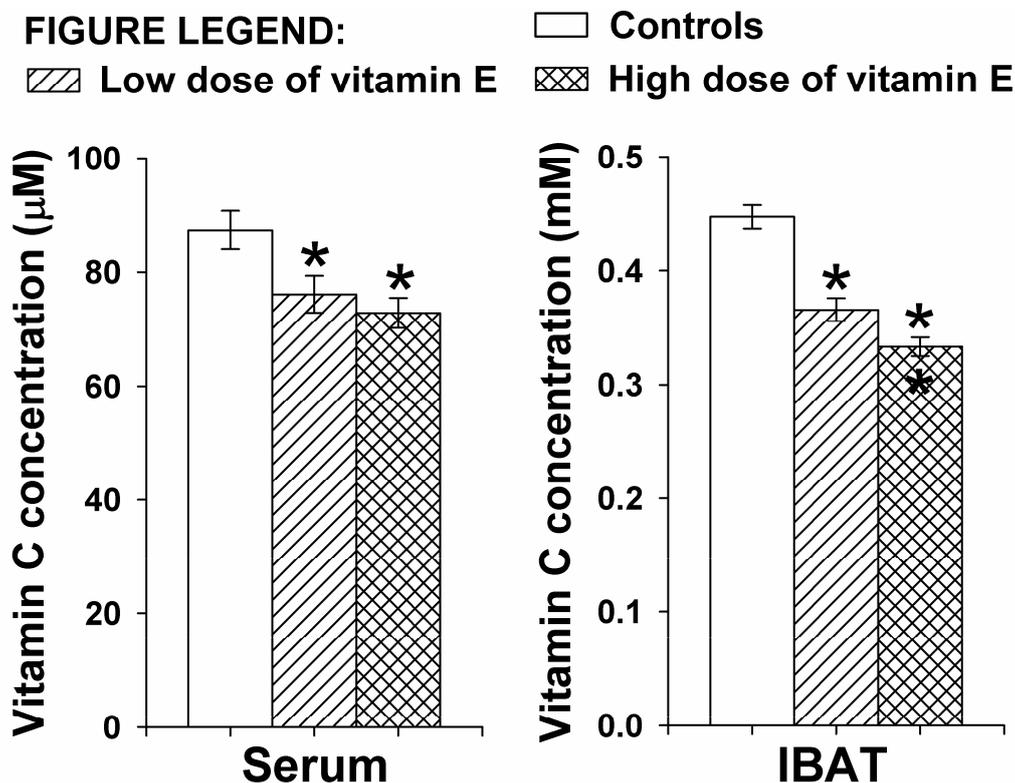


Fig. 2. Influence of low and high doses of vitamin E supplementation on the concentration of vitamin C in rat serum (left) and IBAT (right). Statistically significant differences ($P < 0.05$) in relation to the control are marked with an asterisk above the columns, while statistically significant differences between two doses of the vitamin E are marked with an asterisk inside the columns.

In the serum and IBAT of rats fed by vitamin E, the endogenous concentration of ascorbate decreases, in the latter in a dose-dependent fashion (Fig. 2).

DISCUSSION

In agreement with the literature data (Prasad and Kalra, 1989), our results show that vitamin E supplementation decreases the tissue activities of both CuZnSOD and MnSOD. According to these results, the antioxidant exerted the same effects in different cell compartments of the IBAT.

Mitochondria are considered to be the major source of superoxide radicals in eukaryotic cells, and vitamin E is known to accumulate in the inner mitochondrial membranes (Ibrahim et al., 2000) where it protects them against respiratory oxidative stress (Ham and Liebler, 1995). From this point of

view, the decreased MnSOD activity confirmed that the antioxidant supplementation improved mitochondrial antioxidative defense.

Through the prevention of the “oxidative stress transfer” from mitochondria to cytoplasm (Tanaka et al., 1997), vitamin E is probably able also to protect the IBAT cell cytoplasm against mitochondrially generated ROS. Based on our results, this was concluded not only from the decrease in CuZnSOD activity, but also from the observed decrease in the tissue lipid peroxidation level.

The function of SODs is a catalytic dismutation of $O_2^{\bullet-}$ with hydrogen peroxide as the final product. Thus, the obtained decrease in SOD activities suggests reduced H_2O_2 production, followed by consequent catalase activity decrease, as was confirmed by our results.

Vitamin E decreased the endogenous vitamin C content in the blood, which is in agreement with the literature data showing that tocopherol supplementation has negative effects on liver ascorbate synthesis (Moreau and Dabrowski, 2003).

The fall in blood vitamin C causes the same changes at the IBAT level — a tissue decrease in ascorbate content. This result shows the antioxidative effect of vitamin E in the IBAT, since the tissue vitamin C content could be the hallmark of its oxidative status. For example, it has been shown that the concentration of ascorbate in IBAT is generally higher in hibernators than in non-hibernators, probably due to preparation of the organism for oxidative stress during awakening (Buzadzic et al., 1990).

In conclusion, our results confirmed that vitamin E supplementation decreased the IBAT lipid peroxidation level, the hydrogen peroxide concentration, and SODs and catalase activity level, thus improving the tissue antioxidative protection and sparing its vitamin C content.

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