

Research article

EFFECTS OF PALM VITAMIN E SUPPLEMENTATION ON EXERCISE-INDUCED OXIDATIVE STRESS AND ENDURANCE PERFORMANCE IN THE HEAT

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ABSTRACT

This study investigates the effects of tocotrienol-rich palm vitamin E supplementation on exercise-induced lipid peroxidation and endurance performance in the heat. In a double blind, cross-over study, eighteen healthy, male recreational athletes completed two endurance running trials, until exhaustion, on a motorized treadmill at 70% VO_{2max} on two separate occasions following a 6-week supplementation regimen of either tocotrienol-rich palm vitamin E (E) or placebo (P). Both trials were conducted in the heat (31°C, 70% relative humidity). During the trials, rectal temperature (T_{rec}), ratings of perceived exertion (RPE) and oxygen uptake (VO_2) were recorded. Blood samples were collected for the determination of plasma volume changes (PVC), malondialdehyde (MDA), creatine kinase (CK), total antioxidant status (TAS) and vitamin E. After the supplementation regimen, serum alpha-tocopherol increased ~33% but serum concentrations of tocotrienols were negligible. No significant differences were evident in mean T_{rec} , RPE, VO_2 or in the time to exhaustion between the E-supplemented and the placebo-supplemented groups. Similarly, mean PVC, CK and TAS were also not different between the two groups. Resting plasma mean MDA concentration in the E-supplemented group was significantly lower than that in the placebo-supplemented group. At exhaustion, plasma mean MDA was higher than the resting values in both groups. Although tocotrienol-rich palm vitamin E supplementation decreased lipid peroxidation at rest and, to some extent, during exercise in the heat, as evident from the lower MDA levels, it however did not enhance endurance running performance or prevent exercise-induced muscle damage or influenced body core temperature or plasma volume changes during exercise in the heat.

KEY WORDS: Oxidative stress, endurance, heat, malondialdehyde, palm vitamin E.

INTRODUCTION

Generation of reactive oxygen species (ROS) increases during exercise in both animal (Davies et al., 1982; Gohil et al., 1986) and man (Dillard et al., 1978; Kanter et al., 1988). Under normal circumstances, however, ROS are neutralized by an elaborate endogenous antioxidant system,

comprising of enzymatic and non-enzymatic antioxidants (Gohil et al., 1986; Urso and Clarkson, 2003). However, during increased oxygen utilization, as happens during strenuous exercise, the rate of ROS production may overwhelm the body's capacity to detoxify them (Sjodin et al., 1990; Urso and Clarkson, 2003), which can lead to increased oxidative stress and subsequent lipid peroxidation

(Davies et al., 1982; Jenkins 1988; Kanter et al., 1988). Free radical production reaches the highest level when exercise is exhaustive (Ji et al., 1998; Li et al., 1998; Sastre et al., 1992). In addition, strenuous physical activity is also associated with an increase in body temperature and elevated body temperature has also been shown to increase the rate of free radical production (Altan et al., 2003; Salo et al., 1991).

There is evidence that free radical production during exercise contributes to muscle fatigue (Barclay and Hansel, 1991; Novelli et al., 1990; Shindoh et al., 1990). Moreover, oxidative stress that results from antioxidant deficiency has been suggested to reduce endurance performance during exhaustive exercise (Coombes et al., 2002; Davies et al., 1982; Gohil et al., 1986). In this regards, high pre-race plasma vitamin E level has been associated with enhanced physical endurance in dogs (Piercy et al., 2001). But most human studies, however, have failed to demonstrate any performance enhancement associated with vitamin E supplementation (Lawrence et al., 1975; Rokitzki et al., 1994b; Sharman et al., 1971) except when exercise was performed at high altitude (Simon-Schnass and Pabst, 1988). Its role in enhancing endurance performance therefore remains debatable and unclear, particularly when exercising at high ambient temperature and humidity. Supplementation of vitamin E, on the other hand, has been consistently demonstrated to increase tissue resistance to exercise-induced lipid peroxidation (Dillard et al., 1978; Itoh et al., 2000; Kanter et al., 1993; Rokitzki et al., 1994a; Sumida et al., 1989). Moreover, vitamin E supplementation has also been shown to reduce the leakage of creatine kinase, a marker of exercise-induced muscle damage following exhaustive cycle exercise (Rokitzki et al., 1994a) and endurance running (Itoh et al., 2000). Whether these effects are also evident during exercise, in particular, when exercising in heat is unknown.

Vitamin E refers to the eight structural isomers of tocopherols and tocotrienols: alpha (α), beta (β), gamma (γ) and delta (δ) tocopherols and tocotrienols (Brigelius-Flohe and Traber, 1999; Kamal-Eldin and Appelqvist, 1996). However, most sports-related studies mentioned earlier have used alpha-tocopherols as the form of vitamin E. To our knowledge, to date, no scientific studies have examined free radical activity during exercise in a hot and humid environment, particularly, in heat-adapted recreational athletes, or examined the benefits of tocotrienol-rich palm vitamin E supplementation in these individuals when exercising in a hot and humid environment. Palm

vitamin E contains natural tocotrienol-rich fractions dissolved in palm olein (Hood, 1996). Compared to α -tocopherol, α -tocotrienol is reportedly more effective in the inhibition of lipid peroxidation, being 40-60 times more potent than α -tocopherol (Serbinova et al., 1991). In addition, tocotrienol has also been shown to possess hypocholesterolaemic, anti-thrombotic and anti-tumour effects, indicating that tocotrienol may serve as an effective agent in the prevention and/or treatment of cardiovascular disease and cancer (Theriault et al., 1999). The present study therefore investigates the effects of tocotrienol-rich palm vitamin E supplementation on exercise-induced lipid peroxidation and endurance performance in the heat in heat-adapted recreational athletes.

METHODS

Eighteen, male heat-adapted recreational athletes participated in this study. Their age, height, weight, percent body fat and VO_{2max} were 24.9 ± 1.4 yrs, 1.69 ± 0.01 m, 59.6 ± 1.5 kg, 18.0 ± 0.9 % and 57.7 ± 1.5 ml·kg⁻¹·min⁻¹ respectively. An informed consent was obtained from each subject and the experimental procedures were approved by the Research and Ethics Committee of University Sains Malaysia.

After familiarization with treadmill running, the subjects performed two tests to determine the relationship between running speed and oxygen uptake and their maximum oxygen uptake (VO_{2max}). From these data, running speeds during warm-up at 50% VO_{2max} and endurance running performance at 70% VO_{2max} were established. All subjects were required to undertake a 60-minute trial run on a motorized treadmill at 70% VO_{2max} one week before the first experimental trial to familiarize with the experimental protocol.

A double blind, placebo-controlled, randomized crossover design was used to control within subject variability of all physiological measurements and the effect of order of the administration of the two supplements (tocotrienol-rich palm vitamin E and placebo). There was a washout period of 2 weeks between the two regimens. After a 6-week supplementation regimen with either tocotrienol-rich palm vitamin E (E) or placebo (P), endurance running performance tests were conducted at 31°C and 70% relative humidity for both the groups. Each subject was required to run to exhaustion at 70% VO_{2max} on a motorized treadmill on two different occasions.

Palm vitamin E was supplemented in the form of capsules, provided by the Malaysian Palm Oil Board (MPOB). Each capsule contained 60 mg of

palm vitamin E, a highly concentrated fraction of tocotrienols (33% alpha-tocopherol and 33% alpha-tocotrienol, 24% gamma-tocotrienol, 10% delta-tocotrienol) in 240 mg of superolein while each placebo capsule contained 300 mg of superolein. Participants were instructed not to take other forms of antioxidant supplements during the course of the study and to maintain their physical activity level throughout the supplementation period.

To minimize differences in resting muscle glycogen concentrations, subjects recorded their food intake for 3 days before the first experimental trial and were then instructed to follow the same diet before the second trial. They were also required to abstain from training the day before each trial and to observe a 10 h fast before their arrival to the laboratory. Upon arrival at the laboratory they were given a standardized breakfast, after which they were asked to empty their urinary bladder, and their nude body weight was recorded (Tanita, Japan). A rectal thermistor (Yellow Springs Instrument, USA) was inserted to a depth of 10 cm beyond the anal sphincter for the measurement of core temperature. In addition, skin thermistors (Yellow Springs Instrument, USA) were attached to the chest, biceps, thigh and calf for the measurement of mean skin temperature (Ramanathan, 1964). An indwelling cannula (Vasocan[®], B. Braun, Malaysia) was inserted into a forearm vein for repeated blood withdrawals. Patency of the cannula was maintained with heparinized saline (10 IU heparin sodium in 1 ml 0.9% NaCl, B. Braun, Malaysia).

After standing on the treadmill for 5 minutes, a resting blood sample was collected and CO₂ and O₂ concentrations in the expired air were measured (SensorMedics 2900, USA). Following a 5 minute warm-up run at 50% VO_{2 max} on the treadmill, the speed of the treadmill was increased to elicit 70% VO_{2 max} and the subjects were asked to run until exhaustion. Oxygen and CO₂ in the expired air samples were recorded immediately after warm-up, at 10 minutes into exercise and every 20 minutes thereafter until exhaustion. Venous blood (3 ml) was collected at rest, after warm-up, at 20-minute intervals during the trials, at exhaustion, and at 1 and 24 h post-exercise. Heart rate (Sport Tester PE 3000, Polar, Finland), ratings of perceived exertion (RPE), core and skin temperatures (Libra Medical ET 300R, USA), room temperature and relative humidity (whirling hygrometer, Brannan, England) were recorded every of 10 minutes throughout the exercise test. After completion of the run, the subjects towel-dried themselves and post-exercise nude body weight was measured.

Hematocrit was estimated in EDTA-treated

blood on a microhematocrit centrifuge (Hettich-Haematokrit 20, Germany) and reader (Hawksley, England), and the percent change in plasma volume was calculated using the formula of van Beaumont et al. (1981). For the estimation of malondialdehyde and creatine kinase, 1 ml of EDTA-treated blood was centrifuged at 4000 rpm (Hettich-Rotina 46RS, Germany) and at 4°C for 10 minutes, and the plasma from this sample was then divided into two equal portions and stored at -80°C (Heto Ultra Freeze 3410, Denmark) for subsequent analysis of malondialdehyde and creatine kinase. Plasma malondialdehyde was determined as thiobarbituric acid reactive substances (TBARS) using high performance liquid chromatography (HPLC) (Nielsen et al., 1997). Plasma creatine kinase was analysed using a commercially available kit (Randox, U.K.) and determined colorimetrically (Hitachi Automatic Analyzer 912, Bohringer Mannheim, Germany). The remainder of the blood sample was allowed to clot and then centrifuged at 4,000 rpm for 10 minutes and 4°C. The supernatant was divided into two portions and stored at -80°C for the analysis of serum vitamin E and total antioxidant status. Serum total antioxidant status was analysed colorimetrically (Hitachi Automatic Analyzer 912, Bohringer Mannheim, Germany) using a reagent kit (Randox, U. Kingdom). Serum vitamin E was determined using HPLC (Agilent 1100 series, Agilent Technologies, Germany) after extraction with hexane. Briefly, 50µl of the sample was introduced through the injector into 2 normal phase Zorbax 5 micron (250 X 4.6 mm) columns connected in series. The mobile phase was 99.25:0.75 mixture (hexane: 2-propanol); which was set at a flow rate of 2 ml·min⁻¹. Hexane and 2-propanol mixture used in conjunction with Zorbax silica column has been found to be the optimal normal-phase system for the analysis of tocopherol and tocotrienol isomers (Tan and Brzuskiwicz, 1989). Isomers of vitamin E were detected with a fluorescence detector at EX (excitation wavelength) of 295 nm and EM (emission wavelength) of 325 nm. Standards for tocopherols/tocotrienols were purchased from Merck, Germany.

Statistical analysis

Two-way analysis of variance (ANOVA) with repeated measures was used to compare differences between groups. Bonferroni post-hoc test was used to determine significant mean differences. Comparison of the ratings of perceived exertion was done using Wilcoxon Signed-Rank test. The accepted level of significance was set at $p < 0.05$. Data are expressed as means \pm SE.

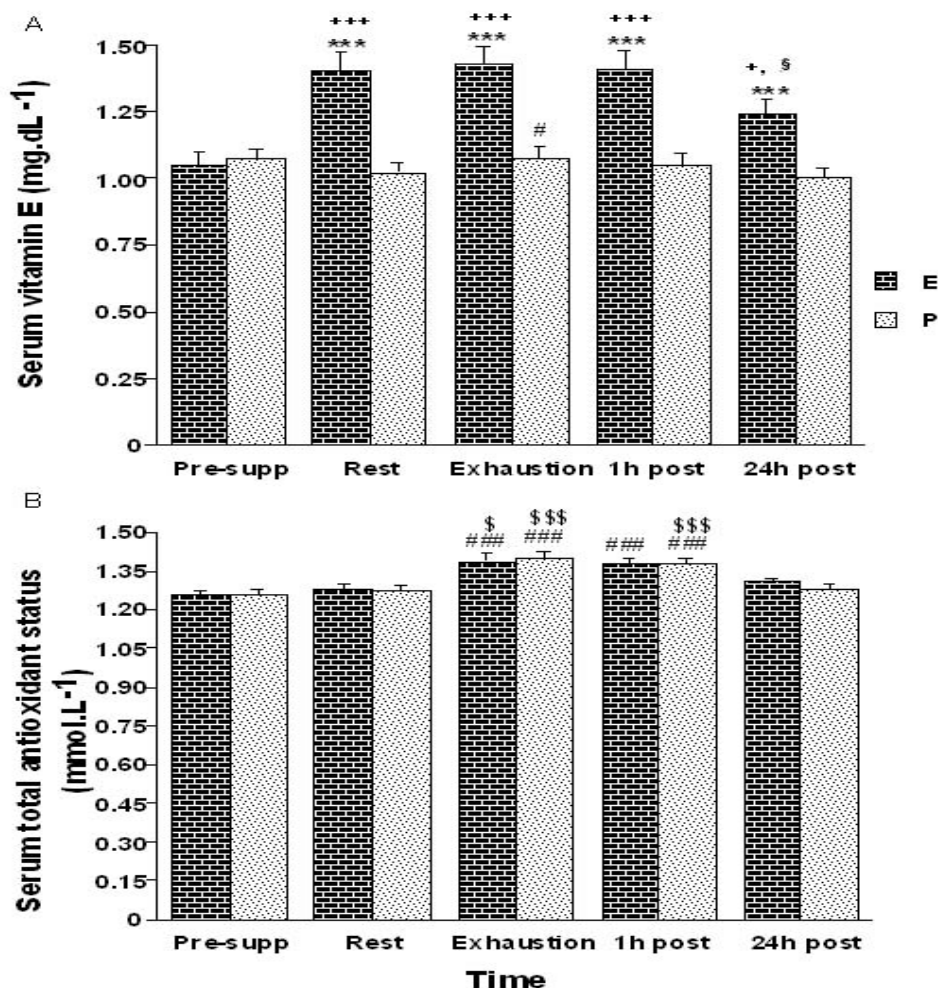


Figure 1. A) Serum vitamin E (alpha-tocopherol, mg·dL⁻¹) and B) Total antioxidant status (mmol·L⁻¹) during and after exercise in the palm vitamin E-supplemented (E) and placebo-supplemented (P) groups.

*** significantly different from corresponding values in P ($p < 0.001$).

+, *** significantly different from corresponding pre-supplementation value ($p < 0.05$ and < 0.001 , respectively).

significantly different from respective resting value ($p < 0.05$).

§ significantly different from respective exhaustion and 1h post-exercise values ($p < 0.05$).

§, §§§ significantly different from corresponding 24h post-exercise values ($p < 0.05$ and < 0.001 , respectively).

RESULTS

Mean room temperature and relative humidity (RH) in the groups were well maintained at $30.9 \pm 0.1^\circ\text{C}$, $70.1 \pm 0.2\%$ RH and $31.0 \pm 0.1^\circ\text{C}$, $70.3 \pm 0.3\%$ RH respectively. Mean oxygen consumption during exercise was similar in both the groups (40.1 ± 0.4 ml·kg⁻¹·min⁻¹ and 40.6 ± 0.5 ml·kg⁻¹·min⁻¹ in the E-supplemented and placebo-supplemented groups respectively). These figures correspond to $70.1 \pm 0.6\%$ and $70.6 \pm 0.7\%$ of $\text{VO}_{2\text{max}}$, which was maintained during exercise in the E-supplemented and placebo-supplemented groups respectively. Running time of heat-adapted individuals to exhaustion was not significantly different between the E-supplemented and placebo-supplemented

groups (81.1 ± 4.5 min vs. 76.9 ± 4.5 min respectively).

Serum vitamin E concentrations increased significantly ($p < 0.001$) after six weeks of tocotrienol-rich palm vitamin E supplementation (E-supplemented group; Figure 1A). In contrast, there were no significant differences in serum vitamin E before and after placebo supplementation. Serum vitamin E concentrations at exhaustion were higher when compared to their respective resting values in both groups but it was only statistically significant ($p < 0.05$) in the placebo-supplemented group. All differences calculated for serum vitamin E remained statistically significant even after the data were adjusted for plasma volume changes except between exhaustion and resting values in the placebo-supplemented group.

Resting serum total antioxidant status (TAS) in both groups was not different from their respective pre-supplementation values. However, serum TAS was significantly higher ($p < 0.001$) at exhaustion and at 1 h post-exercise when compared to their corresponding resting levels in both the groups (Figure 1B). Serum TAS at 24 h post-exercise was significantly ($p < 0.05$) lower than at exhaustion in the E-supplemented group. Similarly, in the placebo group serum TAS levels at exhaustion and 1 h after exercise were significantly higher than that at 24 h post-exercise ($p < 0.001$). In the E-supplemented group however, significant difference was only evident between the levels at exhaustion and 24 h after exercise ($p < 0.05$) but not between 1 and 24 h post exercise. There were however no significant differences in serum TAS between the two groups.

Resting plasma malondialdehyde (MDA)

levels were significantly lower ($p < 0.05$) after supplementation in the E-supplemented group when compared to the placebo-supplemented group (Figure 2A). Although at exhaustion, mean MDA level was lower in the E-supplemented group compared to the placebo-supplemented group, this difference, however, was not statistically significant ($p = 0.090$).

Creatine kinase (CK) levels at exhaustion, 1 h and 24 h post-exercise were significantly ($p < 0.001$) higher than their corresponding resting values in both groups (Figure 2B). Plasma CK levels, although were higher in the placebo-supplemented group during and after exercise, but when compared to the E-supplemented group, the differences were however not statistically significant. All statistically significant differences for the plasma CK levels were maintained even after correction for changes in plasma volume.

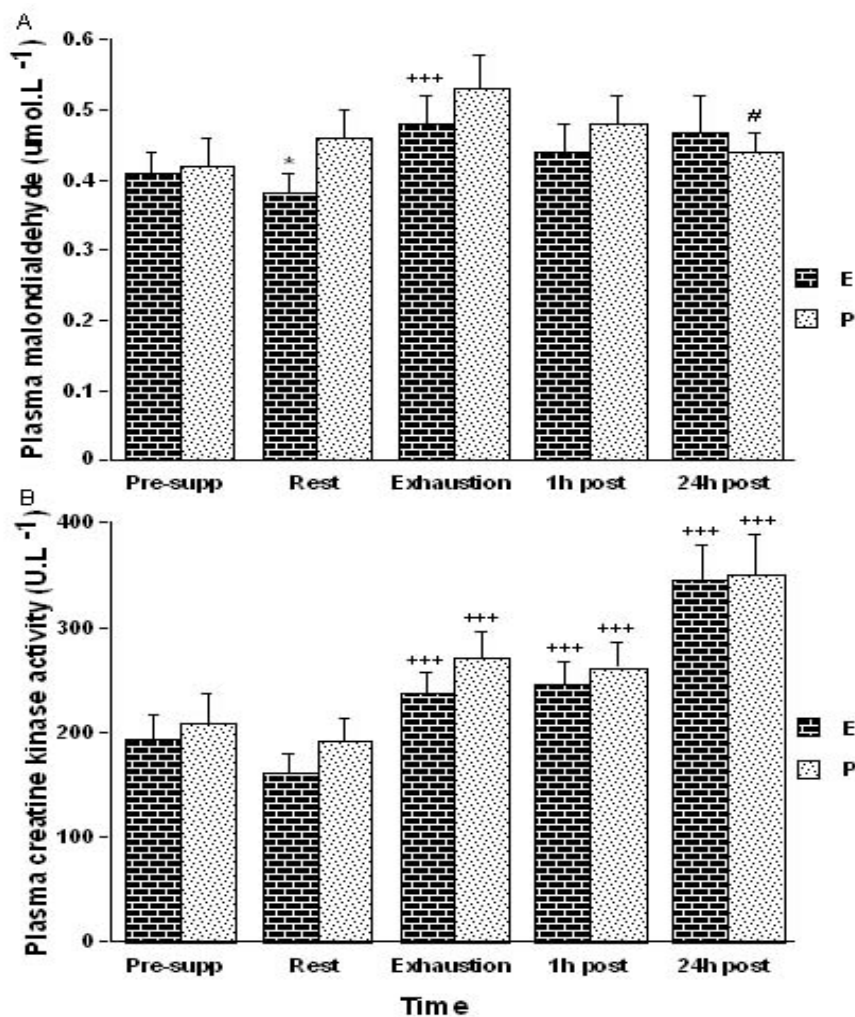


Figure 2. A) Plasma malondialdehyde ($\mu\text{mol}\cdot\text{L}^{-1}$) and B) Plasma creatine kinase activity ($\text{U}\cdot\text{L}^{-1}$) during and after exercise in the palm vitamin E-supplemented (E) and placebo-supplemented (P) groups.

* significantly different from P at $p < 0.05$.

+++ significantly different from respective resting value ($p < 0.001$).

significantly different from corresponding value at exhaustion ($p < 0.05$).

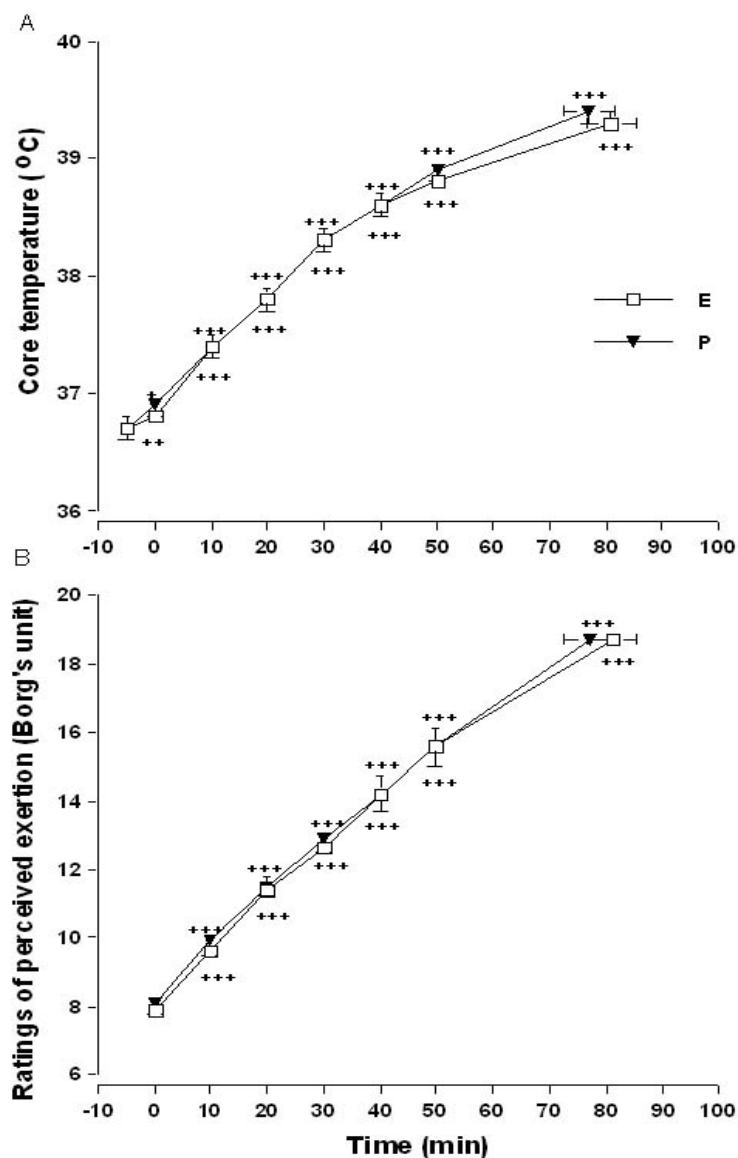


Figure 3. A) Core temperature ($^{\circ}\text{C}$) and (B) Ratings of perceived exertion (Borg's unit) during exercise in the palm vitamin E-supplemented (E) and placebo-supplemented (P) groups. +, ++, +++ significantly different from respective resting values ($p < 0.05$, < 0.01 and < 0.001 , respectively).

Core temperature increased significantly ($p < 0.001$) over time from rest until exhaustion in both the groups (Figure 3A). However, there were no significant differences in core temperature between the two groups. Core temperature at exhaustion was $39.3 \pm 0.1^{\circ}\text{C}$ and $39.4 \pm 0.1^{\circ}\text{C}$ in the E-supplemented and placebo-supplemented groups respectively. RPE increased significantly from rest until exhaustion ($p < 0.001$) in both the groups (Figure 3B). However, RPE scores were similar throughout exercise in both groups. RPE at exhaustion was 18.7 ± 0.2 in both groups.

Plasma volume decreased over time during exercise in both groups ($p < 0.001$) (Table 1). Compared to the resting level, plasma volume in the E-supplemented groups was not different at 1 h post-

exercise but was higher ($p < 0.001$) at 24 h post-exercise. Plasma volume in the placebo-supplemented group was not different at 1 h and 24 h post-exercise when compared to the resting level. The plasma volume changes during and after exercise were similar between E-supplemented and placebo-supplemented groups.

DISCUSSION

Although the subjects in this study were given a tocotrienol-rich palm vitamin E supplement, the concentrations of serum tocotrienol were very low despite a 6-week supplementation regimen. The reason for this remains unclear although similar findings have also been reported before (Mensink et

Table 1. Plasma volume changes (PVC, %) in the palm vitamin E-supplemented and placebo-supplemented groups

| Trials | | Rest | After warm-up | 20 min | 40 min | Exhaustion | 1 h post-ex | 24 h post-ex |
|---------|---|------|---------------|--------------|--------------|--------------|-------------|--------------|
| PVC (%) | E | 0 | -2.3 (.2)*** | -4.4 (.2)*** | -4.7 (.5)*** | -6.2 (.3)*** | 1.2 (.5) | 3.9 (.7) |
| | P | 0 | -2.5 (.2)*** | -4.4 (.3)*** | -5.0 (.4)*** | -6.6 (.4)*** | .2 (.6)*** | 2.6 (1.1)*** |

*, ** and *** denote significantly ($p < 0.05$, < 0.01 and < 0.001 , respectively) difference from corresponding resting values.

al., 1999; Khor et al., 1995). The fate of supplemented tocotrienols and the relationship between its intestinal absorption, blood levels, and tissue distribution are still not fully understood (Watkins et al., 1999). It is possible tocotrienol is either metabolized immediately, or converted to alpha-tocopherol, or rapidly taken up by the adipose tissue and displacing alpha-tocopherol from these tissues. In this study, the supplementation of tocotrienol-rich palm vitamin E resulted in a significant increase in alpha-tocopherol but not in tocotrienol. Thus, serum vitamin E concentrations reported in this study are concentrations of alpha-tocopherol.

Pre-supplementation serum alpha-tocopherol levels (Figure 1A) indicate that the subjects in this study were not vitamin E-deficient. Supplementation of tocotrienol-rich palm vitamin E resulted in ~33% increase in serum alpha-tocopherol. Similar increases in serum alpha-tocopherol concentrations, after vitamin E supplementation have also been reported before (Dillard et al., 1978; Itoh et al., 2000; Rokitzki et al., 1994a; Sumida et al., 1989). Owing to its fat solubility alpha-tocopherol is easily retained in the body after absorption.

No significant difference was evident in the endurance running performance in heat following tocotrienol-rich palm vitamin E supplementation when compared to the placebo-supplemented group (81.1 ± 4.5 min vs 76.9 ± 4.5 min in the E-supplemented and placebo-supplemented groups respectively). Similar findings have also been reported before (Lawrence et al., 1975; Sharman et al., 1971; 1976), where vitamin E supplementation was also found not to improve endurance performance, although these studies were all done in thermoneutral environment and none were on heat-adapted individuals exercising in heat.

Apart from having no effect on endurance running performance, interestingly, tocotrienol-rich palm vitamin E supplementation also did not increase serum TAS (Figure 1B) and this despite a significant rise in serum alpha-tocopherol (Figure 1A) during the same period. The reason for this is not clearly apparent although it has also been reported before following supplementation of vitamins A and E (Jakeman and Maxwell, 1993;

Schroder et al., 2000). One possible explanation for this observation is that the measurement of serum TAS reflects the overall antioxidant capacity of the serum, which includes both enzymatic (superoxide dismutase, catalase and glutathione peroxidase) and non-enzymatic antioxidants (vitamins A, C and E, glutathione, ubiquinones, α -lipoic acid, flavonoids and uric acid). Palm vitamin E supplementation to non-deficient individuals under resting conditions may be downgrading the synthesis and possibly the activity of some of these endogenous antioxidants, but with little net change in overall TAS activity. Clearly, more studies are needed to ascertain these possibilities. It nevertheless appears that significant elevation of serum vitamin E following supplementation in normal individuals with normal vitamin E levels does not necessarily correlate to overall serum TAS.

Although tocotrienol-rich palm vitamin E supplementation did not alter resting TAS, the concentration of MDA, a marker of lipid peroxidation, was nevertheless significantly lower ($p < 0.05$) in the E-supplemented group when compared to that in the placebo-supplemented group at rest (Figure 2A), suggesting an increased antioxidant activity at rest. Similar decrease in MDA at rest following supplementation with antioxidant vitamins has been reported before (Itoh et al., 2000; Kanter et al., 1993; Sumida et al., 1989). Interestingly, although tocotrienol-rich palm vitamin E supplementation decreased resting MDA levels, it however did not prevent exercise-induced increase in lipid peroxidation. The levels of plasma MDA at exhaustion increased in both groups (Figure 2A), although statistically significant increase was only evident in the E-supplemented group ($p < 0.001$) and not in the placebo-supplemented group ($p = 0.081$). Nevertheless, plasma MDA level in the E-supplemented group at exhaustion was slightly lower than that in the placebo-supplemented group, although the difference did not reach statistical significance ($p = 0.099$). The reason for this is also not apparent. Compared with the placebo-supplemented group, plasma MDA in the E-supplemented group was 11.1% and 6.7% lower at exhaustion and 1 h post-exercise respectively, suggesting that the positive antioxidant effect that

was apparent at rest was carried over during exercise. When viewed together, these results indicate that tocotrienol-rich palm vitamin E supplementation only decreases lipid peroxidation at rest and, perhaps to a very small extent, during exercise in the heat. This evident benefit, albeit small, of tocotrienol-rich palm vitamin E supplementation on lipid peroxidation during exercise appears in contrast to some other studies, which found no beneficial effects of vitamin E supplementation on lipid peroxidation during exercise (Maxwell et al., 1993; Vasankari et al., 1997). The reason for this is unclear but it may be related to the type of vitamin E supplemented or due to different techniques used for the measurement of TBARS in plasma after exercise. However, more studies are needed to verify the benefit of vitamin E supplementation on lipid peroxidation during exercise, possibly measuring other markers of lipid peroxidation at the same time.

Increased oxidative stress is believed to be responsible for the slight muscle damage that follows exhaustive exercise and increases in plasma creatine kinase are considered to indicate muscle damage (McBride et al., 1998; Kanter et al., 1993). We estimated plasma creatine kinase activity to see if tocotrienol-rich palm vitamin E supplementation reduced muscle damage during exercise. Supplementation of tocotrienol-rich palm vitamin E, did not result in any significant changes in plasma creatine kinase (CK) activity from pre-supplementation values in both groups (Figure 2B) and plasma CK activity at exhaustion and post-exercise (1 and 24 hours) increased significantly ($p < 0.001$) from the resting values and somewhat equally in both groups (Figure 2B). The increase in plasma CK activity was particularly more marked after 24 hours. The delay is possibly due to the length of time it takes for creatine kinase released from damaged muscle fibres to enter the blood. These data suggest that muscle damage did occur during exercise in both trials, and although it was slightly lower in the E-supplemented group, supplementation of tocotrienol-rich palm vitamin E did not prevent muscle damage during strenuous exercise. This is in contrast to what has been observed before where vitamin E supplementation was found to prevent the elevation of enzyme leakage during exhaustive exercise in man and animal (McBride et al., 1998; Kanter et al., 1993). Subjects supplemented with antioxidant vitamins had a significantly lower creatine kinase value at 24 h post-race compared to non-supplemented subjects (Rokitzki et al., 1994a; 1994b). Vitamin E supplementation has also been shown to reduce the leakage of CK following 6 successive days of

endurance running (Itoh et al., 2000) or after a 40-min run (Gillam et al., 1992). The reason for the difference in our findings and those mentioned above is unclear but it may be related to the duration of vitamin E supplementation and the hot and humid environment of exercise. In the study of Rokitzki et al. (1994a) α -tocopherol was supplemented for a period of 5-months, whereas tocotrienol-rich palm vitamin E was only supplemented for 6 weeks in this study.

Endurance performance is influenced by a number of other factors including changes in body core temperature and plasma volume during exercise. We also examined the effects of tocotrienol-rich palm vitamin E supplementation on these. During exercise, body core temperature increased significantly in both the groups reaching values of 39.3° and 39.4° C at exhaustion in the E- and placebo-supplemented groups respectively (Figure 3A), and the changes in body core temperature of the subjects were similar in both groups. The rise in body temperature during exercise is well established and the similar rise in both groups in this study is probably due to the same environmental temperature and relative humidity during the trials.

As was the case with body core temperature, there was also no significant difference in plasma volume changes during exercise in both the groups (Table 1). Similar significant reductions ($p < 0.001$) in plasma volume, from pre-exercise values, were evident in both groups. Reduction in plasma volume during exercise is a well-established occurrence and, has been attributed to redistribution of body fluids and loss of fluids through sweat and breathing during exercise. Of importance from this study are the findings that tocotrienol-rich palm vitamin E supplementation does not significantly affect changes in body core temperature and plasma volume before, during or after exhaustive exercise.

CONCLUSION

This is the first study carried out to investigate the effects of tocotrienol-rich palm vitamin E supplementation on lipid peroxidation and endurance running performance in the heat in heat-adapted individuals. It appears that 6 weeks of tocotrienol-rich palm vitamin E supplementation to heat-adapted recreational athletes with normal serum vitamin E levels does not improve endurance running performance. This is in agreement with a number of other studies although in most other studies α -tocopherol was the main source of vitamin E (Lawrence et al., 1975; Sharman et al., 1971; 1976). Despite the claims that tocotrienol is a more

potent antioxidant than tocopherol (Serbivona et al., 1991), the present study showed that, as was the case with studies using alpha-tocopherol, endurance running performance was not enhanced after 6-weeks of tocotrienol-rich palm vitamin E supplementation. The reason for the lack of a positive effect of tocotrienol-rich palm vitamin E on endurance performance may be because the subjects in this study had adequate vitamin E stores and therefore additional supplementation did not bring any extra benefit to them. It is possible potential benefits may only be evident in vitamin E deficient individuals. The results of the present study however do indicate that supplementation with tocotrienol-rich palm vitamin E decreases the level of lipid peroxidation at rest.

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REFERENCES

- Altan, O., Pabuccuoglu, A., Altan, A., Konyalioglu, S. and Bayraktar, H. (2003) Effect of heat stress on oxidative stress, lipid peroxidation and some stress parameters in broilers. *British Poultry Science* **44**, 545-550.
- Barclay, J.K. and Hansel, M. (1991) Free radicals may contribute to oxidative skeletal muscle fatigue. *Canadian Journal of Physiology and Pharmacology* **69**, 279-284.
- Brigelius-Flohe, R. and Traber, M.G. (1999) Vitamin E: function and metabolism. *FASEB Journal* **13(10)**, 1145-1155.
- Coombs, J.S., Rowell, B., Dodd, S.L., Demirel, H.A., Naito, H., Shanley, R.A. and Powers, S.K. (2002) Effects of vitamin E deficiency on fatigue and muscle contractile properties. *European Journal of Applied Physiology* **87**, 272-277.
- Davies, K.J.A., Quintanilha, A.T., Brooks, G.A. and Packer, L. (1982) Free radicals and tissue damage produced by exercise. *Biochemistry Biophysics Research Communications* **107**, 1198-1205.
- Dillard, C.J., Litov, R.E., Savin, W.M., Dumelin, E.E. and Tappel, A.L. (1978). Effects of exercise, vitamin E and ozone on pulmonary function and lipid peroxidation. *Journal of Applied Physiology* **45**, 927-932.
- Gillam, I., Skinner, S. and Telford, R. (1992) Effect of antioxidant supplements on indices of muscle damage and regeneration. *Medicine and Science in Sports and Exercise* **24** (Suppl), S17.
- Gohil, K., Packer, L., DeLumen, B., Brooks, G.A. and Terblanche, S.E. (1986) Vitamin E deficiency and vitamin C supplements: Exercise and mitochondrial oxidation. *Journal of Applied Physiology* **60**, 1986-1991.
- Hood, R.L. (1996) Tocotrienol and cholesterol metabolism. In: *Nutrition, lipids, health and disease*. Eds: Ong, A.S.H., Niki, E. and Packer, L. Illinois: AOCS Press. 96-103.
- Itoh, H., Ohkuwa, T., Yamazaki, Y., Shimoda, T., Wakayama, A., Tamura, S., Yamamoto, T., Sato, Y. and Miyamura, M. (2000) Vitamin E supplementation attenuates leakage of enzymes following 6 successive days of running training. *International Journal of Sports Medicine* **21**, 369-374.
- Jakeman, P. and Maxwell, S. (1993) Effects of antioxidant vitamin supplementation on muscle function after eccentric exercise. *European Journal of Applied Physiology* **67**, 426-430.
- Jenkins, R.R. (1988) Free radical chemistry: Relationship to exercise. *Sports Medicine* **5**, 156-170.
- Ji, L.L., Bejma, J., Ramires, P.R. and Donahue, C. (1998) Free radical generation and oxidative stress in the heat are intensified during aging and exhaustive exercise. *Medicine and Science in Sports and Exercise* **30**, S322.
- Kamal-Eldin, A. and Appelqvist, L.A. (1996) The chemistry of antioxidant properties of tocopherol and tocotrienol. *Lipids* **31(7)**, 671-701.
- Kanter, M.M., Nolte, L.A. and Holloszy, J.O. (1993) Effects of an antioxidant vitamin mixture on lipid peroxidation at rest and postexercise. *Journal of Applied Physiology* **74**, 965-969.
- Kanter, M.M., Lesmes, G.R., Kaminsky, L.A., Ham-Saeger, J.L. and Nequin, N.D. (1988) Serum creatine kinase and lactate dehydrogenase changes following an eighty kilometer race. *European Journal of Applied Physiology* **57**, 60-63.
- Khor, H.T., Chieng, D.Y. and Ong, K.K. (1995) Tocotrienols inhibit liver HMG CoA reductase activity in the guinea pig. *Nutrition Research* **15**, 537-544.
- Lawrence, J.D., Bower, R.C., Riehl, W.P. and Smith, J.L. (1975) Effects of alpha-tocopherol acetate on the swimming endurance of trained swimmers. *American Journal of Clinical Nutrition* **28**, 205-208.
- Li, J.X., Chan, K.M., Xin, D., and Li, H. (1998) A study of generation of oxygen free radicals in skeletal muscle during and after exercise. *Medicine and Science in Sports and Exercise* **30**, S321.
- Maxwell, S.R., Jakeman, P., Thomason, H., Leguen, C. and Thorpe, G.H. (1993) Changes in plasma antioxidant status during eccentric exercise and the

- effect of vitamin supplementation. *Free Radical Research Communication* **19**, 191-202.
- McBride, J.M., Kraemer, W.J., Triplett-McBride, T. and Sebastianelli, W. (1998) Effects of resistance exercise on free radical production. *Medicine and Science in Sports and Exercise* **31**, 67-72.
- Mensink, R.P., van Houwelingen, A.C., Kromhout, D. and Hornstra, G. (1999) A vitamin E concentrate rich in tocotrienols had no effect on serum lipids, lipoproteins, or platelet function in men with mildly elevated serum lipid concentrations. *American Journal of Clinical Nutrition* **69**, 213-219.
- Nielsen, F., Mikkelsen, B.B., Nielsen, J.B., Andersen, H.R. and Grandjean, P. (1997) Plasma malondialdehyde as biomarker for oxidative stress: reference interval and effects of life-style factors. *Clinical Chemistry* **43**, 1209-1214.
- Novelli, G.P., Bracciotti, G. and Falsini, S. (1990). Spin trappers and vitamin E prolong endurance to muscle fatigue in mice. *Free Radical Biology and Medicine* **8**, 9-13.
- Piercy, R.J., Hinchcliff, K.W., Morley, P.S., Disilvestro, R.A., Reinhart, G.A., Nelson, S.L. Jr., Schmidt, E. and Craig, A.M. (2001) Association between vitamin E and enhanced athletic performance in sled dogs. *Medicine and Science in Sports and Exercise* **33**, 826-833.
- Ramanathan, N.L. (1964) A new weighing system for men surface temperature of the human body. *Journal of Applied Physiology* **19**, 531-533.
- Rokitzki, L., Logemann, E., Huber, G., Keck, E. and Keul, J. (1994a) Alpha-tocopherol supplementation in racing cyclists during extreme endurance training. *International Journal of Sports Nutrition* **4**, 253-264.
- Rokitzki, L., Logemann, E., Sagredos, A.N., Murphy, M., Wetzel-Roth, W. and Keul, J. (1994b) Lipid peroxidation and antioxidative vitamin under extreme endurance stress. *Acta Physiologica Scandinavica* **151**, 149-158.
- Rowell, L.B., Brengelmann, G.L., Blackmon, J.R., Twiss, R.D. and Kusumi, F. (1968) Splanchnic blood flow and metabolism in heat-stressed man. *Journal of Applied Physiology* **24**, 475-484.
- Salo, D.C., Donovan, C.M. and Davies, K.J. (1991) HSP70 and other possible heat shock or oxidative stress proteins are induced in skeletal muscle, heart, and liver during exercise. *Free Radical Biology and Medicine* **11**, 239-246.
- Sastre, J., Asensi, M., Gasco, E., Pallando, F.V., Ferrero, J.A., Furukawa, T. and Vina, J. (1992) Exhaustive physical exercise causes oxidation of glutathione status in blood: prevention by antioxidant administration. *American Journal of Physiology* **263**, R992-R995.
- Schroder, H., Navarro, E., Tramullas, A., Mora, J. and Galiano, D. (2000) Nutritional antioxidant status and oxidative stress in professional basketball players: Effects of a three compound antioxidative supplement. *International Journal of Sports Medicine* **21**, 146-150.
- Serbinova, E., Kagan, V., Han, D. and Packer, L. (1991) Free radical recycling and intra membrane mobility in the antioxidant properties of alpha-tocopherol and alpha-tocotrienol. *Free Radical Biology and Medicine* **10**, 263-275.
- Sharman, I.M., Down, M.G. and Norgan, N.G. (1976) The effects of vitamin E on physiological function and athletic performance of trained swimmers. *Journal of Sports Medicine and Physical Fitness* **16**, 215-225.
- Sharman, I.M., Down, M.G. and Sen, R.N. (1971) The effects of vitamin E and training on physiological function and athletic performance in adolescent swimmers. *British Journal of Nutrition* **26**, 265-276.
- Shindoh, C., DiMarco, A., Thomas, A., Manubay, P. and Supinski, G. (1990) Effect of N-acetylcysteine on diaphragm fatigue. *Journal of Applied Physiology* **68**, 2107-2113.
- Simon-Schnass, I. and Pabst, H. (1988) Influence of vitamin E on physical performance. *International Journal of Vitamin Nutrition Research* **58**, 49-54.
- Sjodin, B., Hellsten-Westing, Y. and Apple, F.S. (1990) Biochemical mechanisms for oxygen free radical formation during exercise. *Sports Medicine* **10**, 236-254.
- Sumida, S., Tanaka, K., Kitao, H. and Nakadomo, F. (1989) Exercise-induced lipid peroxidation and leakage of enzymes before and after vitamin E supplementation. *International Journal of Biochemistry* **21**, 835-838.
- Tan, B. and Brzuskiwicz, L. (1989) Separation of tocopherol and tocotrienol isomers using normal- and reverse-phase liquid chromatography. *Analytical Biochemistry* **180(2)**, 368-373.
- Theriault, A., Chao, J., Wang, Q., Gapor, A. and Adeli, K. (1999). Tocotrienol: A review of its therapeutic potential. *Clinical Biochemistry* **32(5)**, 309-319.
- Urso, M.L. and Clarkson, P.M. (2003) Oxidative stress, exercise, and antioxidant supplementation. *Toxicology* **189**, 41-54.
- van Beaumont, W., Underkofler, S. and van Beaumont, V. (1981) Erythrocyte volume, plasma volume and acid-base changes in exercise and heat dehydration. *Journal of Applied Respiratory Environmental Exercise Physiology* **50**, 1255-1262.
- Vasankari, T.J., Kujala, U.M., Vasankari, T.M., Vuorimaa, T. and Ahotupa, M. (1997) Increased serum and low-density-lipoprotein antioxidant potential after antioxidant supplementation in endurance athletes. *American Journal Clinical Nutrition* **65**, 1052-1056.
- Watkins, T.R., Bierenbaum, M.L. and Giampaolo, A. (1999) Tocotrienol: biological and health effects. In: *Antioxidant status, diet, nutrition and health*. Ed: Papas, A.M. Washington DC: CRC Press. 479-495.

KEY POINTS

Tocotrienol-rich palm vitamin E supplementation for 6 weeks:

- reduced lipid peroxidation at rest.
- did not enhance endurance running performance in the heat.
- did not prevent exercise-induced muscle damage as indicated by CK activity.

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