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Original Paper

Vitamin E Supplementation Attenuates Strenuous Exercise Induced DNA Damage and Lipid Peroxidation of the Liver in Rats

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Abstract

This study was designed to investigate the effects of vitamin E supplementation on exercise – induced DNA damage and lipid peroxidation in rat livers. Male Wistar rats were divided into three groups: a sedentary control (CON) group fed a control diet, and two exercise groups, one fed a control diet (EX) and the other fed a vitamin E supplemented diet (EX+VE). The exercise groups performed treadmill runs for 4 weeks, 5 days a week for 30 minutes per day. Thiobarbituric acid reactive substance (TBARS) levels in the EX+VE group were significantly lower than the EX group ($p<0.05$). Hepatic 8 – hydroxydeoxyguanosine (OHdG) in the EX+VE group was also significantly lower than the EX group ($p<0.05$). The results showed that there was oxidative damage to lipids and DNA due to exhausting exercise together with a decrease in α-tocopherol levels in the liver due to lipid peroxidation. However, when high levels of vitamin E were added to the diet, TBARS levels in the liver significantly decreased and the increase in hepatic 8-OHdG levels was suppressed. These results suggest that intake of antioxidants plays an important role in protection against oxidative stress of the liver when performing strenuous exercise.

Introduction

Physical exercise is associated with increased free radical generation primarily due to a dramatic increase in oxygen uptake both at the whole body and local tissue levels [1]. The productions of these deleterious free radical reactions are different depending on a variety of exercises, such as intensity, frequency, and duration. Oxidative stress induced by exercise is almost always compromised by the antioxidant activity [2]. Therefore, antioxidant activity may be important in the defense system against free radicals.

Vitamin E has been shown to act as a powerful antioxidant in membranes, and may also be required as a structural component in membranes containing polyunsaturated fatty acids [3]. The vitamin E contents in tissues may have some influences, not only on lipid peroxide levels, but also on the level of oxidative DNA damage. However, there were no statistical differences in 8-OHdG levels, a biomarker of oxidative DNA damage, among different dietary groups divided into a low vitamin E diet and a high vitamin E diet [4]. The report suggests that there would be no relation between 8-OHdG levels and dietary vitamin E content

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under usual situations without physical activity. Vitamin E reduces exercise induced DNA damage as determined in the alkaline comet assay [5], and it has shown that muscles or other tissues consume vitamin E during increased physical activity [6]. A few reports investigate the relationship between 8-OHdG levels and physical activity [7, 8]. Asami et al. [9] demonstrated that 8-OHdG levels in the liver, lung, and heart DNA of rats forced to exercise significantly increased compared with those in rats spontaneously exercised. In the tissue, the relationship between 8-OHdG levels and antioxidants is not clear when performing exhaustive exercise. However, Hruszkewycz [10] reported evidence of induced mitochondrial DNA damage by lipid peroxidation. Thus, we suggest that hepatic 8-OHdG levels during exercise may also be inhibited by vitamin E supplementation, because vitamin E supplements can reduce exercise-induced lipid peroxidation. Moreover, the antioxidant defense system would be highly influenced by hepatic vitamin E mobilization, because regulation of vitamin E homeostasis occurs in the liver [11]. The liver is also one of the most sensitive organs for exercise – induced oxidative stress, and therefore adaptation [12].

There is consistent evidence from human and animal studies that strenuous physical exercise may induce a state wherein the antioxidant defenses of several tissues are overwhelmed by excess reactive oxygen [13]. The purpose of this study was to test a possible association between oxidative DNA damage in the liver of rats and vitamin E status. We investigated the effects of strenuous endurance exercise-induced oxidative DNA damage on vitamin E supplementation using 8-OHdG as a marker in the liver of rats, and the protection offered by dietary supplementation of vitamin E.

Materials and Methods

Animals, Strenuous Endurance Exercise Protocol and Experimental Diets

Seven-week-old male Wistar rats weighing 180-200 g (CLEA JAPAN) were used. All experimental protocols were performed in accordance with the Guiding Principles for the Care and Use of Animals approved by the Council of the Physiological Society of Japan. They were acclimatized for one week during which each rat was exercised three times on an animal treadmill (KN-73, Natsume) for 10 minutes at a running speed of 5 m/min. After the one week preliminary experiment, the rats were divided into three groups: a sedentary control group (CON, n=7) fed a control diet (4.5 mg/kg vitamin E acetate, Wako Pure Chemical Industries), and two exercise groups, one fed a control diet (EX, n=7) and the other fed a vitamin E supplemented diet (EX+VE, n=7, 45 mg/kg vitamin E acetate). All groups were fed either the control diet or the vitamin E supplemented diet for 4 weeks. The exercised groups eventually ran for 30 minutes a day, 5 days per week for 4 weeks. The treadmill (set at a 10% incline throughout) was set at 80% $\dot{V}O_2$max intensity (a speed of 28.3 m/min) for maximum oxygen uptake ($\dot{V}O_2$max) [14].

The dietary composition was: 50% sucrose, 20% casein, 15% cornstarch, 5% fat eliminated vitamin E, 5% cellulose, 3.5% salt mixture (AIN-76), 1% vitamin mixture (AIN-76) without vitamin E, 0.3% DL-methionine, and 0.2% choline bitartrate [4]. Although purified lard was used as a dietary fat, no α-tocopherols could be detected.

The rats in all groups were euthanized with pentobarbital sodium (100 mg/kg, Abbott Lab.) within 24 hours after the last experimental period, and blood samples were drawn from the abdominal vena cava. The liver samples were immediately removed and stored at -80℃ until analysis.

Measurement of α-Tocopherol and Thiobarbituric Acid Reactive Substance Levels

Using the method introduced by Ueda and Igarashi, α-tocopherol contents in the plasma and liver were analyzed using a NUCLEOSIL $5NH_2$ column (Chemco Scientific) and high-performance liquid chromatography with a fluorometric detector (SHIMADZU, LC-10A) with excitation at 298 nm and emission at 325 nm [15]. To evaluate TBARS levels in rats, their livers were measured using an absorption spectra assay [16] with 2-thiobarbituric acid (4,6-Dihydroxy-2-mercaptopyrimidine, Wako Pure Chemical Industries) as a standard.

Isolation and Hydrolysis of DNA from the Rat Livers and Quantification of 8-OHdG

DNA extraction and hydrolysis was performed using the method described by Kaneko and Tahara [17]. DNA was isolated by the sodium iodide and polyethylene glycol method. Subsequently, the reactant was analyzed using an 8-OHdG assay system (Japan Institute for the Control of Aging), which is a competitive enzyme-linked immunoassay for determining the levels of 8-OHdG in biological samples, and quantified spetrophotometrically by absorbance at 450 nm. This ELISA method using monoclonal antibodies specific to 8-OHdG was established as described previously [18, 19].

Statistical Analysis

All data were expressed as Mean ± SD. Overall differences were determined by one-way analysis of variance (ANOVA, Stat View 5.0, SAS Institute Inc.) with the level of significance set at 0.05. Fisher's PLSD was used whenever a significant difference between the two groups was shown by ANOVA.

Results

The rats fed the vitamin E supplemented diet in the exercise group were significantly lower in body weight than in those fed the control diet in the control group ($p<0.05$). Liver weight significantly decreased in the exercise groups compared to the sedentary control group ($p<0.05$, Table 1). α-Tocopherol contents in the liver and serum were significantly higher in the EX+VE group than in the CON and EX groups ($p<0.05$, Table 2). Therefore, we measured α-tocopherol contents per liver in each group. The α-tocopherol contents per liver significantly decreased in the EX group compared to the CON group. TBARS levels in the liver were significantly lower in the EX+VE group compared to the EX group (Fig. 1). Vitamin E

Table 1 Effects of vitamin E supplementation and strenuous endurance exercise on body weight and liver weight in rats

	Body weight		Liver			
			g		g/100g B.W.	
CON	332.2± 7.3	a	14.6±0.8	a	4.4±0.3	a
EX	324.5±10.0	a,b	12.1±0.6	b	3.7±0.2	b
EX+VE	321.7± 8.7	b	11.6±1.1	b	3.6±0.3	b

B.W. ; body weight
Values are presented as means ± SD.
Differences between values within a column not associated with a common superscript letter are significantly different ($p<0.05$).

supplementation resulted in a significant decrease of 8-OHdG in the EX+VE group when compared to the EX group ($p<0.05$, Fig. 2). The 8-OHdG levels in the EX group tended to increase compared to the CON group.

Table 2 Effects of vitamin E supplementation and strenuous endurance exercise on α-tocopherol levels in the liver and serum of rats

	Hepatic α-tocopherol		Serum α-tocopherol
	(μg / g)	(μg / liver)	(μg / ml)
CON	39.6 ± 8.8 ^{b}	577.1 ± 133.0 ^{b}	13.6 ± 2.6 ^{b}
EX	45.5 ± 2.8 ^{b}	547.9 ± 26.0 ^{b}	10.0 ± 2.1 ^{b}
EX+VE	148.1 ± 20.9 ^{a}	1707.9 ± 263.8 ^{a}	17.8 ± 5.2 ^{a}

Values are presented as means ± SD.
Differences between values within a column not associated with a common superscript letter are significantly different ($p<0.05$).

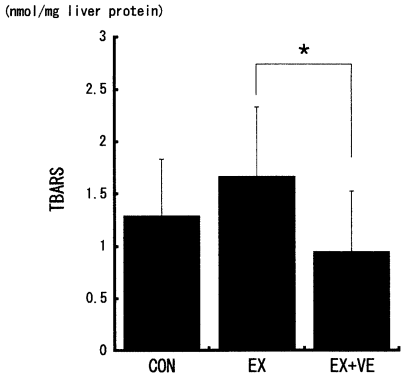

Fig. 1 Effects of vitamin E supplementation and strenuous endurance exercise on TBARS in the rat liver. Values are presented as means ± SD. *Significantly different ($p<0.05$).

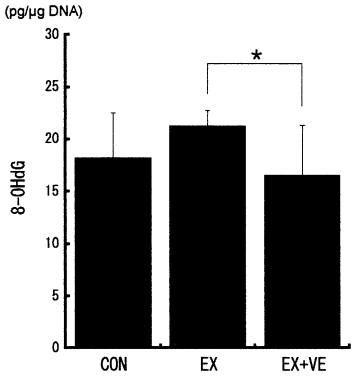

Fig. 2 Effects of vitamin E supplementation and strenuous endurance exercise on 8-OHdG levels in the rat liver. Values are presented as means ± SD. *Significantly different ($p<0.05$).

Discussion

Numerous studies have reported that strenuous exercise induces DNA damage in humans [5, 8]. Treadmill running to exhaustion in subjects caused DNA damage to the leukocytes in a single cell gel test [5]. Exercise-induced DNA damage was also detected after a short-distance triathlon competition [8].

8-OHdG is excreted into the urine as the repair product of DNA [8]. Therefore, it seems that urinary 8-OHdG excretions reflect DNA damage and restorable function. However, the exercise-induced reactive oxygen species production is thought to cause oxidative DNA damage to several types of tissue. After all, 8-OHdG is useful as a means of clarifying DNA damage, but the origin of the urinary 8-OHdG excretions cannot be specified. We found that 8-OHdG levels did not change significantly between the sedentary control group and the exercised group which was fed a control diet. Thus, we suggest that strenuous endurance exercise has little oxidative effect on hepatic DNA, and that the antioxidant capacity that the hepatic DNA received did not necessarily increase only by strenuous endurance exercise.

In the present study, there were significant differences in the vitamin E contents included in the diets. Therefore, hepatic 8-OHdG levels significantly decreased in the EX+VE group when compared to the EX group. Our results demonstrated that strenuous endurance exercise does not affect 8-OHdG levels in the liver when compared with the CON and EX groups using the same dietary vitamin E contents as in the CON group and the EX group. Hartmann et al. [5] and Sumida et al. [20] reported that antioxidant supplementation prevented oxidative DNA damage after a single bout of exhaustive exercise. In contrast, Umegaki et al. [4] reported that a change in vitamin E had little influence on the level of oxidative DNA damage in the liver, and thought that glutathione levels and glutathione-related enzyme activities in vitamin E-deficient rats were higher than those of vitamin E-supplemented rats. We agree with the opinion that endurance exercise could be suppressing 8-OHdG by revitalizing anitioxidative enzyme activities. It is thought, however, that the existence of vitamins further improves antioxidative capability against exercise-induced DNA damage.

It is known that DNA damage frequently occurs when cells are exposed to oxidative stress. Halliwell and Aruoma [21] explained the ability of oxidative stress to cause DNA damage as follows: DNA damage is due to oxygen-containing radical formation and triggers a series of metabolic events within the cell. Itoh et al. [22] reported that the basal level of hydroxyl radicals decreased in the liver after training, but the difference was not significant. Although the mechanism of hydroxyl radicals or singlet oxygen which vitamin E has is unknown, vitamin E must play an important role in protecting against the oxidative DNA damage following high-intensity endurance exercise.

Exhaustive exercise, such as acute bouts of exhaustive treadmill running, enhances lipid peroxidation in the liver [23]. Nevertheless, Alessio and Goldfarb [24] found that the sedentary group demonstrated increases in lipid peroxidation levels in the liver and white skeletal muscle after exercise, whereas the endurance-trained group did not. Almost all studies have reported that the overall antioxidant capacities of tissues are significantly higher in trained rats [12, 25]. It is consistent with the view that a protective effect of training is associated with increased cellular antioxidant defenses and that antioxidant nutrients prevent exercise-induced oxidative damage [26]. Our results showed that TBARS contents in the EX group tended to increase when compared to the CON group and significantly decrease in the EX+VE group. Ji [27] considered that free radical species that escape antioxidant enzymes are quenched by vitamin E, and incidentally formed hydroxyl radicals ($OH^{\cdot}$) are scavenged by vitamin E. Even if the hepatic TBARS of control rats fed a low vitamin E diet increased, there were no changes in hepatic 8-OHdG in these rats [4]. We found that antioxidant substances, especially vitamin E, were indispensable when maintaining defense functions to oxidization, because a low vitamin E diet disturbed the balance of the antioxidant defense

system. We suggest that not all vitamin E is consumed as an antioxidant and is exhausted in the liver, since vitamin E is absorbed into the liver from food, even if it is used for the suppression of exercise-induced oxidative stress. Oxidative stress results when reactive oxygen species are not adequately removed inside generated tissues. This can happen if antioxidants are depleted and/or if the formation of reactive oxygen species is elevated beyond the ability of the defense system to cope with them [28]. Therefore, it is clear that most of the exercise-induced reactive oxygen species are eliminated by an antioxidant chain reaction of vitamin E.

Strenuous endurance exercise is associated with a remarkable increase in oxygen consumption. In other words, elevated oxygen consumption may increase free radical activity, whereas regular exercise leads to an increase in the antioxidant defense system against free radical damage. Furthermore, the antioxidant defense system against oxidative stress consists of a synergy between antioxidant enzymes and antioxidant nutrients. When hepatocytes are exposed to oxidative stress, DNA damage frequently occurs. However, dietary antioxidant supplementation reduces the reactive oxygen species levels as well as the reactive oxygen species induced damage. The present study indicates that hepatic TBARS levels significantly decreased and the increase in hepatic 8-OHdG levels was suppressed when vitamin E was added to the diet. These results suggest that vitamin E is a useful supplementation for defending liver DNA damage against oxidative stress during strenuous exercise.

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