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Original article

The influence of α-tocopherol supplementation on plasma concentration of this vitamin and insulin in sedentary or physically trained rats

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Abstract

Male young growing rats of Wistar strain (n=54) were fed a vitamin E deficient diet for 3 days of adaptation period and then 10 days of experimental period. After adaptation half of the rats ran, once per a day, on a treadmill with a rate of 2.0 m/s for 15 minutes for the following 10 days. Animals were given orally 0, 0.5, 1.0 or 4.0 mg/d/rat of α-tocopherol. The aim of the study was to evaluate the influence of physical training on tocopherol and insulin concentrations in the blood plasma of rats treated with different doses of vitamin E. The concentrations of α-tocopherol (HPLC-UV method used) and insulin (rat insulin specific RIA method used) were estimated in the blood plasma. Lower concentration of vitamin E in physically trained animals was observed, which may suggest increased needs of organisms for antioxidants, as a result of increased free radical production. In trained rats a lower concentration of plasma insulin was also observed. This effect is probably related to improvement in insulin action observed in organisms under physical exercise. The most significant impact on plasma α-tocopherol was due to its supplementation level. The treatment of rats with different doses of α-tocopherol was found to be not related to insulin concentration in plasma. A significant increase in feed conversion factor was noted in vitamin E treated animals. The obtained results suggest that the increased requirement of trained rats for antioxidants might be covered by α-tocopherol supplementation.

Key words: vitamin E deficiency, α-tocopherol, physical training, insulin, rat

Introduction

It has been shown that plasma vitamin E concentration is positively associated with insulin sensitivity and pancreatic compensation for insulin resistance (Costacou et al. 2008). An increased risk of type 2 diabetes in patients with low vitamin E plasma concentrations has also been observed (Salonen et al. 1995, Ylonen et al. 2003). Free radical mechanisms are involved in causation of diabetes mellitus. Diabetes can be induced by treatment with free radicals producing substances such as: alloxan, streptozotocin, catalytic iron. The involvement of free radicals in the genesis of diabetes is also supported by effective use of free radical scavengers in the prevention of this disease. Davison et al. (2008) observed that diabetic patients (type 1 of diabetes mellitus) were more susceptible to oxidative stress induced during exercise than healthy individuals, but oral vitamin C supplementation attenuated the stress response.

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Physical exercise dramatically increases the oxygen needs of locomotive muscles and the body consumption of O_2 is more than 10 times greater compared to the resting level. Exercise is considered to cause increased production of free radicals and appearance of oxidative stress (Bloomer et al. 2006). In exercised rats fed a vitamin E deficient diet an interactive effect on oxidative stress and the activity of antioxidant enzymes were noted (Chang et al. 2007). Vitamin E is the term used to describe a group of related fat-soluble tocochromanols, including eight naturally occurring components, all exhibiting antioxidant activity. Two major homologous series of tocochromanols are tocopherols and tocotrienols. Both groups of these compounds are synthesized by higher plants and cyanobacteria.

The vitamin E activity is expressed as *RRR*-α-tocopherol equivalents, which accounts for about 90% of the activity in human tissue; the relative potency of α, β, γ, and δ-tocopherol is reported to be approximately 100:50:25:1. The commercially available synthetic form is all rac-α-tocopheryl acetate with the activity of 0.67 x *RRR*- α-tocopherol. For practical purposes, 1 International Unit (IU) of vitamin E is defined as 1 mg of all rac-α-tocopheryl acetate (Schefer and Elmada 1984).

Particles of vitamin E (α-tocopherol) protect the biological membranes of cells from damaging factors. This is due to one of its main properties – an antioxidant property (Fang et al. 2002, Bansal et al. 2005). It can react with free radicals and protect membrane unsaturated lipids and proteins from oxidation (Clycombe and Meydani 2001, Gürbay et al. 2006). The antioxidative effect of tocopherol results from its participation in scavenging free radicals and termination of lipid peroxidation (Thakur and Srivatsava 1996) and it can protect cells and their structures from oxidation (Kalender et al. 2004). The efficiency of tocopherol in the reduction of oxidative stress is depended on the dose of vitamin used. The highest dose is the most effective, both in humans and animals (Sacheck and Blumberg 2001).

Physical training normally attenuates hyperglycemia; however, when connected with vitamin E deprivation, it may increase oxidative stress. Exhaustive exercise was found to decrease body stores of antioxidant vitamins, including α-tocopherol (Faf 2003). It is suggested that the needs of animals undergoing high physical exercise, such as working and race horses or sled dogs, are much higher than sedentary subjects (Avellini et al. 1999, Raila et al. 2004).

The aim of this study was to evaluate the levels of α-tocopherol in sedentary and physically trained rats fed a diet supplemented with different doses of this vitamin and its relation to plasma insulin.

Materials and Methods

54 white male albino rats (Wistar strain) with initial body weight of 77±4 g were used. The animals were kept individually in metal cages at room temperature and constant humidity with a 12 h/12 h light/dark cycle. The animals were divided into eight groups of six rats each. The rats had free access to water and food. The body weight was checked every second day, and diet intake every day. All procedures were carried out in compliance with ethical requirements and were approved by the Local Ethical Commission in Warsaw. During the adaptation period (3 days) and experimental period (10 days) the animals were provided with a diet without vitamin E, consisting of wheat starch (53%), casein (20%), saccharose (10%), lard (7%), potato starch (5%), mineral mix (4%), vitamin mix (1%), l-methionine (0.3%) and choline (0.3%) (Brylinska and Kwiatkowska 1996). Water was available *ad libitum.*

Rats from four groups ran on a motorized treadmill at a rate of 2.0 m/s for 15 minutes per day for 10 days. Tocopherol acetate was served *per os* in different doses 20 minutes before running and simultaneously in groups of sedentary animals. The following doses of tocopherol were used: 0, 0.5, 1.0 and 4.0 mg/d/rat. Arachidonic oil was used for dilutions of tocopherol acetate (concentration 300 mg/ml) produced by Terpol S.A., Poland. A final α-tocopherol concentration was obtained by dilution of this preparation with an adequate amount of soybean oil to final volume of 50 μL given to rats.

On the last day of the experimental period the rats were deprived of food overnight. The rats were sacrificed under anesthesia (i.m. injection of ketamine and xylazine mixture) and blood from the heart was collected. Plasma was obtained by centrifugation of blood using vacuum heparin sodium blood collection test tubes with 4000 x g for 15 minutes, and then frozen.

The plasma samples were analyzed for α-tocopherol concentration using the HPLC-UV method of Van Vliet et al. (1991). Samples were deproteinized with ethanol with BHT (as an antioxidant), followed by chloroform extraction. A Gilson chromatograph equipped with a Hypersil RP-C 18 column was used. The mobile phase was a mixture of acetonitrile, hexane and isopropanol (65:14:21), delivered with a flow rate of 0.8 mL/min. Tocopherol estimation was performed using a wavelength of 295 nm. The obtained results were referred to standard curve plots of α-tocopherol (Sigma-Aldrich) in a range of 1÷10 μg/mL. Analytical recovery of α-tocopherol was 90±2%, and the detection limit 60 ng/mL.

For insulin estimation a Rat Insulin Radioimmunoassay Kit (LINCO Research, Mi, USA) was used. In this test primary antibodies against highly purified rat insulin were raised in guinea pigs. The limit of sensitivity for rat insulin assay was 0.1 ng/mL.

The results of the experiment were evaluated statistically using one-way and two-way analysis of variance (ANOVA) to test the effect of tocopherol on oxidative stress reduction. The relationships between tocopherol dose and its concentration in blood plasma were described using exponential regression analysis using SPSS 12.0 PL. For estimating the significance of differences among means the Ryan-Einot-Gabriel-Welsh modification of test F and Tukey's honest significance test were applied. In all cases, $p \leq 0.05$ was used as the criterion of statistical significance.

Results

Feed conversion ratio in rats was 2.90 in sedentary and 2.69 in trained animals (Table 1). The feed conversion ratio was significantly improved by training ($p \leq 0.001$). These data show that utilization of feed was more efficient in animals subjected to the running test every day. The rats supplemented with α-tocopherol revealed better feed conversion than the control animals ($p \leq 0.001$).

Table 1. Influence of physical exercise and tocopherol supplementation of rats (mg/d/rat) on the feed conversion ratio of rats. (n=6).

Treatment		Physical exercise	
		with	without
Vitamin E	0	2.58 ± 0.11^{a}	2.64 ± 0.19^{a}
	0.5	2.73 ± 0.10^{a}	3.08 ± 0.16^{c}
	1.0	2.70 ± 0.13^{a}	$2.82 \pm 0.16^{a,b}$
	4.0	2.73 ± 0.15^{a}	$3.05 \pm 0.17^{b,c}$
Σ		2.69 ± 0.15^{a}	2.90 ± 0.15^{b}

Means ±SD
Statistical evaluation concerns all values given in table.
Values with different superscript letter differ at $p \leq 0.05$.

The level of α-tocopherol in the plasma of sedentary rats before the experimental period was only 8% higher than after the 10 d period of feeding with the α-tocopherol deficient diet (Table 2). In physically trained animals the decrease was over 50% of the initial value. Moreover, training significantly influenced the correlation between vitamin E dose and plasma α-tocopherol (Fig. 1). Only trained rats fed a diet supplemented with 4.0 mg of vit. E/d/rat were found to have a significantly higher concentration of plasma α-tocopherol as compared to sedentary animals receiving the same amount of supplemental vitamin E.

Table 2. Concentration of tocopherol in plasma of rats (mg/L) treated with 0, 0.5, 1.0 or 4.0 mg of tocopherol/d/rat. (n=6).

Treatment		Physical exercise	
		with	without
Control group – before experimental period		1.51 ± 0.08^{b}	
Vitamin E	0	0.68 ± 0.14^{a}	1.39 ± 0.16^{b}
	0.5	4.43 ± 1.22^{c}	4.01 ± 0.75^{c}
	1.0	5.16 ± 1.07^{c}	5.43 ± 1.13^{c}
	4.0	9.71 ± 1.12^{e}	7.74 ± 0.66^{d}

Means ±SD
Statistical evaluation concerns all values given in table.
Values with different superscript letter differ at $p \leq 0.05$.

Physical training caused a decrease of plasma insulin concentration in rats ($p \leq 0.001$). Apparently supplementation of vitamin E was found not to have any influence on the insulin concentration (Table 3).

Table 3. Influence of physical exercise and tocopherol supplementation of rats (mg/d/rat) on the concentration of plasma insulin of rats (ng/mL) (n=6).

Treatment		Physical exercise	
		with	without
Control group – before experimental period		1.81 ± 0.11^{b}	
Vitamin E	0	1.44 ± 0.16^{a}	1.85 ± 0.18^{b}
	0.5	1.34 ± 0.18^{a}	1.78 ± 0.15^{b}
	1.0	1.41 ± 0.09^{a}	1.82 ± 0.15^{b}
	4.0	1.46 ± 0.14^{a}	1.87 ± 0.12^{b}
Σ		1.43 ± 0.15^{a}	1.83 ± 0.14^{b}

Means ±SD
Statistical evaluation concerns all values given in table.
Values with different superscript letter differ at $p \leq 0.05$.

Discussion

The Nutritional Requirement of Laboratory Animals (NRC, 1995) estimated a rat's requirement for vitamin E at an amount of 18 mg/kg diet. For rats consuming on average 14.3 g per day that is ca.

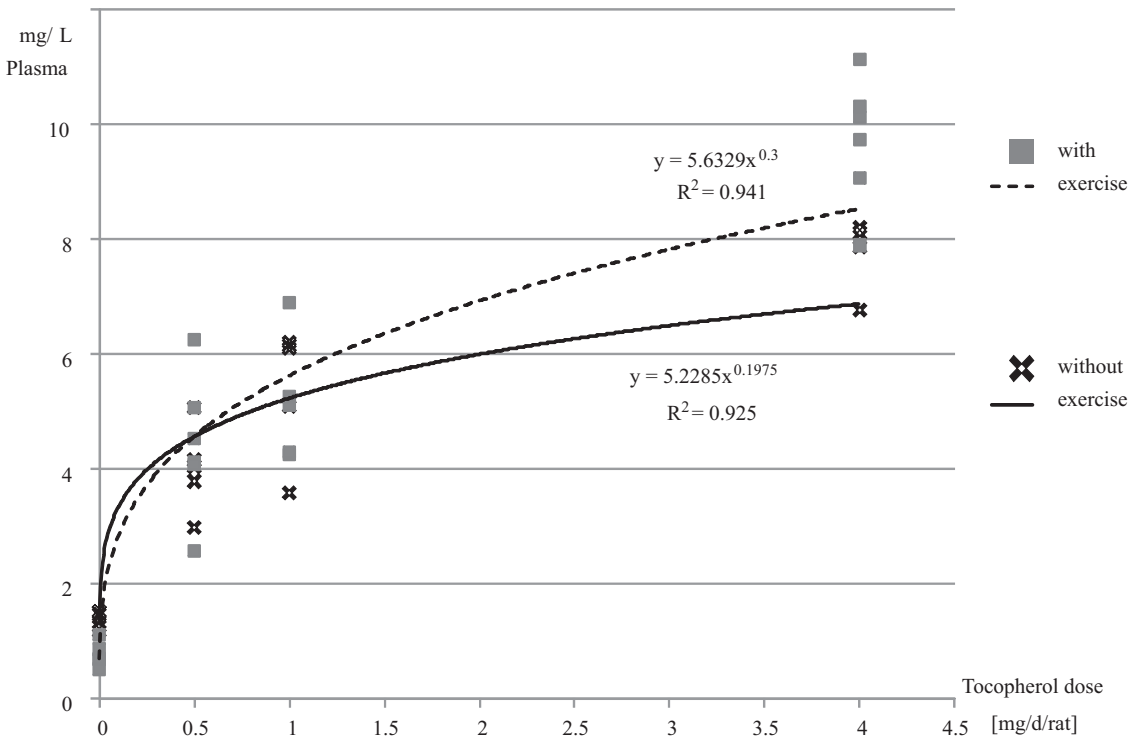

Fig. 1. Correlation between the dose of tocopherol supplementation (treatment of 0, 0.5, 1.0 or 4.0 mg of tocopherol/d/rat) and its concentration in blood plasma of rats (mg/L).

0.26 mg of vitamin E, which equals 2.16 mg/kg BW. In our experiment rats received 3 levels of supplementation: 0.5, 1.0 and 4.0 mg/rat/day, equivalent to 4.19, 8.4 and 33.6 mg/kg BW/day, respectively. Although the use of α-tocopherol in the prevention of cardiovascular diseases is essential for humans, it has been suggested that a high dose of this vitamin (44 mg/kg BW) may increase blood pressure and may influence the CNS in an adverse manner in stroke prone spontaneously hypertensive rats (Miyamoto et al. 2008). In rats given very high doses of h-tocopheryl acetate by gavages ≥125 mg/kg BW/day, TSH levels were elevated by 30-100%. At a very high dose of about 500 mg/kg BW/day biochemical indices of hepatotoxicity (serum alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase) were elevated (Gürbay et al. 2006).

Trained rats had a significantly lower concentration of insulin in blood plasma. A similar effect was observed in a previous experiment with rats receiving vitamin A supplementation (Debski et al. 2009). Also, in obese rats Morris and co-workers (2008) observed that physical training of rats caused a drop of serum insulin by 77%. It is well known that physical exercise can improve insulin sensitivity, even in muscles previously resistant to this hormone (Thyfault 2008). Ryan et al. (2000) noted that in menopausal women resistive training led to increased effectiveness of insulin action, which may be related to an observed decrease in plasma leptin concentration. In individuals suffering from type 2 diabetes physical training reduced fasting insulin concentration and caused an improvement in insulin sensitivity (Evans et al. 2001). It has also been observed that physical exercise caused a significant decrease in insulin and glucose concentration in the blood of OLETF rats trained on running wheels. The voluntary running of these animals, which are a model of hyperphagic obesity, was associated with decreased lipid peroxidation in skeletal muscle (Morris et al. 2008).

Vitamin E given to the rats either subjected to physical training or sedentary had no impact on the concentration of insulin in blood plasma. This observation, together with the positive influence of training on feed conversion, may suggest that the intensity of physical exercise was not too high even in such a condition as insufficient dietary α-tocopherol intake. The feed conversion rate was significantly influenced by training. These data show that utilization of feed was more efficient in animals subjected to the running test every day. In rats treated with vitamin E the feed conversion ratio was significantly increased compared to control animals. It was shown that 10 days of physical exercise, connected with the use of a vitamin E deficient diet, leads to a decrease in both plasma α-tocopherol (which may suggest decreased body stores) and insulin concentration.

Diets rich in vitamins possessing an antioxidant capacity have a potential health benefit in diseases caused by oxidative stress. The function of α-tocopherol as an antioxidant is based on its oxidation to tocopheroxyl radical (reaction with first free radical), which is more stable, has low activity, and can react with the next free radical creating new product. In this way one molecule of α-tocopherol can eliminate two free radicals (Stahl et al. 2002). Enhanced formation of ROS has been noted during hepatic ischemia-reperfusion and depletion of hepatic ATP concentration; dietary α-tocopherol supplementation caused faster recovery of ATP level and improved the antioxidant defense system of hepatocytes (Codoner-Franch et al. 2008). The enhanced formation of reactive oxygen species is considered to be responsible for triggering cardiovascular diseases, neoplastic processes, inflammations and many others. α-tocopherol is an essential component of a normal mammalian diet and oral supplementation is well tolerated. About 90% of the free α-tocopherol is transported via the lymphatic system into the bloodstream, where it is distributed into lipoproteins. The plasma concentration alone usually does not directly reflect the intake of vitamin E, and there is a strong correlation between vitamin E intake and fat intake (Bramley et al. 2000).

Rats fed for 13 days (3 days – preliminary period, 10 days experimental period) a diet deficient in this vitamin and subjected to physical training showed a significant decrease in plasma α-tocopherol of 51%. In sedentary rats only a slight (11%) decrease of α-tocopherol in plasma was observed. A lower concentration of plasma α-tocopherol in physically trained, but not supplemented animals, is apparently related to its higher use in oxidative stress action appearing during training, and also to redistribution of the vitamin among tissues (Antosiewicz et al. 2002). Redistribution of α-tocopherol among organs might be the reason for the higher concentration observed in our experiment of this vitamin in the plasma of trained rats receiving the highest dose of vitamin E (compared with sedentary rats). The content and distribution of α-tocopherol in tissues is connected with their antioxidant capacity and protection of cells, and their structures (El Demerdasch 2004, Gulec et al. 2006). A deficiency of tocopherol can cause a disturbance in organism oxidative balance (Winklhofer-Roob et al. 2003), and eventually to the appearance of degenerative diseases symptoms.

The influence of physical exercise on plasma α-tocopherol in all groups of rats (n = 24) was statistically not significant. However, the two way analysis of variance indicates that the influence of physical training plus vitamin E dose was significant. It seems that the most significant impact on plasma α-tocopherol was due to its supplementation level, and that the increased requirement of trained rats for antioxidants might be covered by α-tocopherol supplementation.

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