LAPORAN AKHIR PROJEK PENYELIDIKAN
R & D JANGKA PENDEK

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ABSTRACT

INTRODUCTION: The increase in oxygen consumption during endurance exercise leads to free radical (FR) production and subsequent lipid peroxidation (LIPOX). Raised body temperature has also been reported to increase the rate of FR production. This oxidative stress may impair endurance performance since FRs can cause cell damage and has been implicated in muscular fatigue. Vitamin E supplementation has been shown to attenuate FR-induced LIPOX. It is however unclear if vitamin E supplementation could decrease LIPOX and improve endurance running performance of heat-adapted recreational athletes in the heat. **PURPOSE:** This study examined the effects of tocotrienol (Palm Vitee) supplementation on exercise-induced LIPOX and endurance performance in the heat. **METHODS:** 18 healthy, male recreational athletes (aged: 24.9 ± 1.4 yrs; body weight: 59.6 ± 1.5 kg; VO_{2max}: 57.7 ± 1.5 $ml.kg^{-1}.min^{-1}$) completed two endurance running trials until exhaustion on a motorised treadmill at 70% VO_{2max} on two separate occasions following a 6-week supplementation of either vitamin E (E) or placebo (P). Both trials were conducted at an ambient temperature of 31°C and a 70% RH. During the trials, rectal temperature (T_{rec}), skin temperature (T_{sk}), heart rate (HR) and ratings of perceived exertion (RPE) were recorded at 10-min intervals while oxygen uptake (VO_2) was recorded every 20 min. Blood samples were collected every 20 min during the running trials for the determination of plasma volume changes (PVC), lactate (LAC), glucose (GLU), free fatty acid (FFA), triglyceride (TRI), malondialdehyde (MDA), creatine kinase (CK), total antioxidant status (TAS) and vitamin E. **RESULTS:** No significant differences were evident in T_{rec}, T_{sk}, HR, RPE, VO_2 or in the time to exhaustion between the E and P trials (81.1 ± 4.5 *vs* 76.9 ± 4.5 min respectively). Similarly, PVC, CK, LAC, GLU, FFA, TRI and TAS were also not different between the two trials. Vitamin E supplementation, however, resulted in a significantly higher ($p<0.001$) mean serum vitamin E concentration at rest and during post-exercise compared to that in the placebo

trial. Resting plasma MDA concentration in the E trial was significantly lower than that in the P trial (0.38 *vs* 0.46 $\mu mol.l^{-1}$; $p<0.05$). At exhaustion, plasma MDA was higher than the resting values in both trials and it was higher in the P trial compared to the E trial although the difference did not reach statistical significance ($p=0.090$). CK activity at exhaustion, 1 h and 24 h post-exercise was not different during the two trials but was significantly higher ($p<0.001$) than the corresponding resting values in both trials. **CONCLUSION:** 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, does not enhance endurance running performance or prevent exercise-induced muscle damage during exercise in the heat. In addition, vitamin E supplementation did not influence the changes of some of the physiological parameters (e.g. PVC, LAC, GLU, FFA, TRI, CK and TAS) that occurred during exercise in the heat.

Key Words: Endurance running performance, lipid peroxidation, muscle damage, heat, vitamin E

1. INTRODUCTION

During exercise, oxygen uptake can be elevated 10 - 20 times to meet the increased metabolic demand of the exercising muscles (Astrand & Rodahl, 1986). Under normal circumstances, a small amount of univalently produced oxygen intermediates, termed free radicals, leak out of the electron transport chain during this process (Chance *et al.*, 1979) and results in the production of free radicals like superoxide, peroxyl and hydroxyl radicals (Chance *et al.*, 1979; Jenkins, 1988; Holley & Cheeseman, 1993; Gutteridge & Halliwell, 1994; Sen 1995). Free radicals are unstable molecules or fragments of molecules with unpaired electrons in their outer orbits (Sjodin *et al.*, 1990; Clarkson & Thompson, 2000; Powers *et al.*, 2004). They strive to balance their unpaired electrons by combining with electrons with opposite spins in other molecules that are important for cellular function (Sjodin *et al.*, 1990). By being unstable, free radicals are also highly reactive and can cause damage in the cells and tissues by initiating chemical chain reaction like lipid peroxidation (Jenkins, 1988; Duthie, 1993; Gutteridge & Halliwell, 1994; Packer, 1997). Membrane lipid peroxidation may alter fluidity and permeability of the membrane and thus compromise the integrity of the membrane barrier resulting in a loss of cellular function (Sen, 1995; Tiidus & Houston, 1995; Dekkers *et al.*, 1996; Evans, 2000; Powers *et al.*, 2004) and even cell death (Hollan, 1996).

Reactive oxygen species (ROS) represent a broad spectrum of species, including non-radical derivatives of oxygen (hydrogen peroxide, singlet oxygen, hydroperoxides) that are also capable of inciting oxidative tissue damage (Sen, 1995). Most cells in the body, including skeletal muscle cells, contain several naturally occurring mechanisms for the protection against injuries caused by ROS (Laughlin *et al.*, 1990). The ROS are neutralised by an elaborate antioxidant system comprising of enzymes such as superoxide dismutase, catalase, glutathione peroxidase and non-enzymatic antioxidants such as vitamins A, E and C, glutathione, ubiquinone, α-lipoic acid and flavonoids (Gohil

et al., 1988; Kanter, 1998a; Kanter 1998b; Criswell *et al.*, 1993; Goldfarb, 1993; Urso & Clarkson, 2003, Powers *et al.*, 2004). Therefore, these enzymatic and non-enzymatic antioxidant defence systems protect the membranes and other cell organelles from the damaging effects of free radical reactions (Gohil *et al.*, 1988; Goldfarb, 1993; Yu, 1994; Kanter, 1998a; Kanter 1998b; Goldfarb, 1999; Evans, 2000; Powers *et al.*, 2004).

During increased oxygen utilisation, as happens during exercise, the rate of production of these free radical species may exceed the body's capacity to detoxify them (Sjodin *et al.*, 1990). This can lead to increased oxidative stress and subsequent lipid peroxidation and cell damage (Davies *et al.*, 1982; Jenkins, 1988; Kanter *et al.*, 1988; Alessio, 1993; Goldfarb, 1993; Kanter, 1994; Sen, 1995; Tiidus & Houston, 1995; Dekkers *et al.*, 1996; Leaf *et al.*, 1997; Packer, 1997; Alessio *et al.*, 1998; Zoppi *et al.*, 1998; Evans, 2000; Mastaloudis *et al.*, 2001). Free radical production reaches the highest level when the exercise is exhaustive (Sastre *et al.*, 1992; Ji *et al.*, 1998, Li *et al.*, 1998). Several studies have investigated the formation of free radicals during exercise and its relation to exercise-induced muscle damage (Clarkson & Tremblay, 1988; Kanter *et al.*, 1988; Barclay & Hansel, 1991; Vina *et al.*, 2000).

Under normal circumstances, increased oxidative stress induced by exercise is equally matched by concomitant increase in antioxidant activity (Salminen & Vihko, 1983; Alessio & Goldfarb, 1988; Ji, 1993; Dekkers *et al.*, 1996; Powers *et al.*, 1999; Evans, 2000). However, there is evidence to suggest that the antioxidant activity is not always adequate in preventing exercise-induced lipid peroxidation (Inal *et al.*, 2001; Urso & Clarkson, 2003) and damage to cell membranes (Alessio, 1993; Jenkins & Goldfarb, 1993; Sen, 1995; Takanami *et al.*, 2000). The oxidative stress that results from the deficiency of antioxidant nutrients has been shown to increase cell damage and reduce endurance capacity during strenuous physical activity in animal studies (Davies *et al.*, 1982; Gohil *et al.*, 1986; Packer *et al.*, 1994).

One of the nutrients that have shown promise as a protective antioxidant against free radical induced stress is vitamin E (Dillard *et al.*, 1978; Simon-Schnass & Pabst, 1988; Sumida *et al.*, 1989; Cannon *et al.*, 1990; Meydani *et al.*, 1993; Rokitzki *et al.*, 1994a; Itoh *et al.*, 2000; Schroder *et al.*, 2000; Jessup *et al.*, 2003). Vitamin E is the generic name describing bioactivities of both α-tocopherol and tocotrienol derivatives (Kayden & Traber, 1993; Kamal-Eldin & Appelqvist, 1996; Brigelius-Flohe & Traber, 1999; Theriault *et al.*, 1999). Vitamin E reportedly reduces exercise-induced increase in lipid peroxidation (Dillard *et al.*, 1978; Simon-Schnass & Pabst, 1988; Sumida *et al.*, 1989; Rokitzki *et al.*, 1994a; Evans, 2000). Furthermore, 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).

Despite the ability of vitamin E to reduce free radical induced cell damage during exercise, its role in enhancing endurance performance remains debatable. High pre-race plasma vitamin E level has been associated with enhanced physical endurance in dogs (Piercy *et al.*, 2001). Another study showed that running time to exhaustion was reduced by approximately 30-40% for untrained rats fed with a vitamin E-free diet (Gohil *et al.*, 1986). Most human studies, however, have failed to demonstrate any performance enhancement associated with vitamin E supplementation (Sharman *et al.*, 1971; Shephard *et al.*, 1974; Lawrence *et al.*, 1975; Rokitzki *et al.*, 1994b; Nielsen *et al.*, 1999) except when exercise was performed at high altitude (Simon-Schnass & Pabst, 1988). Interestingly, however, little has been studied on free radical activities during exercise in the heat and the influence of vitamin E supplementation on free radical activities and performance in the heat.

Elevated body temperature has been shown to increase the rate of free radical production (Kanter, 1994; Dekkers *et al.*, 1996; Clanton *et al.*, 1999; Di Meo & Venditti,

2001; Altan *et al.*, 2003) and there is growing evidence that free radical production during exercise contributes to muscular fatigue (Novelli *et al.*, 1990; Shindoh *et al.*, 1990; Barclay & Hansel, 1991; Reid *et al.*, 1992; O'Neill *et al.*, 1996).

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. Furthermore, most studies investigating the effect of vitamin E on performance have used tocopherol and none have used combined α-tocopherol and tocotrienol supplements. The present study therefore investigates the effects of Palm Vitee (tocotrienol-rich fractions) supplementation on exercise-induced lipid peroxidation and endurance performance in the heat of heat-adapted recreational athletes.

2. METHODS

2.1 SUBJECTS

Twenty-five male recreational athletes were recruited as subjects in this double-blind, placebo-controlled, randomised cross-over study (Fig. 2. 1). Participation in regular physical activity of the subjects was determined through a biodata form (Appendix A). The experimental protocols were explained to them, in addition to what was required of them before they were asked to sign a consent form (Appendix B). The study protocol was approved by the Research and Ethics Committee of Universiti Sains Malaysia (Appendix C).

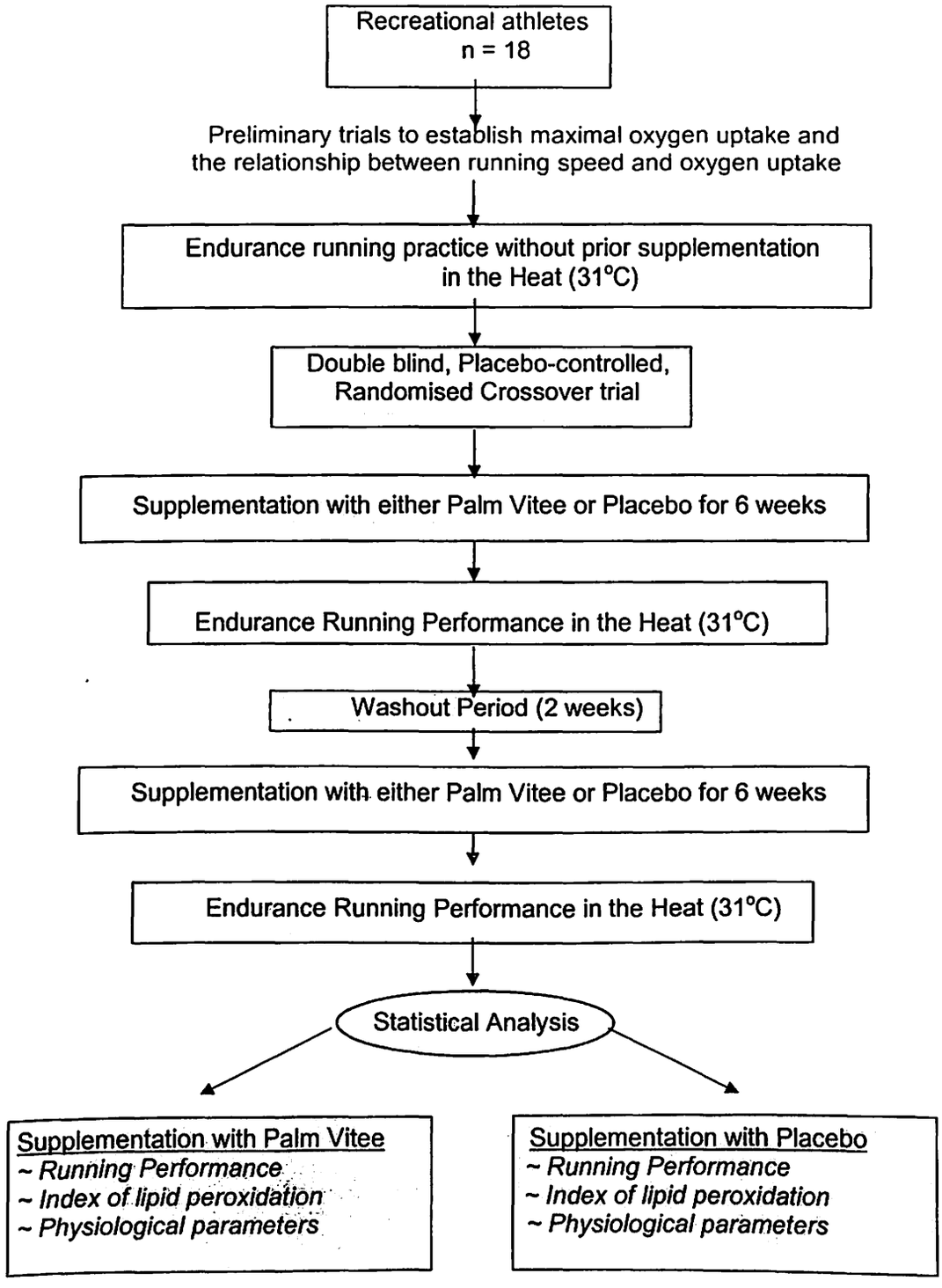

Figure 2.1. Experimental design of the study.

2.2 TEST PROCEDURES

2.2.1 Preliminary Measurements

The subjects' body weight and percent of body fat were obtained by using an electronic body composition analyzer (Tanita® TBF-410, Japan). A telescoping measuring rod (Seca 220, Germany) was used to measure the height of the subjects.

After familiarisation with treadmill running, the subjects performed two tests:

i) A 16-min incremental sub-maximal running test to determine the relationship between running speed and oxygen uptake.

ii) An uphill incremental treadmill-running test to determine maximum oxygen uptake (VO_{2max}).

For the sub-maximal test, the subjects were fitted with a heart rate sensor (Sport Tester PE3000, Polar, Finland), a mouthpiece and a nose clip. A head gear was fitted to support a two-way non-re-breathing valve (Hans Rudolph 2700 series, USA) attached to the mouthpiece. The subjects then ran on a motorised treadmill (Quinton 18-60, USA) for four minutes at four different speeds (7, 8, 9 and 10 $km.h^{-1}$) over a period of 16 minutes. All expired air during the tests was passed through a mixing chamber where sensors to the pre-calibrated paramagnetic oxygen and infrared carbon dioxide analysers (SensorMedics 2900, USA) were used to determine the percentages of oxygen and carbon dioxide respectively in the expired air. Both analysers were calibrated using two nitrogen-based calibration gases (26% oxygen in nitrogen mixture, and 4% carbon dioxide and 16% oxygen in nitrogen mixture). The output from the gas analysers was processed using a computer for the calculation of oxygen consumption (VO_2) and carbon dioxide production (VCO_2). On the average, these measurements were recorded every

20 seconds. VO_2 values in $ml.kg^{-1}.min^{-1}$ were the averages of the highest values measured during the final 60 seconds of each measurement.

Maximum oxygen uptake was determined using a modified Astrand protocol (Heyward, 1991). This test required the subjects to run to volitional exhaustion during a continuous incremental run on a motorised treadmill. Subjects were initially allowed to warm-up for ~5 minutes at a low speed (6-7 $km.h^{-1}$). After the warm-up, the subjects were fitted with the headgear, mouthpiece, nose-clip and heart rate sensor as in the sub-maximal test. An appropriate speed (8-12 $km.h^{-1}$) was selected and the test began with a grade of 0% for 3 minutes. Thereafter, the grade was increased 2 1/2% every 2 minutes and the subjects were encouraged to run until exhaustion. Expired air samples and heart rate responses were measured at the end of each 2-minute stage. The VO_{2max} value was accepted to have been reached when there was a plateau in oxygen uptake despite increasing workload (American College of Sports Medicine, 2000). Other criteria used to indicate the attainment of VO_{2max} were:

i) Failure of heart rate to increase with increases in exercise intensity.

ii) A respiratory exchange ratio of >1.15 (American College of Sports Medicine, 2000).

From on the data obtained, running speeds during warm-up (50% VO_{2max}) and during endurance running performance (70% VO_{2max}) were established from a regression equation with speed and oxygen uptake. After these preliminary tests, all the subjects were required to come and train on the motorised treadmill in the laboratory over a two to three week period before they were put on the first supplementation regimen. This was done to familiarise the subjects with the experimental protocol and to eliminate any possible 'learning effect' during the actual experimental trials. Five of the subjects dropped out for various reasons and twenty were put on the supplementation regimen. Two of the subjects withdrew from the study after the first supplementation period. The

final data analysis for the second phase was derived from eighteen subjects who completed both the trials.

2.2.2 Experimental Trials

The randomised trials were conducted in an improvised climatic chamber where halogen lamps (Philips - 500W, France) were used to raise the ambient temperature to ~31°C in both the trials. Relative humidity in both the trials was maintained at 70% by using a heated water-bath (Memmert W350t, Germany) placed within the chamber.

To minimise differences in resting muscle glycogen concentrations, subjects recorded their food intake for 3 days before the first experimental trial in a food diary. They were then instructed to follow the same diet before the second trial. They were also required to refrain from training the day before each trial and to observe a 10-12 h fast before their arrival to the laboratory.

Upon arrival at the laboratory, a standardised breakfast consisting of a slice of white bread (Gardenia®, Malaysia) and a glass of water (300 ml) was given approximately half an hour before the experimental trial. The subjects then emptied their urinary bladder. Nude body weight of the subjects was recorded using an electronic body composition analyzer (Tanita® TBF-410, Japan). Following this, 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). Core and skin temperatures were recorded on a temperature monitor (Libra Medical ET 300R, USA). The heart rate was monitored throughout the trial by a heart rate sensor (Sport Tester PE3000, Polar, Finland), which was fitted onto the chest wall. An indwelling cannula

(Vasocan® – 22 G, 1", B. Braun, Malaysia) was inserted into a subcutaneous forearm vein and an extension tube (minimum volume extension tubing - 30 cm, B. Braun, Malaysia) was connected to it to facilitate repeated blood withdrawals. Patency of the cannula was maintained with heparinised saline (10 IU heparin sodium in 1 ml 0.9% NaCl, B. Braun, Malaysia). Approximately 0.8ml of heparinised saline was injected into the extension tube after each blood withdrawal.

As the subject was being prepared, the environmental condition of the chamber was continuously monitored. Once the environmental temperature and relative humidity were stable, the subjects then moved into the chamber for the experimental trials. After standing on the treadmill for 5-10 minutes, a resting venous blood sample (8 ml) was collected and oxygen and carbon dioxide concentrations in the resting expired air sample were measured (Fig. 2.2). The subjects then had a warm-up run on the treadmill for 5 minutes at 50% VO_{2max}. This was immediately followed by a run to exhaustion at exercise intensity of 70% VO_{2max}. Exhaustion was considered to have been reached when the subjects could no longer maintain the prescribed running speed.

During each blood collection, the first 1 ml of the blood sample was withdrawn separately using a 5 ml sterile syringe (Becton Dickinson, Singapore). This amount was not included in the blood analysis as it was diluted by the heparinised saline. The syringe was then replaced, and 8 ml of blood was collected in a 10 ml sterile syringe (Becton Dickinson, Singapore). Blood samples were collected at the end of the warm-up period, at 20-minute intervals throughout the trials and at exhaustion while the subject was still on the treadmill. During the trials, oxygen and carbon dioxide concentrations in the expired air were recorded during the final minute of the warm-up period, at 10 minutes into exercise and every 20 minutes thereafter until exhaustion. After completion of the warm-up and at intervals of 20 minutes, 3 $ml.kg^{-1}$ body weight of cooled water (4-8°C)

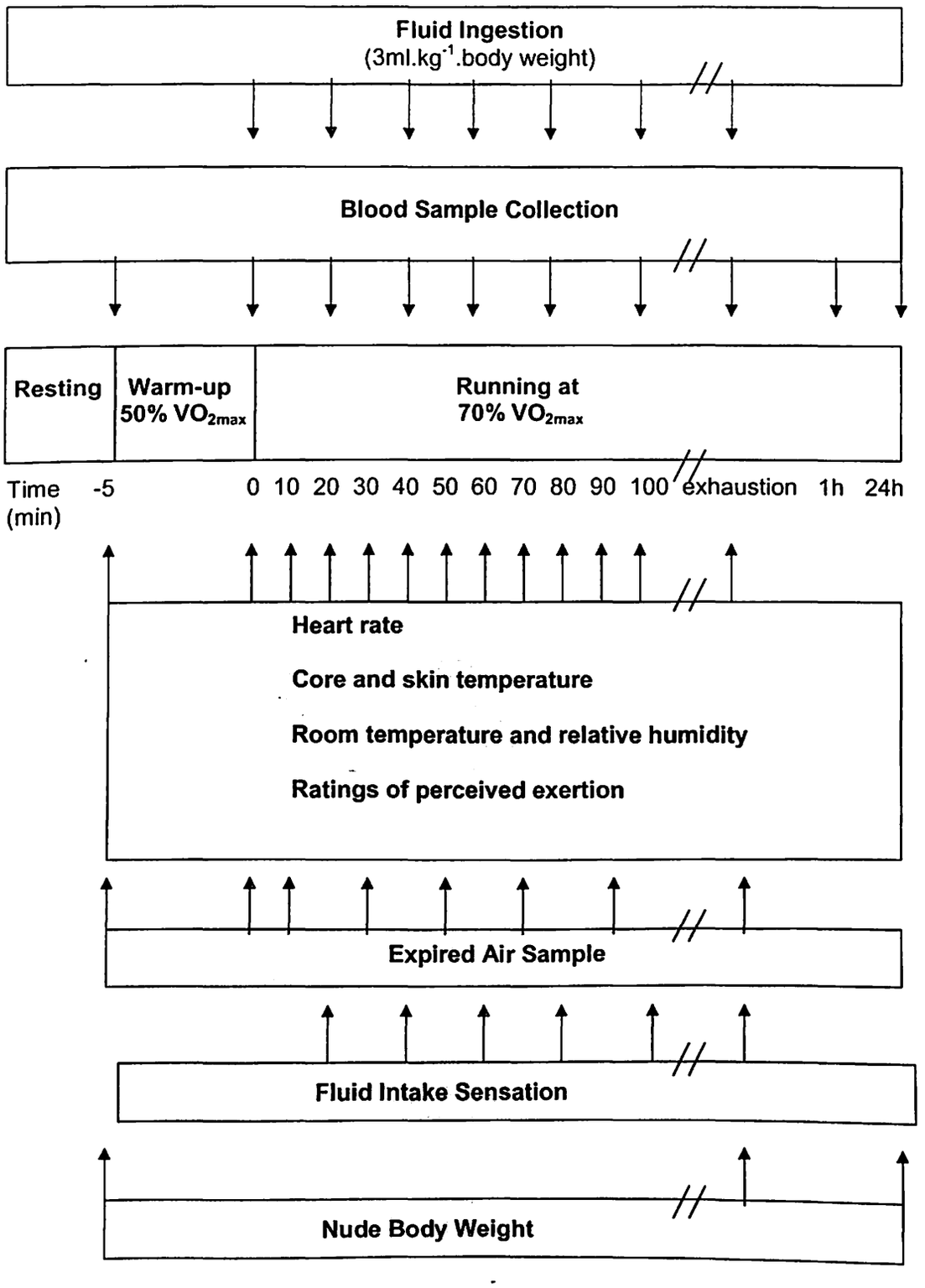

Figure 2.2. Protocol for experimental trials.

was consumed by the subjects to avoid any possible adverse effects of dehydration (Fig. 2.2).

Heart rate, ratings of perceived exertion (RPE), core and skin temperatures, room temperature and relative humidity were recorded at 10-minute intervals throughout the trials. Heart rate responses during the trials, which were recorded via the heart rate monitor, were later downloaded onto a desktop computer for analysis. RPE was obtained using the Borg's scale (Borg, 1998). Fluid sensation was obtained at 20-minute intervals (Fig. 2.2) using a fluid sensation scale (Peryam & Pilgrim, 1957).

At the point of exhaustion, and to ensure that the subjects were truly fatigued, the running speed was reduced to elicit 60% VO_{2max} for 2 minutes. Thereafter, the speed was returned to the prescribed speed (70% VO_{2max}) and the subjects were further encouraged to run as long as possible (Chryssanthopoulos & Williams, 1997; Wee *et al.*, 1999). Verbal encouragement was given to all subjects by the same researcher. All time-keeping devices were kept out of sight of the subjects during these trials. Once the subjects stopped running, they were allowed to cool down for about 2 minutes on the treadmill at a walking pace of 4-6 $km.h^{-1}$.

After the completion of the run, the heart rate sensor and all the thermistors were removed from the subjects. After towel drying themselves, post-exercise nude body weight was measured. The difference in nude body weight and the amount of fluid consumed were then used to determine the sweat rate (Murray, 1996). Subjects were then given 500 ml of water and they rested in the laboratory (~25°C, ~70% RH) before another venous blood sample (8 ml) was collected at 1 hour post-exercise. All the subjects were required to report to the laboratory 24 hours post-exercise when a further venous blood sample (8 ml) was collected. Each subject repeated the same procedure for the subsequent trial.

2.3 Blood Collection and Analyses

During the experimental trials, approximately 8 ml of venous blood was withdrawn during each sample collection. One ml of blood from each sample was transferred to an EDTA (Ethylenediamine tetra-acetic acid) tube and was used for the determination of haematocrit and haemoglobin concentration. Haematocrit was determined by microcentrifugation (Hettich-Haematokrit 20, Germany) at 13,000 RPM for 10 minutes and using a Microhaematocrit Reader (Hawksley, England) while haemoglobin concentration was analysed by the cyanmethaemoglobin method (Drabkin's reagent). Percent change in plasma volume was calculated using a formula of van Beaumont *et al.* (1981).

From the remainder of the blood sample (7 ml), a volume of 0.5 ml was transferred to a natrium flouride containing tube and the plasma was separated by centrifugation (10 minutes, 4,000 rpm, 4°C; Hettich-Rotina 46RS, Germany). The plasma was divided into equal portions and stored at -80°C (Heto Ultra Freeze 3410, Denmark) for subsequent analysis of lactate and glucose. Plasma lactate was estimated using a lactate analyser (Yellow Springs Instrument 1500, USA). Plasma glucose was analysed using a commercial glucose kit (Randox, U. Kingdom) and the concentration determined by a spectrophotometer (Shimadzu CL-750 Micro-Flow, Japan).

Another 2 ml of the blood sample was anticoagulated with EDTA and plasma was separated by centrifugation (10 minutes, 4000 rpm, 4°C; Hettich-Rotina 46RS, Germany). The plasma from this sample was divided into five equal portions and stored at -80°C for subsequent analysis of free fatty acids, malondialdehyde, creatine kinase, triglyceride and cholesterol. Plasma malondialdehyde was determined as thiobarbituric acid reactive substances (TBARS) by using a high performance liquid chromatography (HPLC) technique (Nielsen *et al.*, 1997). Total free fatty acids was analysed by using

commercially available reagent kit (Wako, Japan) and their concentration was determined by using a spectrophotometer (Shimadzu CL-750 Micro-Flow, Japan). Plasma creatine kinase and triglyceride were also analysed using commercially available kits (Randox, U. Kingdom). Plasma creatine kinase was determined by using a chemistry analyser (AMES Quik-Lab, Germany) while plasma triglyceride was determined using a spectrophotometer (Shimadzu CL-750 Micro-Flow, Japan).

The remainder of the blood sample (4.5 ml) was allowed to clot and then centrifuged for 10 minutes at 4,000 rpm and 4°C (Hettich-Rotina 46RS, Germany). The supernatant was divided into two portions and stored at -80°C (Heto Ultra Freeze 3410, Denmark) 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 after extraction with hexane by a HPLC technique modified from Wahlqvist *et al.* (1992).

2.4 Statistical Analysis

All data were examined for normality through the Kolmogorov-Smirnov test. Descriptive statistics were performed on all dependent variables. ANOVA for repeated measures was used to determine the differences in physiological-related parameters over time between trials. The dependent parameters include heart rate, oxygen uptake, percent change of plasma volume, haematocrit, haemoglobin, core and skin temperatures, plasma malondialdehyde, plasma creatine kinase, plasma free fatty acid, plasma lactate, plasma glucose, plasma triglyceride, plasma cholesterol, serum total antioxidant status and serum vitamin E.

Homogenity of variance in the data was determined using Mauchly's test. For data that violated the assumed sphericity, Greenhouse-Geisser correction was used to adjust the significance levels of the test statistics. Bonferroni adjustment for multiple comparisons was used to locate the differences when repeated measures analysis of variance revealed a significant main effect of time. When appropriate, students' paired t-test was used to compare the differences between trials at individual time points. Ratings of perceived exertion and fluid sensation scale were analysed using Wilcoxon Signed-Rank test.

The Statistical Package for Social Sciences (SPSS) Version 10.0 was used for the statistical analysis. The accepted level of significance was set at $p<0.05$. Results were reported as means ± standard error (SE).

3. RESULTS

3.1 SUBJECTS

The subjects' age, height, weight, and % body fat together with maximum heart rates and VO_{2max}, obtained during an uphill incremental treadmill-running test to exhaustion are shown in Table 3.1.

Table 3.1 Physical characteristics and physiological capacities of subjects.

Age (yr)	Height (cm)	Weight (kg)	% Body Fat	Heart $Rate_{max}$ (beats. min^{-1})	VO_{2max} ($ml.kg^{-1}.min^{-1}$)
24.9 ± 1.4	169.2 ± 1.2	59.6 ± 1.5	18.0 ± 0.9	193 ± 2	57.7 ± 1.5

Values are means ± SE

3.2 ROOM TEMPERATURE AND RELATIVE HUMIDITY

The average room temperature and relative humidity in the vitamin E (E) and placebo (P) trials are presented in Table 3.2. Both room temperature and relative humidity were stable during both the trials. There were no significant differences in room temperature and relative humidity between E and P trials.

Table 3.2 Room temperature and relative humidity in the vitamin E supplemented (E) and placebo (P) trials.

Trials	Vitamin E (E)	Placebo (P)
Room Temperature (°C)	30.9 ± 0.1	31.0 ± 0.1
Relative Humidity (%)	70.1 ± 0.2	70.3 ± 0.3

3.3 SERUM VITAMIN E

The composition of each Palm Vitee capsule was 53 mg vitamin E (~33 % alpha-tocopherol and ~67 % tocotrienols). Therefore, the actual supplementation taken by the subjects during the vitamin E supplementation regimen was 318 mg (6 capsules x 53 mg) of vitamin E (tocotrienol-rich fractions) per day for 6 weeks.

From ANOVA with repeated measures, it was found that there was a significant main effect of supplementation (F= 62.03; df= 1, 17; $p<0.001$) and a significant main effect of time (F= 13.21; df= 4, 68; $p<0.001$) on serum vitamin E during the trials. There was also a significant interaction between supplementation and time on serum vitamin E concentrations during the trials (F= 20.12; df= 2.25, 38.20; $p<0.001$).

Compared to pre-supplementation levels, serum vitamin E concentrations were significantly higher ($p<0.001$) in the E trial (Fig. 3.1) after six weeks of supplementation. In contrast, there were no significant differences in serum vitamin E following placebo supplementation in the P trial. Serum vitamin E concentrations at exhaustion were higher when compared to the resting values in both trials but it was only statistically significant ($p<0.05$) in the P trial. Mean 24 h post-exercise serum vitamin E concentration was lower than the corresponding resting levels in both the trials but the differences were not statistically significant. Nevertheless, the 24 h post-exercise serum vitamin E level was significantly ($p<0.05$) lower than the corresponding values at exhaustion and at 1 h post-exercise in the E trial. After the supplementation regimen, serum vitamin E concentrations were significantly higher ($p<0.001$) in the E trial compared to the P trial during and after exercise. All differences calculated for serum vitamin E remained statistically significant after adjusting data for plasma volume changes. However, the statistical significance between exhaustion and resting value in the P trial was lost.

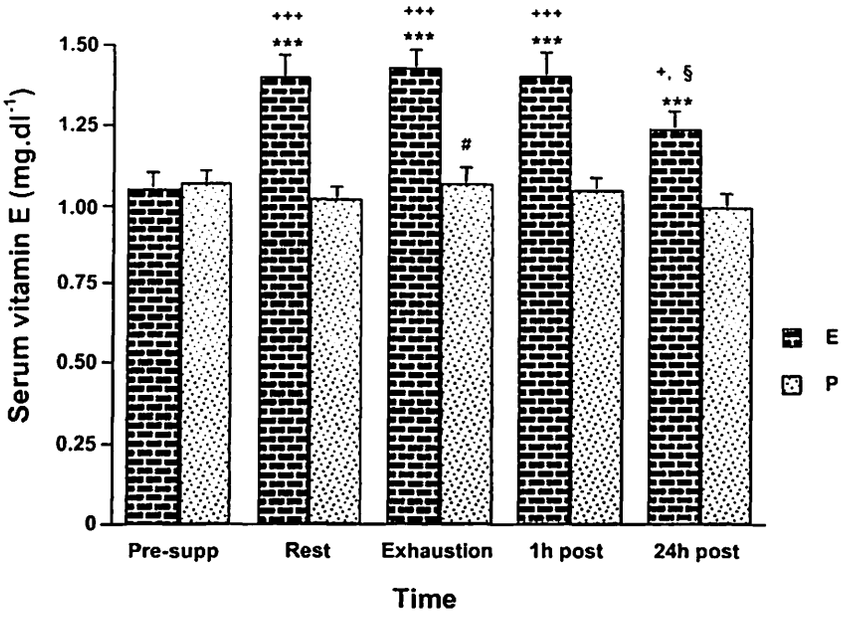

Figure 3.1 Serum vitamin E ($mg.dl^{-1}$) during and after exercise in the vitamin E supplemented (E) and placebo (P) trials.
*** significantly different from corresponding values in P ($p<0.001$).
+, *** significantly different from corresponding pre-supplementation value ($p<0.05$ and $p<0.001$, respectively).
significantly different from respective resting value ($p<0.05$).
§ significantly different from respective exhaustion and 1h post-exercise values ($p< 0.05$).

3.4 SERUM TOTAL ANTIOXIDANT STATUS (TAS)

ANOVA with repeated measures revealed no significant main effect of supplementation ($F= 0.18$; df= 1, 17; $p=0.679$) but a significant main effect of time ($F= 44.45$; df= 4, 68; $p<0.001$) on serum TAS during the trials. There was also no significant interaction between supplementation and time on the level of serum TAS during the experimental trials ($F= 0.97$; df= 2.45, 41.66; $p=0.402$).

Resting serum TAS was not significantly different from pre-supplementation values in both the trials. However, serum TAS was significantly higher ($p<0.001$) at exhaustion and at 1 h post-exercise when compared to the corresponding resting levels

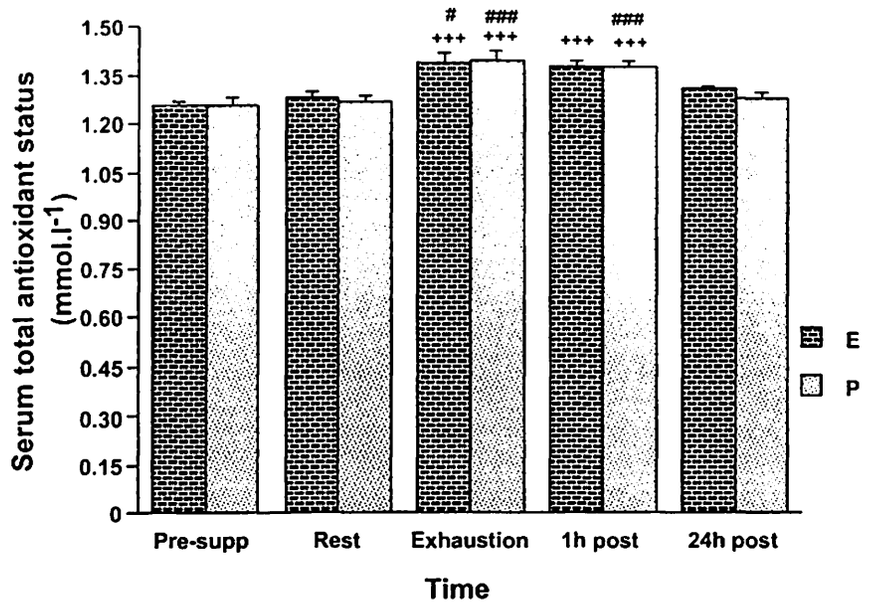

Figure 3.2 Serum total antioxidant status ($mmol.l^{-1}$) during and after exercise in the vitamin E supplemented (E) and placebo (P) trials.
$^{+++}$ significantly different from respective resting values ($p<0.001$).
$^{\#}$, $^{\#\#\#}$ significantly different from corresponding 24h post-exercise values ($p<0.05$ and $p<0.001$, respectively).

in the both the trials (Fig. 3.2). Serum TAS at 24 h post-exercise was not different from corresponding resting values in both trials and it was higher in the E trial compared to the P trial although statistical significance ($p=0.071$) was not found. Serum TAS at 24 h post-exercise was significantly ($p<0.05$) lower than at exhaustion in the E trial. Similarly, serum 24 h post-exercise level in the P trial was significantly ($p<0.001$) lower than levels at exhaustion and 1 h post-exercise. There were no significant differences in serum TAS during and after exercise between the E and P trials. After the data were adjusted for plasma volume changes, serum TAS value at 24 h post-exercise was significantly higher ($p<0.05$) than the corresponding resting value in the E trial. Other calculated differences in serum TAS remained statistically significant except the value between exhaustion and at 24 h post-exercise in the E trial.

3.5 OXYGEN UPTAKE (VO_2)

ANOVA for repeated measures indicated that there was no significant main effect of supplementation (F= 0.24; df= 1, 17; p=0.633) but a significant main effect of time (F= 987.79; df= 2.57, 43.60; p<0.001) on oxygen uptake during the experimental trials. There was no significant interaction between supplementation and time on oxygen uptake during the endurance running trials (F= 0.60; df= 1.92, 32.57; p=0.553).

Separate ANOVA with repeated measures for each trial indicated that oxygen uptake increased over time from rest until exhaustion (p< 0.001) in both the trials (Fig. 3.3). Mean oxygen consumption during exercise was similar during both the trials (40.1 ± 0.4 $ml.kg^{-1}.min^{-1}$ and 40.6 ± 0.5 $ml.kg^{-1}.min^{-1}$ in the E and P trial respectively). These figures correspond to 70.1 ± 0.6% and 70.6 ± 0.7% of VO_{2max} which was maintained during the E and P trials respectively.

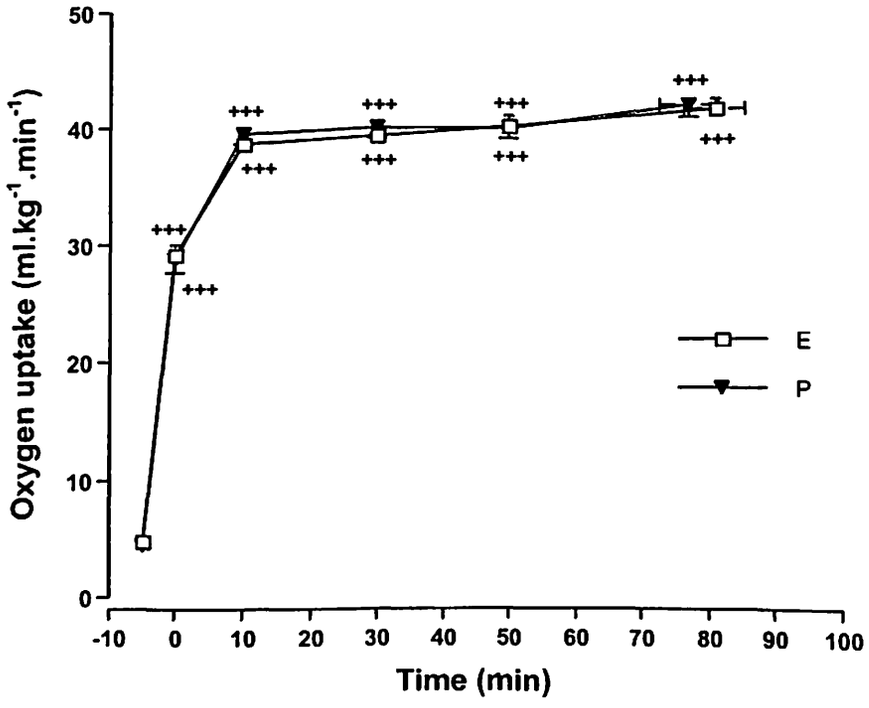

Figure 3.3 Oxygen uptake ($ml.kg^{-1}.min^{-1}$) during exercise in the vitamin E supplemented (E) and placebo (P) trials.
$^{+++}$ significantly different from respective resting values (p<0.001).

3.6 PLASMA MALONDIALDEHYDE (MDA)

ANOVA for repeated revealed no significant main effect of supplementation (F= 2.51; df= 1, 17; p=0.132) but a significant main effect of time (F= 4.10; df= 4, 68; p<0.01) on the levels of plasma MDA during the trials. However, there was no interaction between supplementation and time on plasma MDA levels during the endurance running performance trials (F= 1.77; df= 4, 68; p=0.144).

Separate ANOVA with repeated measures for each trial indicated that there was an increase in the levels of MDA at exhaustion compared to the resting values in both trials but it was only statistically significant (p<0.001) in the E trial (Fig. 3.4). Post-exercise MDA levels at 1 h and 24 h post-exercise were not significantly different when compared to the corresponding resting levels in both trials. Using paired t-tests, it was found that the resting MDA level was significantly lower (p<0.05) in the E trial compared to the P trial. At exhaustion, the MDA level was higher in the P trial (0.48 ± 0.04 vs 0.53 ± 0.05 $\mu mol.l^{-1}$) but this was not statistically significant (p= 0.090). The plasma MDA level at 24 h post-exercise was significantly lower (p<0.05) than the corresponding value at exhaustion in the P trial. MDA levels at other time points were not significantly different between trials. After adjusting the data for plasma volume changes, all significant differences were maintained except the 24 h post-exercise value in the P trial.

3.7 ENDURANCE RUNNING PERFORMANCE

Running time to exhaustion was not significantly different between the E and P trials (81.1 ± 4.5 min vs. 76.9 ± 4.5 min respectively) (Fig. 3.5).

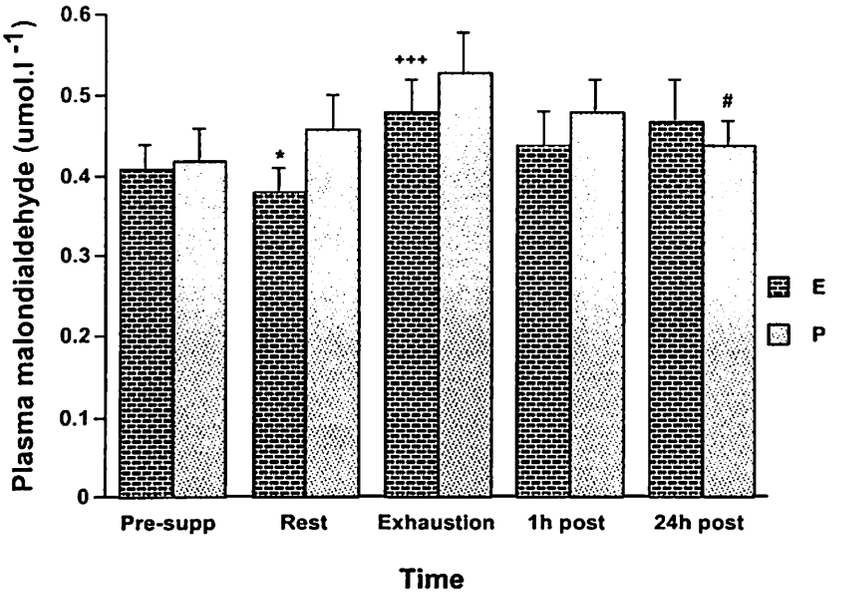

Figure 3.4 Plasma malondialdehyde ($\mu mol.l^{-1}$) during and after exercise in the vitamin E supplemented (E) and placebo (P) trials.
* significantly different from P at $p<0.05$.
$^{+++}$ significantly different from respective resting value ($p<0.001$).
$^{\#}$ significantly different from corresponding value at exhaustion ($p<0.05$).

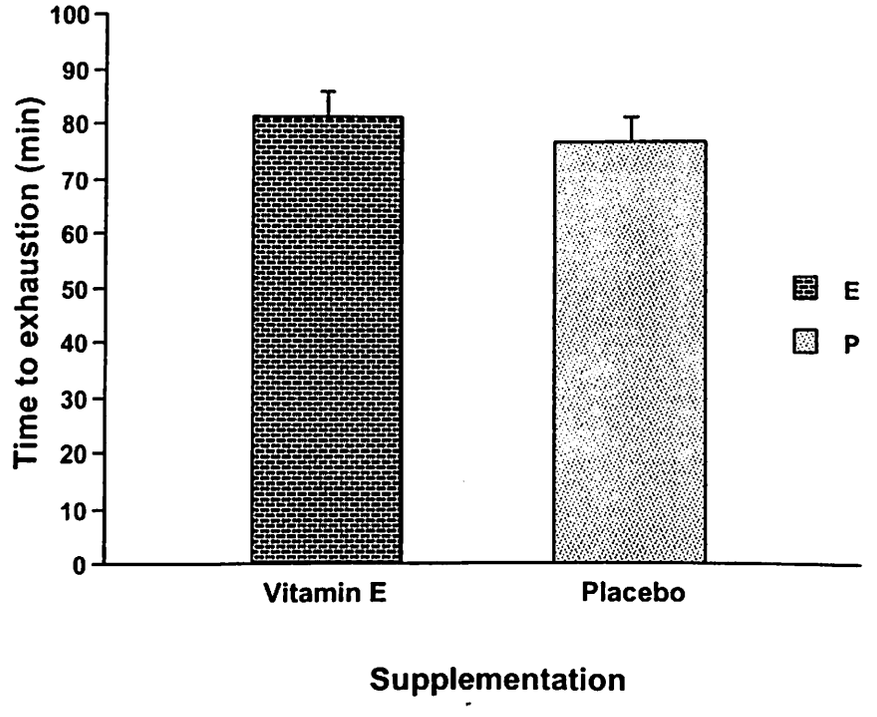

Figure 3.5 Exercise time to exhaustion in the vitamin E supplemented (E) and placebo (P) trials.

3.8 FLUID INTAKE AND SWEAT RATE

The volume of fluid ingested every 20 minutes and the total volume of fluid ingested was similar in both the trials (Table 3.3). Estimated sweat rate was also similar between trials (Table 3.3)

3.9 BODY WEIGHT CHANGES

Pre-exercise body weight, immediate post-exercise body weight, 24 h post-exercise body weight and percentage of body weight loss were similar in both the trials (Table 3.3).

Table 3.3 Pre, post and 24 h post-exercise body weight, percent body weight loss, volume of fluid ingested and estimated sweat loss during exercise in the vitamin E supplemented (E) and placebo (P) trials.

Parameter	Vitamin E (E)	Placebo (P)
Pre-exercise body weight (kg)	60.7 ± 1.5	60.5 ± 1.5
Post-exercise body weight (kg)	59.5 ± 1.5^{+++}	59.3 ± 1.5^{+++}
Body weight loss (%)	1.9 ± 0.1	1.9 ± 0.1
24-hr post-exercise body weight (kg)	60.2 ± 1.5^{+++}	60.0 ± 1.5^{++}
Volume of fluid ingested (ml.20 min^{-1})	182.3 ± 4.6	181.9 ± 4.5
Total volume of fluid ingested (ml)	751 ± 54	697 ± 44
Estimated sweat rate (L.h^{-1})	1.39 ± 0.08	1.39 ± 0.06

Values are means ± SE
$^{++}$, $^{+++}$ significantly different from respective pre-exercise body weight ($p<0.01$ and $p<0.001$, respectively).