Jennifer M. Sacheck · Eric A. Decker · Priscilla M. Clarkson

The effect of diet on vitamin E intake and oxidative stress in response to acute exercise in female athletes

Accepted: 28 April 2000

Abstract Vitamin E is the major lipid-soluble antioxidant found in foods, and its bioavailability is affected by the presence of dietary fats. Athletes often consume low-fat diets and may be more susceptible to the oxidative stress produced by exercise due to the low availability of vitamin E. In this study, the effects of a low-fat diet on vitamin E intake and oxidative stress markers were assessed in collegiate female rowers. All subjects habitually consumed either a low-fat (LF; <40 g fat · day⁻¹) or a high-fat (HF; >60 g fat · day⁻¹) diet. Subjects ran downhill for 45 min at 75% of their age-predicted maximal heart rate. Blood samples were collected immediately pre- and post-exercise, and at 6, 24, and 48 h post-exercise. Subjects in the LF group consumed significantly less vitamin E (2.9 mg vitamin E · day⁻¹) than advised by the Recommended Dietary Allowance (RDA; 8.0 mg vitamin E · day⁻¹) and than those in the HF group (9.8 mg vitamin E · day⁻¹; P < 0.05). Plasma concentrations of vitamin E, malondialdehyde, and conjugated dienes were not significantly different between LF and HF before or after exercise. Creatine kinase became significantly elevated above baseline at 6 h and 24 h post-exercise in both groups (P < 0.05). We can conclude from these data that although the subjects in the LF group were not consuming the recommended amount of vitamin E in their diets, their vitamin E intake appears to be sufficient to protect against the oxidative stress produced by this moderate-intensity exercise.

Key words Lipid peroxidation · Malondialdehyde · Creatine kinase · Eccentric exercise · Dietary fat

Introduction

For energy and performance reasons, many athletes strive to consume high-carbohydrate diets and to have a low fat intake. In the case of the antioxidant vitamin E, its absorption and utilization depends substantially upon the presence of dietary lipids (Dimitrov et al. 1991; Goldfarb 1993). Studies have shown that upon vitamin E supplementation, subjects with a higher fat intake have higher plasma vitamin E concentrations (Dimitrov et al. 1991; Lehmann et al. 1977). A low intake of vitamin E coupled with the oxidative stress of prolonged exercise may further exacerbate the resulting oxidative damage to cellular membranes. Vitamin-E-deficient diets may make athletes more susceptible to muscle damage and injury.

To support this premise, it has been demonstrated that vitamin-E-supplemented athletes show a decrease in plasma creatine kinase (CK, a muscle enzyme used as a measure of muscle damage; Apple and Rhodes 1988) and the lipid peroxidation marker, malondialdehyde (MDA), after strenuous exercise (Bendich 1991; Rokitzki et al. 1994b; Sumida et al. 1989). Rokitzki et al. (1994b) supplemented male cyclists with vitamin E and found a significant reduction in CK and MDA in the serum of those supplemented with vitamin E during aerobic training, thus indicating a protective effect of vitamin E against lipid peroxidation. In another study in which a downhill running protocol was used, both CK and lipid peroxides (measured as thiobarbituric acid reactive substances, TBARS) became elevated post-exercise with a similar time course (Maughan et al. 1989). These results suggest a relationship between free radical production and exercise-induced muscle damage.

In the present study, it was hypothesized that female athletes on a low-fat diet consume less vitamin E in their
daily diets and would show higher indices of oxidative stress and muscle damage after intense exercise. This research is significant in that it examines athletes’ habitual diets in relation to oxidative stress. In addition, because estrogen has been shown to protect against cell damage, females may respond differently to the oxidative stress induced by exercise (Tiidus 1995).

**Methods**

**Subjects**

As part of a pilot study, 105 female varsity athletes (18–25 years old) from the University of Massachusetts [comprised of rowers (n = 50), swimmers (n = 32), runners (n = 9), and soccer players (n = 18)] were asked to complete a health-habit and food frequency questionnaire (NCI Dietary Analysis System. Version 3.7c, Bethesda, MD, USA) to determine vitamin supplementation use and fat intake. Analysis of this data showed that there were enough rowers consuming both low-fat and high-fat diets so that it was possible to examine one athletic population and better control for training status. Twelve rowers who had an average low daily fat intake of less than 40 g fat · day⁻¹ (LF) and another group of 12 rowers with an average high fat intake of greater than 60 g fat · day⁻¹ (HF) participated in this study. There were two dropouts during the study period; one due to illness and one due to a sudden change in dietary habits, therefore yielding a total of 11 subjects in each group (Table 1). All rowers were tested during the months of November through February during their winter training season, which consisted of combinations of ergometer rowing, strength training, calisthenics, and jogging approximately 5–6 days week⁻¹. Athletes were asked to refrain from heavy exercise on the 2 days prior to testing. Prior to the study, the athletes read and signed an informed consent document that had been approved by the Human Subjects Committee at the University of Massachusetts, and completed a physical activity readiness questionnaire and a menstrual status questionnaire. Subjects were eumenorrheic and had their menstrual cycles tracked for 3 months prior to testing. None of the athletes were taking supplements, medications, or oral contraceptives, or consumed alcohol during the study period.

**Experimental design**

A 3-day diet record (1 weekend day and 2 weekdays) was taken within the week prior to the exercise protocol to confirm each athlete’s daily fat intake as being either LF or HF. The diet records were reviewed and analyzed using Nutritionist IV Data Bank (N-squared Computing, First Databank Division, The Heart Corporation, San Bruno, CA, USA). Maintenance of established dietary patterns was strongly encouraged throughout the study. Skinfolds (triceps, suprailiac, and thigh) were also measured in triplicate on each subject using Lange Skinfold Calipers (Model 68902, Country Technology, Gay Mills, WI, USA).

**Exercise protocol**

The study exercise was performed during the early follicular phase (days 2, 3 or 4) of each subject’s menstrual cycle, as estimated by tracking her menstrual cycle for 3 months prior to the study period. The follicular phase was chosen because of the low estrogen levels, and previous research shows that the concentration of estradiol is not affected by endurance exercise during the early follicular phase (Bonen et al. 1979; Loucks 1985). The exercise was performed between 6 a.m. and 10 a.m. and subjects were asked to arrive at the laboratory after an overnight fast.

The exercise consisted of running on a motorized treadmill. For the first 5 min, subjects ran at 0% grade. During this time, the running speed was determined based on the heart rate equivalent to 75% of each subject’s age-predicted maximum (220–subject’s age). Heart rate was measured with the aid of a Polar heart rate telemeter unit (Polar CIC, Port Washington, NY, USA). Subjects then ran at this speed for 45 min, at a downhill grade of ~10°.

**Criterion measures**

Venous blood samples were collected from the antecubital vein into tubes containing ethylenediaminetetraacetic acid, immediately prior to exercise, immediately post-exercise, and at 6, 24, and 48 h post-exercise, while the subject was seated upright with her arm resting on an arm rest. Whole blood was separated out pre- and post-exercise for measurement of hematocrit and hemoglobin. All other blood samples were immediately placed on ice and centrifuged at 3000g and 4 °C, and plasma aliquots were stored at −80 °C until subsequent biochemical analysis. All samples were run in duplicate.

**Hematocrit and hemoglobin**

Hematocrit and hemoglobin were measured on whole blood pre- and post-exercise to account for the effects of hemococoncentration associated with changes in plasma volume during acute exercise. The methods used to measure hematocrit and hemoglobin followed that used by Dill and Costill (1974). Hematocrit was measured on a microcapillary reader and 4% was subtracted to correct for the plasma trapped within red blood cells. Hemoglobin samples were stored at −20 °C until analysis. A Sigma total hemoglobin reagent kit (Sigma, St. Louis, MO, USA) was used to measure blood hemoglobin spectrophotometrically (Bausch and Lomb spectrophotometer, Spectronic 1001, Rochester, NY, USA).

**Creatine kinase**

Plasma CK was measured using a CK reagent kit (Sigma) and measured spectrophotometrically (Bausch and Lomb spectrophotometer) at an absorbance of 340 nm.

**Vitamin E**

Plasma samples of vitamin E were measured by reverse-phase high performance liquid chromatography (HPLC; Hitachi F-2000 Fluorescence Spectrophotometer and D-2500 Chromato-Integrator, Danbury, CT, USA; Waters 510 HPLC Pump, Millipore, Milford, MA, USA). Lipid extraction was performed using a method similar to that described by Zaman et al. (1993). The sample was subsequently reconstituted with 0.2 ml ethanol and then vortexed. The sample was filtered through an end-tipped syringe (0.2-µm filter, Corning Glass Works; Corning, NJ, USA). Prior to analyses, the HPLC apparatus was equilibrated daily by pumping the 100%

| Table 1 Physical characteristics [mean (SD)] of the subjects in the low-fat diet (LF) and high-fat diet (HF) groups. (BMI Body mass index) |
|---|---|---|---|---|---|
| Group | Age (years) | Height (cm) | Weight (kg) | BMI | % Body fat |
| LF (n = 11) | 19.6 (0.9) | 167.2 (5.1) | 61.5 (6.0) | 22.0 (1.3) | 19.0 (3.0) |
| HF (n = 11) | 20.5 (1.7) | 173.5 (3.7)* | 74.9 (11.1)* | 24.8 (2.9)* | 22.0 (3.0) |

* Values significantly greater than LF (P < 0.05)