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EFFECTS OF ACUTE, SUBMAXIMAL EXERCISE ON SKELETAL MUSCLE VITAMIN E

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Vitamin E is the major lipid soluble anti-oxidant and may play an important protective role against free radicals produced during exercise. The purpose of this study was to determine the effect of a submaximal exercise bout on vitamin E levels in selected tissues. Five week- old lean, female Zucker rats were randomly divided into sedentary and run groups. At least 4 days following a maximal VO_2 test, the run group ($n = 7$) ran on a treadmill at 70.3 $\pm$ 1.5% VO_2 max for 34–42 minutes. Duration was varied according to body weight to keep total work constant. Immediately post-exercise, animals were decapitated, exsanguinated and the quadriceps (red and white vastus lateralis), liver and heart quickly excised and stored under liquid nitrogen until analyzed. Lipids were extracted in heptane and alpha-tocopherol levels determined by reverse-phase HPLC with electrochemical detection. Quadriceps vitamin-E levels declined post-exercise 30% compared to sedentary controls. Specifically, in the red quadriceps from 37 $\pm$ 2 to 26 $\pm$ 2 ($n = 7$, $p < 0.01$), and in the white quadriceps from 22 $\pm$ 2 to 16 $\pm$ 2 ($p < 0.05$) nmol/g wet weight. No change in vitamin E content was noted for either heart (113 $\pm$ 6 vs.110 $\pm$ 7, $p > 0.05$) or liver (68 $\pm$ 6 vs. 78 $\pm$ 5, $p > 0.05$). It is concluded that a single bout of submaximal treadmill running can result in a significant depletion of vitamin E in skeletal muscle.

KEY WORDS: Vitamin E, exercise, skeletal muscle.

INTRODUCTION

It is widely reported that free-radical production occurs concomitantly with electron transport in respiring mitochondria.[1] The effect of exercise on the formation of free radicals and subsequent damage is an area of considerable interest because of the increase in respiration associated with exercise. Increased lipid peroxidation products have been observed in the expired air[2] and plasma[3] of exercising humans. Acute exercise in the rat produces increased lipid peroxidation products in red and white quadriceps after both moderate and high intensity treadmill running.[4,5] Treadmill running to exhaustion produces increased free radical concentration, lipid peroxidation and mitochondrial damage in skeletal muscle and liver.[6]

A significant portion of tissue vitamin E is located in the inner mitochondrial membrane in close proximity to the electron transport apparatus[7] rendering it a "first-line" antioxidant.[8] It has been suggested that physically active individuals require increased vitamin E intake based on decreased vitamin E levels in muscles from trained normal and vitamin E deficient rats.[9] However, other investigators have

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shown no decrease in vitamin E content with training in red or white quadriceps[10] or gastrocnemius.[11]

The purpose of this study was to determine if a single, acute bout of submaximal ($\sim$ 70% VO_2 max) treadmill running alters tissue vitamin E levels in the rat.

METHODS

Housing and Care of the Animals

Female lean (Fa/Fa) Zucker rats were obtained from the Animal Model CORE Facility of the Obesity research Center, Vassar College, Poughkeepsie, NY. The rats were housed three to a cage. The temperature of the animal room was maintained at 21°C, and a 12-h light-dark cycle set. Rats were provided water and laboratory chow (Continental Grain Co., Chicago, IL) containing 35 mU/g alpha-tocopherol ad libitum.

Oxygen Consumption

O_2 consumption (VO_2) during treadmill running was determined by the method of Brooks and White,[12] using a modified bottomless plexiglass metabolic chamber (35.6 × 12.7 × 14.3 cm) as described previously.[13] For determination of VO_2 max, all animals ran on a 8% incline at an initial speed of 12–13 m/min. Speed was increased by 3–4 m/min every 3 min thereafter. Animals were considered to have reached their VO_2 max if they ran until they could no longer keep pace with the treadmill and if they achieved a respiratory exchange ratio (R) greater than 1.0. The majority of animals additionally demonstrated a leveling off of VO_2 during the last few stages of the test, confirming that thay had achieved their maximum. Gas values were read from the analyzers during the final 20 s of each stage. VO_2 and CO_2 production (VCO_2) were calculated according to Brooks and White[13] using the Haldane transformation. The R was calculated as VCO_2/VO_2.

Acute Exercise Bout

At least 4 days following the maximal test, both control and run rats were fasted for 4–8 hours. The acute exercise group was then run at a speed determined from their VO_2 max test to elicit approximately 70% of their previously determined VO_2 max (relative to ml/kg/min). Rats ran on an 8% grade, with the time of the run adjusted in order to keep the total amount of work constant at 13 kgm for each rat. Respiratory exchange ratio (R) was determined every 5 min of the run.

Tissue Preparation and Analysis

Within 5 s of completing the acute exercise bout or control period, the animals were decapitated and exsanguinated. Quadriceps, heart and a section of the liver were immediately removed. Quadriceps were immediately immersed in liquid N_2, while the heart and liver section were freeze-clamped with tongs pre-cooled in liquid N_2. All tissues were stored in liquid N_2 until analysis.

On the day of analysis, the quadriceps were thawed on ice and separated into superficial (white quadriceps, WQ) and deep (red quadriceps, RQ) sections. Lipids

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TABLE I
Effects of acute, submaximal exercise on alpha-tocopherol levels in selected tissues

Group	Red Quadriceps	White Quadriceps	Heart	Liver
Control	37 ± 2	22 ± 2	113 ± 6	68 ± 6
Run	26 ± 2**	16 ± 2*	110 ± 7	78 ± 5

Values are means ± SE ($n = 7$), expressed as nmol/g wet weight $^{*}p < 0.05$, $^{**}p < 0.01$ vs. control

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containing alpha-tocopherol (vitamin E) were extracted in heptane from all tissues according to the method of Burton.[14] Extracted lipids were stored under nitrogen at —120°C.

Contents of tissue alpha-tocopherol were determined by the method of Murphy and Kehrer.[15]

Statistical Analysis

Data are given as means ± SE. A one-way analysis of variance was utilized and Fisher's protected least significant difference was used post hoc to determine significant difference between pairs. A level of $p < 0.05$ was set for significance in all tests.

RESULTS

Acute Run Data

Body weights for the acute run and control groups were not significantly different, 210 ± 6 and 205 ± 2 grams, respectively. Maximum VO_2 for the acute run group was 68.5 ± 1.9 ml/kg/min. All animals ran at 20 m/min during the acute run. Total work done during the acute run was 13.1 ± 0.8 kgm. Intensity and duration during the acute run averaged 70.2 ± 1.5% VO_2 max and 39.14 ± 0.99 min, respectively. R values during the run were 0.88 ± 0.01.

Alpha-tocopherol

Alpha-tocopherol contents of selected tissues are summarized in Table I. A single bout of submaximal running significantly decreased alpha-tocopherol levels in both the red and white quadriceps approximately 30%. Alpha-tocopherol content remained unchanged in both heart and liver.

DISCUSSION

A single bout of treadmill running at 70% VO_2 max for ~40 min. resulted in an approximate 30% decrease in skeletal muscle vitamin E stores. This moderate intensity and duration are similar to that commonly prescribed for fitness in humans. The decrease with acute exercise in this study is similar to that seen previously in rested animals after chronic exercise training.[9] However, several other investigators have noted no decrease in vitamin E levels with training.[10,11,16] The decrease in total

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hindlimb vitamin E shown by Aikawa *et al.*[9] may have been an acute result of the last bout of training.

The consequence of decreased muscle vitamin E post-exercise is unclear. Severely vitamin E deficient animals show decreased endurance time[17] and increased intracellular enzyme release with excessive contractile activity.[18] Exhaustive exercise and vitamin E deficiency produce similar increases in free radical concentration, lipid peroxidation and damage to ER, SR and mitochondria.[6] In accordance with our finding, Davies *et al.*[6] showed treadmill running at submaximal levels for one-half the time to exhaustion increased oxidative damage intermediate to that of sedentary and exhausted animals.

Vitamin E supplementation has been reported to increase time to exhaustion in mice.[19] Supplementation in humans prior to acute exhaustive exercise attenuates increases in blood levels of malondialdehyde and enzyme leakage indicating decreased lipid peroxidation and cellular damage.[20] Decreased enzyme efflux directly attributed to vitamin E has also been demonstrated *in vitro*.[21] Calcium induced enzyme efflux in isolated muscle was decreased by alpha-tocopherol, phytol and isophytol. A membrane stabilizing action in addition to an antioxidant effect is indicated by the effectiveness of the latter compounds.

Alpha-tocopherol levels in heart were unchanged by the acute submaximal exercise bout. Swim-training has been shown to decrease vitamin E levels in the right ventricle and subendomyocardium of the rat.[22] Conversely, treadmill training has shown no decrease of vitamin E in hearts of normal rats.[10] Due to the known differing cardiovascular responses and adaptations to swimming and running in the rat,[23] it would appear that the antioxidant capacity of vitamin E is not stressed in the rat heart subjected to submaximal treadmill running.

The present study showed no decline in liver vitamin E levels immediately post-acute submaximal exercise. Davies *et al.*[6] showed increases in lipid peroxidation and mitochondrial damage post-exhaustive exercise. Liver vitamin E levels have been shown to be depleted faster in trained vitamin E deficient rats compared to sedentary vitamin E deficient rats.[9] These latter studies suggest chronic exercise as a factor in depleting liver vitamin E status despite the lack of an acute exercise effect. Possible reasons for this discrepancy include: 1) partial vitamin E deficiency is required for liver vitamin E depletion with exercise, 2) the effect of exercise in depleting liver vitamin E occurs only with chronic training, 3) the liver relies less on vitamin E as an antioxidant during exercise or 4) liver vitamin E levels may change at a later time after stress in muscle.

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