The effects of tocotrienol supplementation on the muscle contractile properties, fatigue and antioxidant enzymes activities of gastrocnemius muscle of trained and untrained rats

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Accepted 23 December, 2010

Several studies have consistently indicated that vitamin E deficiency can lead to enhanced free radical formation resulting in compromised exercise performance and increased tissue lipid peroxidation. The aim of this study is to evaluate the muscle contractile properties and fatigue resistance of gastrocnemius muscle under Tocotrienol supplementation schemes in sedentary and exercise protocols. Rats were divided into four groups which included sedentary Tocotrienol supplementation (ST) and exercise Tocotrienol (ET) groups with feeding dose 8 mg/kg body weight for six weeks, as well as sedentary control (SC) and exercise control (EC) groups. Exercise protocol consisted from 40 jumps for 40 cm in height for a 6-week. The muscle fatigue protocol consisted from trains of pulses at 40 Hz repeated every second for at least 2 min. Significantly, (P<0.05) increased existed in the muscle contractile properties (twitch tension, contraction time and twitch/tetanic tension ratio) comparing with SC and EC, also increased in tetanic tension and electromyography (EMG) amplitude comparing with SC. Decreased EMG failure and increased fatigue index were improved more in ET group. This study revealed supplementation (T) and supplementation with jumping exercise induced muscle contractile properties and decreased fatigue.

Key words: Tocotrienol supplementation, contractile properties fatigue, antioxidant enzymes activities.

INTRODUCTION

Tocotrienols, like tocopherols, are capable of scavenging and quenching reactive oxygen species, also known as free radicals. Their antioxidative activity resides mainly with its “chain-breaking” property, which neutralizes peroxyl and alkoxy radicals generated during lipid peroxidation (Kamal-Eldin and Appelqvist, 1996; Avellini et al., 1999; Avanzo et al., 2001; Beytut and Aksakal, 2003; Chang et al., 2006). Peroxidation of membrane lipids is known to modify and inactivate cellular components that can have damaging effects on crucial cellular factors leading to disease. In the case of LDL-lipids, peroxidation has emerged as the initiating step in the pathogenesis of atherosclerosis. Support of this role found based upon evidence that alpha-tocopherol could inhibit atherosclerosis in both animal and human models (Verlangeri and Bush, 1992; Ravalec et al., 1996; Stephens et al., 1996; Jennifer and Jeffrey, 2001). Serbinova et al. (1991) observed a remarkably higher anti-oxidant activity with tocotrienol against lipid peroxidation in rat liver microsomes than with alpha-tocopherol. Kamat and Devasagayam (1995) also observed similar results in rat brain mitochondria and noted a stronger effect with γ-tocotrienol. Vitamin E-deficient rats exhibit a six fold increase in expired pentane compared with vitamin E-sufficient animals (Dillard et al., 1978, Avellini et al., 1999; El-Demerdash, 2004), and vitamin E-deficient rats consistently have shorter times to exhaustion in treadmill running (Davies et al., 1982; Aikawa et al., 1984; Gohil et al., 1991; Evans, 2000; Fischer et al., 2001; Fiskin et al., 2006). Vitamin E supplemented rats exhibited no greater endurance during
treadmill running than rats on normal diet (Mehlhorn et al., 1989; Alessio, 1993; Evans, 2000; Margaritis et al., 1997; Turan et al., 2001; Marsh et al., 2006) and oxidative stress in physical restraint. Also it has found that 250 IU vitamin E /Kg diet fed to rats can reduce TBARS and lipid peroxide levels in plasma and leg muscles, after one hour of exercise on the treadmill, compared to rats fed a normal diet (Goldfarb et al., 1996; Margaritis et al., 2004; Viitala et al., 2004; Chang et al., 2006; Fiskin et al., 2006).

MATERIALS AND METHODS

This study was conducted in conformity with the policies and guiding principles in the care and use of animals by the animal Research and Ethics Committee of UniversitiSains Malaysia. Sixty-four male Wistar-Kyoto rats of weight between 300 to 350 g were randomized into four groups; there were eight rats per group: (1) Sedentary control; (2) Exercise control; (3) Sedentary supplementation, and (4) Exercise supplementation. The rats will be fed with a dose of (80 µg/Kg/BW) mixed with saline for 6 weeks. Upon completion of the feeding period, animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (40 mg/kg). 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 MG muscle was cut close to the bone and attached to an isometric force transducer (AD Instrument, 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 (AD Instrument, Power Lab® and Chart software™). The exposed portions of the limb were covered by pools of mineral oil at 35 to 37°C, lactated Ringer solution (Otsuka Pharmaceuticals) was infused to maintain the blood pressure. The muscle was washed with 5 ml of 100 mM phosphate buffered saline (PBS, p1-1 7.4). Before registering mechanical properties of MG muscle, we determined optimal muscle length (Lo), the length that generates maximal twitch force, and used this length throughout the protocol. Lo was found by systematically adjusting the length of the muscle while stimulating it with single supramaximal (~150%) twitches and recording the force generated. Peak twitch tension got by single 10-pulse activation at 1 Hz; 0.5 ms duration and variable amplitudes (up to 1 V) were organized in trains. Action potentials from the studied muscle were recorded with a silver bipolar electrode inserted into the medial gastrocnemius and amplified using the low-noise multi-channel preamplifier (AD Instrument, Power Lab® and Chart software™ with a high-pass filter at 0.1 Hz and a low-pass filter at 10 kHz). Force measured under isometric conditions by the force transducer, force sensitivity of 100 µm per 100 mM. For each averaged twitch record. The second stimulation consists from Short (0.5 s) trains of pulses at various (10 to 300 Hz) frequencies with 5 Vand each series of stimulation separated by 10 s to produce peak tetanic tension. Several contractile parameters of motor units measured and calculated: The contraction time (CT), the half- relaxation time (HRT), the peaks twitch tension (to) peaks tetanus tension (Po), the ratio of twitch-to-tetanus forces, as well as the fatigue index (FI). Contraction time (CT) calculated as period from the beginning of mechanical activity to the peak twitch force, and the half-relaxation time (HRT) calculated as the time between the peak twitch force and its decrease to the half of the peak value (Burke et al., 1973).

RESULTS

Our data showed significant (P<0.05) increase in MG muscle length and rate of muscle weight to body weight between sedentary control (SC) group and other experimental groups: In sedentary supplementation (T) groups and exercise supplementation ET groups. A comparison of the contractile properties of the MG of experimental groups with sedentary control animals after stimulated protocol is presented in Table 1 and Figure 1. There were significance increase in peak twitch tensions Pt, Pt/Po ratio and twitch contraction time (CT), as well as twitch EMG amplitude and tetanic EMG amplitude was observed in experimental groups (P<0.05) in both sedentary supplementation (S) and exercise supplementation (SE) groups comparison with SC. Also there were significant (P<0.05) difference in Pt, Pt/Po and CT comparison with EC. Force production during the 2-min fatigue protocol was shown significantly higher (P<0.05) in only exercise supplementation (ET) groups when compared with sedentary control (SC) group. EMG failure was significantly higher (P<0.05) in SC and EC groups when compared with other experimental groups in both sedentary supplementation (ST) and exercise supplementation (ET) groups. There was also significant increase observed in GPX activity between SC and EC compared with exercise supplementation TE (Table 2). Again our result shows there is a significant increase of antioxidant enzymes (GPx) as well as there is decrease in lipid hydroperoxidase (Figure 2).

DISCUSSION

Amelink et al. (1991) reported that VE deficiency enhanced the susceptibility of exercise-induced muscle damage during treadmill exercise in rats. The cellular units that are most affected by Vitamin E deficiency (EDEF) are unknown. Nonetheless, it seems likely that both the mitochondria and/or the SR are subject to oxidative damage in muscle deficient in vitamin E. Davies et al. (1982) reported that (EDEF) resulted in a reduction
Table 1. Muscle contractile properties.

<table>
<thead>
<tr>
<th></th>
<th>Sedentary control (SC)</th>
<th>Exercise control (EC)</th>
<th>Sedentary tocotrienol (ST)</th>
<th>Exercise-tocotrienol (ET)</th>
<th>Sig (P&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MW</td>
<td>2.76±0.29</td>
<td>3.08±0.17</td>
<td>2.76±0.27</td>
<td>2.93±0.11</td>
<td>ns</td>
</tr>
<tr>
<td>ML</td>
<td>33.87±1.24</td>
<td>37±1.41</td>
<td>35.5±1.19</td>
<td>36.37±0.74</td>
<td>*</td>
</tr>
<tr>
<td>MW/ML</td>
<td>0.71±0.05</td>
<td>0.81±0.04</td>
<td>0.75±0.06</td>
<td>0.81±0.01</td>
<td>*</td>
</tr>
<tr>
<td>Tt</td>
<td>62.84±16.9</td>
<td>97.11±9.71</td>
<td>147.26±21.17</td>
<td>172.26±26.0</td>
<td>*, +</td>
</tr>
<tr>
<td>Po</td>
<td>162.11±13.27</td>
<td>209.77±21.27</td>
<td>196.23±34.58</td>
<td>231.4±22.28</td>
<td>*</td>
</tr>
<tr>
<td>Pt/Po ratio</td>
<td>0.38±0.1</td>
<td>0.37±0.02</td>
<td>0.76±0.18</td>
<td>0.76±0.13</td>
<td>*, +</td>
</tr>
<tr>
<td>CT</td>
<td>72.87±4.73</td>
<td>67.5±4.78</td>
<td>98.87±11.87</td>
<td>104.87±12.28</td>
<td>*, +</td>
</tr>
<tr>
<td>HRT</td>
<td>38.12±10.66</td>
<td>28.87±1.72</td>
<td>47.25±8.95</td>
<td>42.25±5.11</td>
<td>ns</td>
</tr>
<tr>
<td>FI</td>
<td>0.2±0.19</td>
<td>0.44±0.23</td>
<td>0.16±0.25</td>
<td>0.52±0.1</td>
<td>*</td>
</tr>
<tr>
<td>EMGF</td>
<td>75.37±34.32</td>
<td>54±35.29</td>
<td>20.91±22.27</td>
<td>10.77±10.25</td>
<td>*, +</td>
</tr>
</tbody>
</table>

* P<0.05 significant with SC; + P<0.05 significant with EC; ns: Not significant.

Figure 1. Muscle contractile properties of the MG of the rats.

in mitochondrial respiratory control that was associated with a two to three fold increase in free radical concentration in skeletal muscle from VE deficient rats.

Chang et al. (2006) showed a significant vitamin E training interaction effect on TBARS and GPX and SOD activities in red quadriceps and white gastrocnemius of rats and indicated that vitamin E status may influence the training-induced adaptation of GPX and SOD activities in rat skeletal muscle. Thiobarbituric acid reactive substance deficiency level was the highest in the Vitamin E deficiency group in gastrocnemius, which resulted from the combination of antioxidant deficiency and intensive exercise.

Avanzo et al. (2001) found the results in chicken breast muscle mitochondria are in agreement with those described in rat heart mitochondria (Scholz et al., 1997), which showed one strong inhibition of lipid peroxidation in mitochondrial membranes from animals fed diets supplemented with vitamin E, and selenium, was the factor that strikingly affected the course of lipid Peroxidation (Itoh et al., 2000; Devi and Kiran, 2004; Chang et al., 2007). The rate of peroxidation was higher in animals fed vitamin E deficient diets, and was not affected by the addition of GSH, GSSG, or both. The susceptibility of membranes to lipid peroxidation is linked to their tocopherol concentrations (Atalay et al., 1996;
Table 2. Antioxidant enzymes activities.

<table>
<thead>
<tr>
<th></th>
<th>Sedentary control (SC)</th>
<th>Exercise control (EC)</th>
<th>Sedentary tocotrienol (ST)</th>
<th>Exercise tocotrienol (ET)</th>
<th>Sig (P&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPx (nmol/min/ml)</td>
<td>10.05 ± 7.12</td>
<td>14.79 ± 11.26</td>
<td>20.58 ± 10.36</td>
<td>50.92 ± 23.65</td>
<td>* +</td>
</tr>
<tr>
<td>SOD (U/ml)</td>
<td>0.20 ± 0.15</td>
<td>0.20 ± 0.12</td>
<td>0.23 ± 0.16</td>
<td>0.21 ± 0.14</td>
<td>ns</td>
</tr>
<tr>
<td>CAT (nmol/min/ml)</td>
<td>0.78 ± 0.37</td>
<td>0.98 ± 0.31</td>
<td>0.79 ± 0.21</td>
<td>1.1 ± 0.23</td>
<td>ns</td>
</tr>
<tr>
<td>LIPO (U/ml)</td>
<td>3.35 ± 0.53</td>
<td>4.03 ± 0.46</td>
<td>2.27 ± 0.61</td>
<td>1.38 ± 0.27</td>
<td>* +</td>
</tr>
</tbody>
</table>

* P<0.05 significant with SC; + P<0.05 significant with EC; ns: Not significant.

Figure 2. Antioxidant enzymes activities of the MG of the rats.

Scholz et al., 1997; Burczynski et al., 1999; Avanzo et al., 2001).

ACKNOWLEDGEMENTS

The authors thank Dr. OoiFoongKiew. Moreover, we are grateful to the Physiology Department, Universiti Sains Malaysia for technical support. We also appreciate the support provided by the Universiti Sains Malaysia for technical support. We also appreciate the

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