Effects of Dietary Vitamin C and E Supplementation on Exercise-Induced Muscle Damage among Young Kelantan Weightlifters

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ABSTRACT

It has been hypothesized that markers of oxidative stress and muscle damage induced by weightlifting training could be decreased by supplementing subjects with dietary vitamin C and E. Hence, this study was carried out to investigate the effects of dietary vitamin C and E supplementation on oxidative stress and muscle damage markers among male and female weightlifters in Kelantan state, Malaysia. For this purpose, thirty two trained weightlifters were recruited and randomly assigned into two groups. The supplement group (n=16, 16.5 ± 2.2 years of age, 162.2 ± 10.4 cm of height, 65.7 ± 26.1 kg of body weight) was given 500 mg of vitamin C and 400 IU of vitamin E per day, while the placebo group (n=16, 15 ± 1.7, 162.2 ± 10.4 cm, 61.5 ± 13.9 kg accordingly) was given maltodextrine, zero calorie per day for 6 weeks. The following parameters were measured before and after intervention to detect the effects of supplementation: muscle circumference of mid-upper arm and calf, serum creatine kinase (CK), lactate dehydrogenase (LDH) and urinary thiobarbituric acid reactive substances (TBARS). Data was expressed as median and interquartile at p < 0.05. There were no significant effects (p>0.05) of dietary vitamin C and E supplementation on the muscle damage markers, CK and LDH, as well as on oxidative stress markers through urinary TBARS analysis, when the two study groups were compared. These results indi-

Key Words: Oxidative stress, Muscle damage, Vitamin C and E supplements, Weightlifting exercise.
icated that vitamin C and E were not effective in ameliorating markers of muscle damage and oxidative stress induced by weightlifting training. It might be possible that these weightlifters already have acquired protection, both structurally and biochemically, resulting from chronic exposure to weightlifting training.

INTRODUCTION

Exercise-induced muscle damage is a tear of muscle fibers and connective tissues. It occurs when a person receives harmful physical, chemical and biological stimuli and also occurs as a result of physical trauma during exercise and physical activity (19). Muscle injury can be categorized into three major types in clinical presentation: Type I injury is characterized by muscle soreness that occurs 24 to 48 hours after unaccustomed exercise, and is known as delayed onset muscle soreness (DOMS); Type II injury is characterized by an acute disabling pain from a muscle tear of a few fibers with fascia remaining intact to a complete tear of the muscle and fascia; in type III injury, muscle soreness or cramping occurs during or immediately after exercise (19, 26).

During weight lifting and resistance exercise, skeletal muscles of bodybuilders and Olympic lifters are subjected to mechanical and oxidative damage. In weight training, the mechanism that causes generation of free radicals does not involve the physiological effects of training, as it appears to involve the effects of recovery after the training as part of the repair process (29). The increased damage during exercise is associated with lipid peroxidation and membrane perturbations, loss of sarcoplasmic reticulum structural integrity, and release of myoglobin and muscle damage enzymes into the circulation (14).

Vitamins are organic compounds found in small amounts in food and classified based on their solubility in water or fat. Since most of the vitamins cannot be synthesized or manufactured in the body they should be acquired from the diet, as they are essential for body functions and are required to support health and well-being (15). Vitamins are important for antioxidant protection. The fat-soluble vitamin E plays an important role in membrane stabilization and synthesis of connective tissues, whereas Vitamin C is water soluble and has an important role in the ubiquinone, mitochondrial oxidative electron transport (8).

Vitamin E supplementation significantly reduces the lactate dehydrogenase (LDH) enzyme activity compared to the placebo (28). A combination of vitamin E (1200 IU) and C (500 mg) supplementation for 37 days prior to 300 eccentric contractions of the knee extensors was reported to attenuate declines in muscle contractile force (27). Furthermore, and in numerous studies, a mixture of vitamin C and E supplementation has been shown to reduce Creatine Kinase (CK) enzyme activity compared to the placebo (4, 25). The addition of selenium to the mixture
of vitamins E and C, and the increases in CK and DOMS were slowed down in untrained females (4).

Hence, this study has been carried out to examine the possible effects of supplementation with vitamin C and E in reducing exercise-induced damage and/or increasing the rate of repair following micro-injury and DOMS. The study proposed to utilize amateur weightlifters in the State of Kelantan, Malaysia.

MATERIAL AND METHODS

Participants

Thirty two subjects were recruited for this study, 20 males and 12 females aged between 13 and 20 years. All subjects were regular and competitive weightlifters who exercised at least 3 hours per session and 5 days per week prior to the study. The subjects were recruited at The Local Sports Council of Kelantan State, Weightlifting Center.

The inclusion criteria for subject participation were healthy weightlifters, age between 13 and 20 years and at least one year of weightlifting training. The exclusion criteria included history of muscle injury, non-compliance with research intervention, and those who had consumed dietary or vitamin supplements or exogenous anabolic–androgenic steroids or other drugs, as stated in the anti-doping regulations, within the 6 weeks that preceded the study.

Subjects were interviewed personally to ensure that they met the inclusion criteria and did not meet any obvious exclusion criteria. The subjects were informed about the experimental design and protocol and possible risks before signing the informed written consent form. This study was approved by the Research and Ethics Committee of the School of Medical Sciences, Universiti Sains Malaysia.

Study design

The study employed a randomised, single-blinded, placebo-controlled, single trial design. Subjects were randomly (gender matched) allocated into one of two groups: vitamin C and vitamin E supplementation (n=16, 16.5±2.2 years of age, 162.2 ± 10.4 cm of height, 65.7 ± 26.1 kg of body weight), or placebo control (n=16, 15 ± 1.7 years, 162.2 ± 10.4 cm, 61.5 ± 13.9 kg accordingly). A dietary vitamin C and E tablets were provided by Kotra Pharma, Malaysia Sdn. Bhd., in the form of capsules. Subjects were given one tablet of vitamin C 500 mg (orange flavored, a mixture of synthetic ascorbic acid & sodium ascorbate) and one tablet of vitamin E 400 IU (α-alpha tochopherols extracted from palm oil provided by the Malaysian Palm Oil Board and approved by Ministry of Health, Malaysia) for a period
of six weeks. Control subjects were given capsules containing maltodextrine, non-caloric filled in empty capsules for six weeks. Both groups were advised to take the supplements and/or placebo capsules on a daily basis and after dinner. To improve the compliance, subjects were contacted more than three times a week to ensure that they had taken the supplements and/or placebo capsules which were provided on the weekly basis.

**Preliminary Tests**

In the preliminary test, baseline blood and urine samples were taken from the subjects before providing them with the supplements and placebo in order to measure the muscle damage markers CK, LDH and urinary TBARS. In addition, anthropometrical measurements, muscle circumferences of mid-upper arm and calf were also obtained.

**Supplementation and post tests**

After preliminary tests and measurements, subjects were asked to perform their daily routine of weightlifting training, 2-3 hours per day, 5 days per week. After 6 weeks of intervention, blood and urine sampling were repeated once again, and tested to determine any effects of the combined antioxidant vitamin C and E supplementation on muscle damage induced by weightlifting training.

**The workout protocol**

Subjects performed 6 sets of classic types of snatch and clean & jerk weight related lifting exercises on the 5 times a week basis. Program included: 3 to 8 exercises per session, load of 80 to 100% of 1RM with number of repetitions of 8 to 1 in a set accordingly. Rest intervals between the sets were from 60 to 120 sec depending on the load fixed on the barbell. One RM for training exercises was determined one week before testing on two separate days. The weight for the lifting tasks was fixed within 75 -100 % for each subject of the 1 RM of the previous week to make them able to perform 6-8 sets before the experimental trial. Rest between sets lasted for 60-120 seconds, and rest between exercises lasted for 5–8 min. The whole program intensity was set at 70%, 80%, 90%, 100% respectively for the first 4 weeks, then decreased back to 70% and 80% for the last 2 weeks of intervention.

**Anthropometric measurements and muscle circumferences**

Muscle circumferences were measured in centimeters by using 100 mm anthropometric tape measure on two parts of the body. The mid-upper arm
circumference was measured halfway between the acromion process of the scapula and the olecranon process distally. The calf circumference measured the proximal circumference of the leg (10).

**Blood Analysis**

Approximately 3 ml of venous blood were taken by using a gold serum separator tube from each subject after fasting during sample collection for both pre-test and post-test. Baseline tests were done after fasting and before giving the vitamins and placebo supplementations to the subjects to determine muscle damage markers of serum CK and LDH, in order to meet the inclusion criteria and to determine the physiological changes before and after taking the supplementations. All blood samples were centrifuged at 4000 RPM for 10 minutes at 4°C by using a Rotina 46 RS centrifuge. The samples were then sent to the laboratory of chemical pathology of Hospital Universiti Sains Malaysia (HUSM) for analysis by using the ARCHITECT c 8000 IL, USA, 2007 autoanalyser.

**Urinary TBARS**

Urine samples were collected from each subject after fasting during the pre-test and post-test. Urine samples were obtained from the first drops of urine void, and all samples were frozen at – 80°C until the assay was performed. The Cayman TBARS Assay kit was used to measure MDA in urine, where the MDA-TBA adduct formed by the reaction of MDA and TBA under high temperatures (90-100°C) and acidic conditions. They were then measured calorimetrically at 530-540 nm.

**Statistical Analyses**

All statistical analyses were performed by using the Statistical Package for Social Sciences (SPSS version 20.0). Normality of the data was examined through Kolmogorov-Smirnov test and the result showed that all data was not normally distributed. The Mann-Whitney Test was used to compare between the two independent groups. All the statistical significance was accepted at $p < 0.05$. All data were expressed as median and interquartile range.

**RESULTS**

In the present study, based on these results, anthropometrical measurements showed no alterations post intervention. While no change appeared in BMI, fat percentage and weight were slightly increased within the placebo group. Muscle
circumferences parameters were significantly decreased within the supplement group and within the placebo group after the intervention. Muscle damage markers such as serum CK and LDH levels showed no significant difference between the two groups before and after the intervention. Oxidative stress markers, Urinary TBARS, also did not show any significant differences between both groups after the intervention.

Table 1.

*Anthropometrical measurements of supplement group and placebo group (n=32).*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Supplement group (n=16)</th>
<th>Placebo group (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
</tr>
<tr>
<td></td>
<td>Pre test</td>
<td>Post test</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>161.5 (16.3)</td>
<td>162.1 (15.4)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>59.0 (28.7)</td>
<td>58.1 (22.4)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.8 (5.5)</td>
<td>21.3 (6.2)</td>
</tr>
<tr>
<td>Fat percentage (%)</td>
<td>25.2 (13.4)</td>
<td>23.7 (12.6)</td>
</tr>
</tbody>
</table>

*a Mann-Whitney Test

Table 2.

*Muscle circumferences of mid-upper arm and calf (cm) of pre- and post-test in supplement group and placebo group (n=32).*

<table>
<thead>
<tr>
<th>Variables</th>
<th>Median (IQR)</th>
<th>Z stata</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Supplement (n=16)</td>
<td>Placebo (n=16)</td>
<td></td>
</tr>
<tr>
<td>Mid-upper arm (right)</td>
<td>Pre test 28.3 (6.1)</td>
<td>26.0 (7.0)</td>
<td>-1.566</td>
</tr>
<tr>
<td></td>
<td>Post test 26.0 (7.0)</td>
<td>26.8 (6.8)</td>
<td>-0.623</td>
</tr>
<tr>
<td>Mid-upper arm (left)</td>
<td>Pre test 28.2 (5.9)</td>
<td>26.0 (6.0)</td>
<td>-1.811</td>
</tr>
<tr>
<td></td>
<td>Post test 25.8 (4.4)</td>
<td>26.3 (6.4)</td>
<td>-0.585</td>
</tr>
<tr>
<td>Calf (right leg)</td>
<td>Pre test 50.3 (10.0)</td>
<td>48.0 (13.5)</td>
<td>-0.735</td>
</tr>
<tr>
<td></td>
<td>Post test 48.8 (8.8)</td>
<td>49.5 (10.1)</td>
<td>-0.208</td>
</tr>
<tr>
<td>Calf (left leg)</td>
<td>Pre test 36.3 (6.8)</td>
<td>33.5 (3.9)</td>
<td>-1.189</td>
</tr>
<tr>
<td></td>
<td>Post test 33.5 (5.9)</td>
<td>33.5 (7.1)</td>
<td>-0.472</td>
</tr>
</tbody>
</table>

*a Mann-Whitney Test
Table 3.
Serum CK, LDH levels (U/L) and Urinary TBARS level (µM) of pre- and post-test in both the supplement group and placebo group (n=32).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Median (IQR)</th>
<th>Z stata</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Supplement (n=16)</td>
<td>Placebo (n=16)</td>
<td></td>
</tr>
<tr>
<td>CK</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre test</td>
<td>162.0 (55.0)</td>
<td>138.0 (49.0)</td>
<td>-0.0943</td>
</tr>
<tr>
<td>Post test</td>
<td>174.0 (63.0)</td>
<td>137.0 (47.0)</td>
<td>-0.057</td>
</tr>
<tr>
<td>LDH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre test</td>
<td>274.0 (101.0)</td>
<td>281.0 (56.0)</td>
<td>-2.070</td>
</tr>
<tr>
<td>Post test</td>
<td>245.0 (125.0)</td>
<td>181.0 (85.0)</td>
<td>-2.186</td>
</tr>
<tr>
<td>TBARS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre test</td>
<td>0.4 (0.3)</td>
<td>0.4 (0.4)</td>
<td>-0.625</td>
</tr>
<tr>
<td>Post test</td>
<td>0.4 (0.4)</td>
<td>0.5 (0.4)</td>
<td>-0.398</td>
</tr>
</tbody>
</table>

a Mann-Whitney Test

DISCUSSION

Muscle circumferences of mid-upper arm and calf

The present study found that there was no significant effect of dietary supplementation of vitamin C and E on mid upper arm and calf circumferences between the supplement group and placebo group during weightlifting training in the trained weightlifters. The results of this study were consistent with a previous study (13) which also did not find any significant difference of fish oil (vitamin E) and isoflavones supplementation on mid upper arm circumference in 50 maximal isokinetic eccentric elbow flexion contractions compared to the placebo. However, many other investigations reported little to no benefit of other nutritional supplements such as β-hydroxy-β-methylbutyrate and creatine monohydrate in relation to muscle circumference, as well as on exercise-induced muscle damage (21, 24) respectively.

Based on the results of the current study, there was no effect of vitamin C and E supplementation on mid upper arm and calf circumferences. However, these circumferences were decreased within the supplement group and did not change within the placebo group after the intervention, while no changes appeared in BMI, fat percentage, as well as body weight slightly increased in the placebo group. Interestingly, the circumference parameters look compatible with those anthropometric measurements such as weight, BMI and body fat percentage, which decreased as well within the supplement group.
However, the circumferences of mid upper arm calf and anthropometric measurements were slightly increased within placebo group after the intervention. It may be that these circumferences were partially affected by vitamin supplementation in the supplement group. It is believed that vitamin C increases the activity of copper-containing hydroxylase, such as dopamine-β-hydroxylase and Phenylalanine hydroxylase. These enzymatic reactions are catalysed by vitamin C (7). As phenylalanine, an essential amino acid which is required for muscle protein is converted to tyrosine by hydroxylation. Therefore, this may decrease muscle protein and in turn, muscle area (23). Moreover, vitamin C is believed to increase acidosis, which will stimulate muscle protein degradation and this may possibly decrease muscle circumference (18).

**CK and LDH enzymes activity**

The present study found that there is no significant effect of dietary supplements of vitamin C and E on muscle damage markers like CK and LDH enzymes activity during weightlifting training in the human model. This result was consistent with [18] which also did not find any significant effect of vitamin C and E supplementation on CK enzyme activity in eccentric exercise compared to the placebo. In another study, it was reported no significant effect of Astaxanthin supplementation on CK and LDH enzymes following exercise in resistance trained men (19). In addition, other investigations showed no benefit of antioxidants supplementation in relation to muscle damage or oxidative stress (1, 31).

It has been demonstrated by other investigations that vitamin C or E in a single supplementation did not influence muscle damage markers and CK activity, but on the contrary, CK activity increased in comparison with the placebo in full body resistance exercise protocol in 30 eccentric exercise of elbow flexor (1, 5) respectively.

In contrast, other studies reported that mixed vitamin C and E supplementation reduced CK enzyme activity compared to the placebo (2, 25, 11) and vitamin E supplementation significantly reduced LDH enzyme activity compared to the placebo (28).

The findings showed that there was no significant decrease in CK and LDH enzymes activity between supplement group and the placebo group. This discovery was consistent with other reports (3, 25, 3, 4, 11).

The recruited subjects for this study were regularly trained, therefore; it might be that individuals, who train regularly, are likely to have higher endogenous antioxidant activity (22). It seems that such adaptations occur and these individuals may not benefit from further exogenous antioxidant intake for purposes of attenuating signs and symptoms of muscle damage, or it might be that the muscle is strong enough and adapted to the training loads. Although, this probably depends on which component of the endogenous antioxidant defense system is positively altered by
weightlifting training; based on the results of this study, it seems to be the case (2). However, this does not mean that the antioxidants provided in this study would not prove beneficial for other purposes (e.g., general health). Moreover, these findings do not indicate that all antioxidant supplements would not influence any effect in regard to the markers assessed in the present investigation. From a training adaptation standpoint, it is uncertain as to whether or not exogenous antioxidant supplementation may decrease the adaptive response to weightlifting training.

**Urinary TBARS**

There was no effect observed for the antioxidant supplementation of dietary vitamin C and E in our sample of weightlifting trained subjects. Based on these results, it appears to be no combined effect of vitamin C and E supplementation on markers of muscle damage or oxidative stress following muscle damage-inducing weightlifting exercise. To assess oxidative damage, we measured urinary TBARS to detect lipid peroxidation.

The study found that there is no significant effect of dietary supplements of vitamin C and E on urinary TBARS activity during weightlifting training in the trained weightlifters. Urinary TBARS response observed in this study is in agreement with some of the previous investigations involving aerobic type exercise (25, 6). It is unclear at this point in time why both weightlifting exercise and aerobic-type exercise would result in no significant effect of vitamin C and E on TBARS and MDA, respectively. Although, in these studies the significant increase in production of free radicals was observed before providing vitamin C and E supplements. It has been reported that that MDA was significantly increased after the intervention with vitamin E for 3 weeks in whole-body resistance exercise among 18 males (1). In contrast, a study by (8) reported that MDA was decreased significantly after 48 hours of vitamin C and E plus selenium supplementation. Other investigations have shown that vitamin E supplementation reduces lipid peroxidation after eccentric contraction in humans (28, 26, 16). Some studies demonstrated that vitamin E provides protection against oxidative damage after downhill running (17). It was suggested that combinations of antioxidants, including vitamin E, reduce oxidative damage after eccentric contraction (29). In regards to the decrease in lipid peroxidation and protein carbonyls, vitamin E has been reported to protect cellular membranes and other fatty cellular components by donating electrons to free radicals (30, 20).

In this study, the lack of physiological stress may be associated with the volume and intensity of exercise, and the amount of muscle activation that the exercise protocol could invoke. Apparently, the intensity of the exercise protocol is vital to cause measurable changes in urinary TBARS. On the other hand, perhaps the subjects were highly adapted to the type of exercise or the endogenous levels
of non-enzymatic antioxidants suppressed by lipid peroxidation in body tissues. It appears as though weightlifting exercise by itself does not stimulate significant levels of increase in TBARS (8). Additional research is needed to further explain the impact of weightlifting exercise and antioxidant effect on altering this biomarker because studies are limited in this area and inconsistent on oxidative stress.

CONCLUSION

In summary, the present study reports no benefit of antioxidant supplementation of vitamin C 500 mg and vitamin E 400 IU on weightlifting training in attenuating markers of muscle damage or oxidative stress in trained weightlifters. It could be possible that tested individuals already have adequate protection, both structurally and biochemically, resulting from chronic exposure to weightlifting training. Additionally, supplements from exogenous antioxidants as provided in the present study, appears not to be beneficial. Therefore, under the current experimental constraints, our findings do not support the use of the specific antioxidant supplements for purposes of decreasing weightlifting-induced muscle damage or oxidative stress in those already considered to be regularly and well-trained.

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AUTHORS CONTRIBUTION

All authors have contributed to the findings of this study. All authors have reviewed and approved the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.
REFERENCES


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