Effects of vitamin E deficiency on fatigue and muscle contractile properties

Authors:
Jeff S. Coombes, Benjamin Rowell, Stephen L. Dodd, Haydar A. Demirel, Hisashi Naito,
R. Andrew Shanely & Scott K. Powers

Abstract

Radical-mediated oxidative damage of skeletal muscle membranes has been implicated in the fatigue process. Vitamin E (VE) is a major chain breaking an-tioxidant that has been shown to reduce contraction-mediated oxidative damage. We hypothesized that VE deficiency would adversely affect muscle contractile function, resulting in a more rapid development of muscular fatigue during exercise. To test this postulate, rats were fed either a VE-deficient (EDEF) diet or a control (CON) diet containing VE. Following a 12-week feeding period, animals were anesthetized and mechan-ically ventilated. Muscle endurance (fatigue) and con-tractile properties were evaluated using an in situ preparationof the tibialis anterior (TA) muscle. Con-tractile properties of the TA muscle were determined before and after a fatigue protocol. The muscle fatigue protocol consisted of 60 min of repetitive contractions (250 ms trains at 15 Hz; duty cycle=11%) of the TA muscle. Prior to the fatigue protocol, no significant differences existed in the force-frequency curves between EDEF and CON animals. At the completion of the fa-tigue protocol, muscular force productionwas signifi-cantly ($P<0.05$) lower inthe EDEF group (reduced by 69%) compared to CON group (reduced by 38%). Following the fatigue protocol, a right shift existed in the force-frequency curve at low stimulation frequencies (£ 40 Hz) inthe EDEF animals compared to the CON animals ($P<0.05$). The stimulated and the contralateral TA muscle from the EDEF animals had significantly higher markers of lipid peroxidationcompared to the same muscles inthe CON animals ($P<0.05$). These data support the hypothesis that VE deficiency impairs muscular endurance and alters muscle contractile prop-erties following a prolonged series of contractions.

Jeff S. Coombes, Benjamin Rowell, Stephen L. Dodd, Haydar A. Demirel, Hisashi Naito,**R. Andrew Shanely** & Scott K.Powers (2002) "Effects of vitamin E deficiency on fatigue and muscle contractile properties" European Journal of Applied Physiology #87 pp.272-277 Version of Record Available @ (DOI 10.1007/s00421-002-0631-3)

Jeff S. Coombes, Benjamin Rowell, Stephen L. Dodd Haydar
A. Demirel, Hisashi Naito
R. Andrew Shanely, Scott K. Powers

Effects of vitamin E deficiency on fatigue and muscle contractile properties

Abstract Radical-mediated oxidative damage of skeletal muscle membranes has been implicated in the fatigue process. Vitamin E (VE) is a major chain breaking antioxidant that has been shown to reduce contraction-mediated oxidative damage. We hypothesized that VE deficiency would adversely affect muscle contractile function, resulting in a more rapid development of muscular fatigue during exercise. To test this postulate, rats were fed either a VE-deficient (EDEF) diet or a control (CON) diet containing VE. Following a 12-week feeding period, animals were anesthetized and mechanically ventilated. Muscle endurance (fatigue) and contractile properties were evaluated using an in situ preparation of the tibialis anterior (TA) muscle. Contractile properties of the TA muscle were determined before and after a fatigue protocol. The muscle fatigue protocol consisted of 60 min of repetitive contractions (250 ms trains at 15 Hz; duty cycle=11%) of the TA muscle. Prior to the fatigue protocol, no significant differences existed in the force-frequency curves between EDEF and CON animals. At the completion of the fatigue protocol, muscular force production was significantly (P<0.05) lower in the EDEF group (reduced by 69%) compared to CON group (reduced by 38%). Following the fatigue protocol, a right shift existed in the force-frequency curve at low stimulation frequencies (£ 40 Hz) in the EDEF animals compared to the CON animals (P<0.05). The stimulated and the contralateral TA muscle from the EDEF animals had significantly higher markers of lipid peroxidation compared to the same muscles in the CON animals (P<0.05). These data support the hypothesis that VE deficiency impairs muscular endurance and alters muscle contractile properties following a prolonged series of contractions.

Keywords Oxidative damage Æ Antioxidants Æ Endurance exercise Æ Redox status

J.S. Coombes (&) Æ B. Rowell Æ S.L. Dodd Æ H.A. Demirel
H. Naito Æ R.A. Shanely Æ S.K. Powers
Departments of Exercise and Sport Sciences and Physiology,
Center for Exercise Science, University of Florida,
Gainesville, FL 32611, USA
E-mail: jcoombes@hms.uq.edu.au
Tel.: +61-7-33656767
Fax: +61-7-33656877

J.S. Coombes
School of Human Movement Studies, Rm 520, Connell Building,
University of Queensland, St Lucia, QLD 4072, Australia

Introduction

Vitamin E (VE) is considered one of the most important dietary antioxidants in biological systems due to its association with the cell membrane and its ability to act directly on reactive oxygen species (ROS) preventing lipid peroxidation (Burton and Traber 1990). During strenuous exercise the augmented generation of ROS in skeletal muscle may increase lipid peroxidation resulting in contractile dysfunction (Davies et al. 1982). Evidence is accumulating that contractile processes such as calcium release at the sarcoplasmic reticulum (SR) and calcium sensitivity of the myofilaments are redox sensitive and disruption of these mechanisms through increased ROS plays a causal role in skeletal muscle fatigue (reviewed in Powers 1998 ; Reid 2001). It follows that a deficiency of VE (EDEF) in skeletal muscle would increase the susceptibility of skeletal muscle to contraction-induced oxidative damage leading to contractile dysfunction.

The possibility that EDEF might impair muscular exercise performance has resulted in four studies investigating this issue (Davies et al. 1982; Gohil et al. 1986; Tiidus and Houston 1993, 1994). Unfortunately, to date, the effect of EDEF on muscle performance remains equivocal. For example, two studies from the same laboratory report a reduction in exercise capacity in VE-deficient rats (Davies et al. 1982; Gohil et al. 1986). In contrast, two studies from another laboratory argue that EDEF does not impair exercise tolerance in rats (Tiidus and Houston 1993, 1994). Careful examination of these reports reveals experimental differences between the

studies but does not provide a definitive answer for the discrepant results. Nonetheless, it seems possible that one or more experimental design factors contribute to the divergent conclusions between these studies. In both of the reports indicating that EDEF impairs exercise tolerance (Davies et al. 1982; Gohil et al. 1986), running time to exhaustion was used to evaluate muscular performance. This type of whole body exercise does not objectively evaluate the contractile performance of individual muscles. Further, exercise time to exhaustion is subject to investigator interpretation and, to date, no data exists concerning the reliability of this type of performance

test in rats. Finally, neither the Gohil et al. (1986) or the Davies et al. (1982) study evaluated muscle levels of VE in their animals to verify that EDEF ex- isted. Hence, experimental design limitations exist in the studies of both Gohil et al. (1986) and Davies et al. (1982). In the two investigations indicating that EDEF does not impair exercise capacity (Tiidus and Houston 1993, 1994), animals did not undergo a performance test but rather performed several weeks of treadmill exercise training. These investigators concluded that EDEF does not impair exercise tolerance. This conclusion was based upon the observation that animals fed a low-VE diet were able to complete the exercise-training program with success equal to control animals on a normal diet containing VE. Similar to the studies of Davies et al. (1982) and Gohil et al. (1986), this type of whole body exercise does not objectively evaluate the contractile performance of individual skeletal muscles. Therefore, additional experiments are required to determine if EDEF impairs skeletal muscle endurance. This forms the basis for the current study.

To determine if EDEF impairs skeletal muscle endurance, we used an in situ preparation of the rat tibialis anterior (TA) muscle to evaluate muscle performance during a series of prolonged contractions. This experimental model has the advantage of an intact blood supply to the muscle and provides an objective tool to evaluate skeletal muscle contractile performance. Based upon evidence that implicates oxidative injury as a contributor to muscular fatigue (Reid et al. 1992; Khawli and Reid 1994), we hypothesized that EDEF would adversely affect muscle contractile performance, resulting in a more rapid development of muscular fatigue during prolonged periods of contractions.

Methods

This study was conducted in conformity with the policies and guiding principles in the care and use of animals of the American College of Sports Medicine and with the approval of the University of Florida's Animal Ethics Committee. Twenty-four female Sprague Dawley rats (4 months old) were divided into two dietary groups: (1) control (CON, n=12) and (2) VE-deficient (EDEF, n=12). CON animals were fed an AIN-93 M purified diet containing 75 IUkg^{-1} diet VE (DL-alpha-tocopheryl acetate). The EDEF animals were fed the AIN-93 M diet prepared with tocopherol stripped corn oil and no VE. Animal diets were professionally prepared by Harlan Teklad (Madison, Wis.). Animals were fed their respective diets for 12 weeks prior to the measurement of muscle contractile properties. To ensure that all animals consumed equal amounts of their respective diets, animals were pair fed. This was achieved by first pairing animals (12 pairs) based on body weight, allocating one to each group and housing them individually. In six of the pairs, each animal in the CON group was given the weight of food that his partner from the EDEF group consumed the previous day. In the other six pairs this was reversed so that the animal from the EDEF group received the weight of food that his partner from the CON group consumed the previous day. Weight gain was equal between groups (data not presented).

Upon completion of the feeding period, animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (40 mgkg$_{-1}$) and ventilated [tidal volume=0.7 ml per100 g body mass; breathing frequency=80 breathsÆmin^{-1} and positive end expiratory pressure (PEEP)=1.0 cmH_2O] with room air using a mechanical ventilator (CIV-101, Columbus, Ohio) to maintain a constant arterial partial pressure of oxygen (P_aO_2) of approximately 90 mmHg. P_aO_2 was determined via analysis of arterial blood using a calibrated blood gas analyzer (IL 1610, Instrumentation Laboratories, Lexington, Mass.). P_aO_2 was maintained by increasing the alveolar ventilation as necessary. Rectal temperature was monitored and body temperature was maintained at 37°C using a heating blanket.

After reaching a surgical plane of anesthesia, the sciatic nerve was located and cut, with the distal stump placed in a bipolar electrode. The tendon of the TA muscle was cut close to the bone and attached to an isometric force transducer (Cambridge, model 400). The transducer output was amplified and differentiated by operational amplifiers and underwent A/D conversion for analysis with a computer-based data acquisition system (GW Instruments-Series II, Somerville, Mass.). The TA muscle was chosen for the experiment because of its mixed fiber-type composition.

Muscle temperature and water content were maintained with a heating lamp and saline-soaked gauze. The origin of the TA muscle was anchored with a bone nail placed in the femur perpendicular to the limb. Previous experiments in our laboratory indicate that circulation to the muscle is not affected by this procedure (unpublished). Calibration of the force transducer was achieved by applying known weights on the lever. The TA muscle was induced to contract via stimulation of the sciatic nerve with a modified Grass Instruments S48 stimulator (Quincy, Mass.). Twitch measurements were used to determine L_o prior to beginning the experiment. Maximal specific tension (P_o), maximal twitch tension (P_t), and a force frequency curve were then determined at L_o before and after a 60 min fatigue protocol. Time to peak tension (t_{pt}), rate of tension development (dP/dt) and half relaxation time ($RT_{0.5}$) were measured during the twitch contractions.

P_o was measured with a series of isometric tetanic contractions using a supramaximal stimulus train (250 ms, 60 V and 150 Hz). Each tetanic contraction was separated by a 2 min recovery period to prevent fatigue between contractions. P_t was measured with a 2 ms twitch contraction at 60 V. The force frequency curve was determined by stimulating the muscle to contract every 2 min at 20, 30, 40, 60, 80, 120, 160 and 200 Hz (60 V, 250 ms trains). The 60 min fatigue protocol consisted of 60 V, 15Hz trains every 2 s. The duty cycle [ratio of the period of muscle contraction (250 ms) to the duration of a cycle of contraction and rest (2,250 ms)] was 1:9 or 11%. The protocol was designed to produce a 40–50% decrease in force output over the 60 min.

Immediately following the contractile measurements the animal was killed$_{-1}$ with a lethal injection of sodium pentobarbital (100 mgkg). Both the stimulated and contralateral TA muscles were removed, trimmed of fat and connective tissue, weighed, frozen in liquid nitrogen, and stored at –80°C for future biochemical analyses.

Measurement of VE levels in muscle

The concentration of VE in the TA muscle was determined by high performance liquid chromatography (HPLC) using the protocol of

Cort et al. (1983). Samples from both the stimulated and the contralateral limb were homogenized separately in acetone using a mechanical homogenizer (Ultra-Turrax T25, IKA Works, Cincinnati, Ohio). Samples were extracted twice with petroleum ether, then reconstituted in isooctane and analyzed for VE content. The isooctane extract (20 ⌋l) was injected onto a 250·4 mm, 10 ⌋m LiChrosorb SI column (Baird and Tatlock, Dagenham, UK).

Determination of antioxidant enzyme activity

To determine if our dietary treatments altered endogenous antioxidant enzyme activity, a small sample of the tibialis anterior from each animal was also assayed for superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT) activities. The tissue was minced and homogenized in cold 100 mM phosphate buffer with 0.05% bovine serum albumin (1:20 w/v; pH 7.4). Homogenization with a mechanical homogenizer was followed by centrifugation at 400 *g* for 10 min. The supernatant was then removed and assayed for SOD, GPX and CAT activity. SOD and GPX activities were determined using a modification of the procedures described by Sun and Zigman (1978), and Flohe and Gunzler (1984), respectively. CAT was assayed using the procedure described by Aebi (1984). The coefficients of variation for SOD, GPX and CAT and were rv4%, 5% and 3% respectively. Total protein content of the muscle homogenate was determined with the dye-binding technique described by Bradford (1976). All biochemical analyzes were performed in duplicate at 25°C and samples from all animals were assayed on the same day to avoid interassay variation.

Lipid peroxidation measurements

To determine the amount of oxidative damage in the TA muscle, levels of lipid peroxidation were measured by two methods. First, malondialdehyde levels were measured spectrophotometrically using the thiobarbituric acid-reactive substance (TBARS) method described by Mihara and Uchiyama (1978). 1,1,3,3-Tetraethoxypropane was used as the standard for this assay. Secondly, lipid hydroperoxides were quantified using the ferrous oxidation/xylenol orange technique described by Hermes-Lima et al. (1995). Cumene hydroperoxide was used as the standard for this assay and hydroperoxide values are expressed in cumene hydroperoxide equivalents (CHE). In our hands, the coefficients of variation for the TBARS and the lipid hydroperoxide assays are rv3% and 4% respectively.

Data analysis

Biochemical data were analysed with a two-way ANOVA for differences between groups and muscles. Contractile measurements (P_o, P_t, tetanic/twitch ratio, t_{pt}, $RT_{0.5}$ and dP/dt) were analysed with independent two-tailed *t*-tests for differences between groups. Force frequency and fatigue data were analyzed with a two-way ANOVA with a Bonferroni correction for multiple analyses. ANOVA differences were evaluated with a Scheffé post hoc test. All data conformed to parametric analysis and significance was es- tablished a priori at $P<0.05$.

Results

EDEF rats had significantly lower levels of VE and GPX activity in the TA muscle than did control diet animals (Table 1) ($P<0.05$). Note that the stimulation protocol did not alter the content or activity of the antioxidants measured in the TA muscle.

A comparison of the contractile properties of the TA from EDEF and CON animals before and after the fatigue protocol is presented in Table 2. There were no significant differences between P_o, maximal P_t, t_{pt}, $RT_{0.5}$ or dP/dt between EDEF and CON rats before the fatigue protocol. However, following the fatigue protocol there was a significant decrease in P_t and the P_o/P_t ratio in EDEF compared to CON ($P<0.05$).

Figure 1 illustrates the force/frequency response before and after the fatigue protocol. There were no significant differences in muscle force production within each group (pre- vs post-fatigue). Between the two groups no significant differences existed in muscle force production before the fatigue protocol at any stimulation frequency. In contrast, following the fatigue protocol, muscle force production was significantly depressed at stimulation frequencies £ 40 Hz in EDEF animals compared to CON ($P<0.05$).

Force production during the 60-min fatigue protocol is shown in Fig. 2. No differences existed in the relative force production between the experimental groups through the first 19 min of contractions. However, compared to CON, EDEF animals generated significantly less force from 20 min until the completion of the 60-min fatigue protocol ($P<0.05$). At the completion of the fatigue protocol, relative muscle force production was significantly lower in the EDEF (69%) compared to the CON group (38%).

Two markers of lipid peroxidation were used to determine the effects of the contractile protocol on oxidative damage of the TA muscle. Figure 3 contains the values for TBARS and CHE in both experimental groups. Note that similar results were obtained using the

Table 1. Effects of vitamin E deficient (*EDEF*) and control (*CON*) diets on antioxidants from either the stimulated or contralateral tibialis anterior muscle. Values are group means±SEM

Antioxidant	CON		EDEF		*P* value
	Stimulated (*n*=12)	Contralateral (*n*=12)	Stimulated (*n*=12)	Contralateral (*n*=12)	
Vitamin E (⌋g/g wet weight)	23.5±1.4	23.2±1.3	7.9±1.2*	7.6±1.2*	<0.001
Glutathione peroxidase (⌋M/min/100 mg protein)	50.6±3.2	62.0±4.3	32.3±2.4	40.1±3.5*	<0.01
Total superoxide dismutase (units/100 mg protein)	74.9±3.0	70.1±4.3	73.0±5.0	69.2±4.5	0.42
Cu-Zn superoxide dismutase (units/100 mg protein)	58.4±2.2	53.3±2.7	54.3±2.8	53.2±3.3	0.36
Mn superoxide dismutase (units/100 mg protein)	16.5±1.8	16.8±2.6	18.7±2.5	16.0±1.8	0.33
Catalase (units/100 mg protein)	6.7±2.1	7.4±2.7	4.5±0.5	6.1±0.5	0.21

*Significantly lower than the same limb muscle from control diet animals ($P<0.05$)

Table 2. Contractile measurements from the tibialis anterior of rats fed either a control diet (*CON*) or a vitamin E deficient (*EDEF*) diet before and after the fatigue protocol. Values are means±SEM

	CON	EDEF	*P* value
Pre fatigue protocol			
Max. tetanic (Nm)	1062±42.1	984±36.2	0.12
Max. twitch (Nm)	393.3±15.6	342.4±28.3	0.17
Tetanic/twitch ratio	2.70±0.44	2.88±0.41	0.25
Time to peak tension (ms)	20.2±1.6	20.5±1.8	0.62
1/2 Relaxation time (ms)	21.1±1.4	23.7±2.3	0.18
d*P*/d*t* (N/s)	14.4±0.4	10.9±1.1	0.12
Post fatigue protocol			
Max. tetanic (Nm)	1014±36.9	948±33.8	0.15
Max. twitch (Nm)	410.5±26.9	341.7±18.3	0.03*
Tetanic/twitch ratio	2.47±0.18	2.78±0.23	0.04*
Time to peak tension (ms)	23.1±2.6	24.4±2.4	0.35
1/2 Relaxation time (ms)	26.1±2.9	28.4±3.3	0.24
d*P*/d*t* (N/s)	10.2±1.8	8.4±1.3	0.18

*Different from control (*P*<0.05)

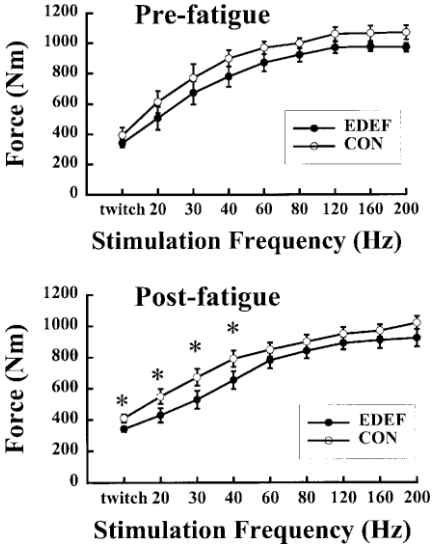

Fig. 1. Force/frequency curves for the tibialis anterior muscle of rats fed either a control (*CON*) diet or a vitamin E deficient (*EDEF*) diet measured before and after a fatigue protocol. Values are means±SEM. An *asterisk* indicates different from EDEF animals (*P*<0.001)

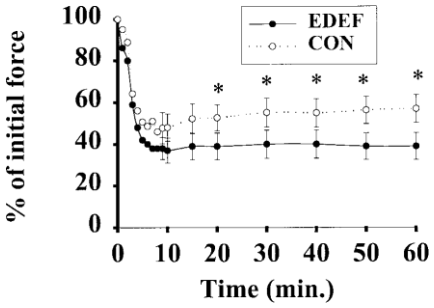

Fig. 2. Mean force production of the tibialis anterior muscle during a 60-min fatigue protocol from rats consuming either a control (*CON*) diet or a vitamin E deficient (*EDEF*) diet. Values are means±SEM. An *asterisk* indicates different from EDEF animals (*P*<0.001)

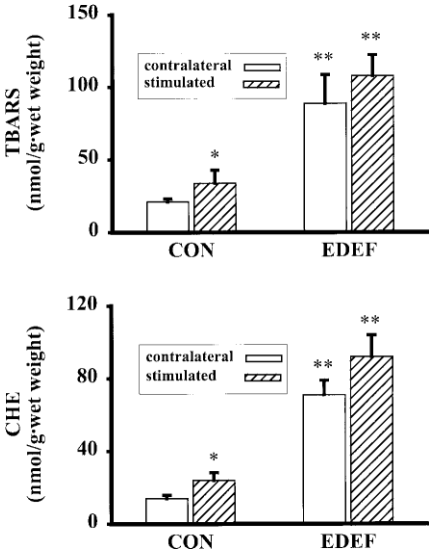

Fig. 3. TBARS and CHE concentrations in the tibialis anterior muscle of rats consuming either a control (*CON*) diet or a vitamin E deficient (*EDEF*) diet. Values are means±SEM. An *asterisk* indicates different from the contralateral limb of CON diet animals (*P*<0.05). A *double asterisk* indicates different from the same limb muscle in CON diet animals (*P*<0.05)

two markers of lipid peroxidation. In the CON diet group, the contractile protocol resulted in a significant increase in both TBARS and CHE levels in the stimulated TA compared to the contralateral muscle (*P*<0.05). An important finding was that both the stimulated and the contralateral TA from the EDEF animals had significantly higher TBARS and CHE levels than did the same muscles in the CON animals (*P*<0.05).

Discussion

Overview of principal findings

The present study reveals that a deficiency of VE in skeletal muscle is associated with an increase in contraction-mediated lipid peroxidation in skeletal muscle and a decrease in muscular force production during a 60-min in situ fatigue protocol. Furthermore, contractile measurements after the fatigue protocol demonstrated that the TA muscle from EDEF rats generated less tetanic force at submaximal stimulation frequencies (i.e. £ 40 Hz). Hence, these data clearly indicate that a VE-deficient diet decreases antioxidant protection in skeletal muscle resulting in a more rapid onset of muscular fatigue during prolonged exercise. A detailed discussion of these and related issues follows.

VE deficiency results in muscle fatigue

Our finding that EDEF results in in situ muscular fatigue agrees with previous work using a whole body

exercise model (Davies et al. 1982; Gohil et al. 1986). Using electron spin resonance it has been demonstrated that muscular exercise results in an increased production of free radicals in skeletal muscle which results in an elevation in muscle lipid peroxidation (Davies et al. 1982). Our data reveal that an EDEF diet exacerbates the contraction-induced increase in lipid peroxidation in muscle. It seems likely that this damage is occurring in regions of the fiber important for the regulation of muscular contraction and, therefore, may contribute to the impaired ability to maintain muscle force production during the fatigue protocol. This postulate is supported by the work of Amelink et al. (1991),who reported that EDEF enhanced the susceptibility of exercise-induced muscle damage during treadmill exercise in rats.

The cellular units that are most affected by EDEF are unknown. Nonetheless, it seems likely that both the mitochondria and/or the SR are subject to oxidative damage in muscle deficient in VE. Davies et al. (1982) reported that EDEF resulted in a reduction in mitochondrial respiratory control that was associated with a two- to threefold increase in free radical concentration in skeletal muscle from VE-deficient rats. These findings suggest that EDEF may lead to progressive lipid peroxidation of the mitochondrial membrane resulting in a decline in energy coupling efficiency over the fatigue protocol.

A second possible site of fatigue is the SR, which contains several redox-sensitive skeletal muscle proteins that play active roles in the contractile process. For example, the dihydropyridine-sensitive voltage sensor, the ryanodine-sensitive calcium release channel of the SR, the SR calcium-dependent ATPase, troponin, and myosin are each sensitive to redox modulation by ROS or reactive nitrogen species (reviewed in Powers 1998; Reid 2001). For example, it is well known that modification of the sulfhydryl groups in myosin has significant functional effects on muscle contractile properties (Crowder and Cooke 1984). Furthermore, it has been postulated that excitation-contraction coupling depends on the redox balance of the SR calcium release channel (Salama et al. 1992). Indeed, the calcium release channel has multiple sulfhydryl populations with distinct sensitivities to redox status (Aghdasi et al. 1997). Combined, these data indicate that optimal contractile function depends on the redox state of some or all of the cellular elements that participate in muscular contraction.

In support of the notion that oxidative modification of the SR is involved in the accelerated fatigue is the observation that after the fatigue protocol we found a decrease in force production in EDEF animals at frequencies equal to 40 Hz. At these frequencies, oxidative fibers would be primarily recruited and it would be these fibers that were also recruited during the 15 Hz fatigue protocol. This implies that VE deficiency affects oxidative fibers and supports the postulate of a redox-regulated alteration in calcium handling by the SR at low stimulation frequencies. Additional studies are required to determine the molecular mechanisms responsible for these observations.

The present study did not determine the VE content of the mitochondria or the SR. Nonetheless, given the high lipid content of these organelles and the lipophilic nature of VE, it seems likely that the EDEF diet would have affected VE availability at these sites; this could have modified redox balance and impacted contractile function. Indeed, previous work from our laboratory has shown that dietary supplementation with high doses of VE also negatively effects skeletal muscle contractile function (Coombes et al. 2001).

VE deficiency decreases GPX activity

Our data reveal that the EDEF diet had no effect on skeletal muscle SOD or CAT activities; however GPX activity was significantly decreased in EDEF animals. The decrease in GPX activity agrees with Salminen et al. (1984), who reported a significant decrease in GPX activity in mouse red quadriceps femoris after 12 weeks on an EDEF diet. Similarly, Chow (1977) also found decreased GPX activity in erythrocytes and plasma from EDEF rats. However, the present findings do not agree with work from Tiidus and Houston (1994), who found no changes in antioxidant enzyme activities in soleus, plantaris, gastrocnemius or red and white vastus muscles from rats fed an EDEF diet for 16 weeks. Conflicting results have also been presented between GPX activities and VE content in liver (Chow et al. 1973). Several factors may be responsible for the discrepant findings including the content of selenium in the diets of the different studies as selenium is a required co-factor for GPX. Further support for the relationship between VE and GPX was demonstrated in a previous study from our laboratory that found VE supplementation increased GPX activity in the myocardium of rats (Coombes et al. 2000). Collectively these findings are consistent with the notion that VE provides post-transcriptional stabilization of GPX mRNA (Li et al. 1996).

Relevance to human physiology

Evidence is accumulating that optimum muscle contraction depends on the redox state of some or all of the cellular elements that participate in muscular contraction. Major perturbations in antioxidant defenses, either through supplementing or through deficiency, can have a negative impact on skeletal muscle function. These data support the recommendation that individuals involved in physical activity require a balanced nutritious diet to avoid antioxidant deficiencies that may impact sporting performance.

Summary and conclusions

Up until the present time, the effect of EDEF on muscle performance has remained equivocal. The purpose of

these experiments was to determine the effects of a EDEF diet on skeletal muscle fatigue using a rigorous and objective model of rodent muscular performance. Our experiments clearly demonstrate that a EDEF diet results in a decrease in antioxidant protection in muscle and increased muscular fatigue that is associated with an increase in contraction-induced lipid peroxidation. Further, contractile measurements performed after the fatigue protocol indicate that muscle force production was compromised at low stimulation frequencies. This observation is consistent with the notion that EDEF is associated with a reduced calcium release from the SR during submaximal stimulation frequencies in fatigued muscle.

References

Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121–126

Aghdasi B, Zhang JZ, Wu Y, Reid MB, Hamilton SL (1997) Multiple classes of sulfhydryls modulate the skeletal muscle Ca^{2+} release channel. J Biol Chem 272:3739–3748

Amelink GJ, van der Wal WA, Wokke JH, van Asbeck BS, Bar PR (1991) Exercise-induced muscle damage in the rat: the effect of vitamin E deficiency. Pflugers Arch 419:304–309

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Burton GW, Traber MG (1990) Vitamin E: antioxidant activity, biokinetics, and bioavailability. Annu Rev Nutr 10:357–382 Chow CK (1977) Dietary vitamin E and levels of reduced glutathione, glutathione peroxidase, catalase and superoxide dismutase in rat blood. Int J Vitam Nutr Res 47:268–273

Chow CK, Reddy K, Tappel AL (1973) Effect of dietary vitamin E on the activities of the glutathione peroxidase system in rat tissues. J Nutr 103:618–624

Coombes JS, Powers SK, Hamilton KL, Demirel HA, Shanely RA, Zergeroglu MA, Sen CK, Packer L, Ji LL (2000) Improved cardiac performance after ischemia in aged rats supplemented with vitamin E and alpha-lipoic acid. Am J Physiol 279:R2149–2155.

Coombes JS, Powers SK, Rowell B, Hamilton KL, Dodd SL, Shanely RA, Sen CK, Packer L (2001) Effects of vitamin E and alpha-lipoic acid on skeletal muscle contractile properties. J Appl Physiol 90:1424–1430.

Cort W, Vicente TS, Waysek EH, Williams BD (1983) Vitamin E content of feedstuffs determined by high-performance liquid chromatographic fluorescence. J Agric Food Chem 31:1330–1333

Crowder MS, Cooke R (1984) The effect of myosin sulphydryl modification on the mechanics of fibre contraction. J Muscle Res Cell Motil 5:131–146

Davies KJ, Quintanilha AT, Brooks GA, Packer L (1982) Free radicals and tissue damage produced by exercise. Biochem Biophys Res Commun 107:1198–1205

Flohe L, Gunzler WA (1984) Assays of glutathione peroxidase. Methods Enzymol 105:114–121

Gohil K, Packer L, de Lumen B, Brooks GA, Terblanche SE (1986) Vitamin E deficiency and vitamin C supplements: exercise and mitochondrial oxidation. J Appl Physiol 60:1986–1991

Hermes-Lima M, Willmore WG, Storey KB (1995) Quantification of lipid peroxidation in tissue extracts based on Fe(III) xylenol orange complex formation. Free Radic Biol Med 19:271–280

Khawli FA, Reid MB (1994) N-acetylcysteine depresses contractile function and inhibits fatigue of diaphragm in vitro. J Appl Physiol 77:317–324

Li RK, Cowan DB, Mickle DA, Weisel RD, Burton GW (1996) Effect of vitamin E on human glutathione peroxidase (GSH-PX1) expression in cardiomyocytes. Free Radic Biol Med 21:419–426

Mihara M, Uchiyama M (1978) Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 86:271–278

Powers SK (1998) The diaphragm and oxidative stress. In: Reznick A, Packer L, Sen CK, Holloszy J, Jackson M (eds) Oxidative stress in skeletal muscle. Birkhauser, Basel, pp 223–238

Reid MB (2001) Nitric oxide, reactive oxygen species, and skeletal muscle contraction. Med Sci Sports Exerc 33:371–376.

Reid MB, Haack KE, Franchek KM, Valberg PA, Kobzik L, West MS (1992) Reactive oxygen in skeletal muscle. I. Intracellular oxidant kinetics and fatigue in vitro. J Appl Physiol 73:1797–1804

Salama G, Abramson JJ, Pike GK (1992) Sulphydryl reagents trigger Ca2+ release from the sarcoplasmic reticulum of skinned rabbit psoas fibres. J Physiol (Lond) 454:389–420

Salminen A, Kainulainen H, Arstila AU, Vihko V (1984) Vitamin E deficiency and the susceptibility to lipid peroxidation of mouse cardiac and skeletal muscles. Acta Physiol Scand 122:565–570

Sun M, Zigman S (1978) An improved spectrophotometric assay for superoxide dismutase based on epinephrine autoxidation. Anal Biochem 90:81–89

Tiidus PM, Houston ME (1993) Vitamin E status does not affect the responses to exercise training and acute exercise in female rats. J Nutr 123:834–840

Tiidus PM, Houston ME (1994) Antioxidant and oxidative enzyme adaptations to vitamin E deprivation and training. Med Sci Sports Exerc 26:354–359