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ORIGINAL ARTICLE

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Effect of vitamin E supplementation on post-exercise plasma lipid peroxidation and blood antioxidant status in smokers: with special reference to haemoconcentration effect

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Abstract The oxidative effects were investigated of exhausting exercise in smokers, and the possible protective role of 400 mg · day^{-1} vitamin E (Vit E) supplementation over a period of 28 days. The subjects exercised to exhaustion including concentric-eccentric contractions following maximal cycling. The haematocrit and haemoglobin, leucocyte (WBC), plasma lactic acid (La) and malondialdehyde (MDA), erythrocyte superoxide dismutase (SOD) and glutathione peroxidase (GPx), serum Vit E and ceruloplasmin (CER) concentrations were measured pre and post exercise. Supplementation increased Vit E concentrations 28% and 31% in the controls and the smokers, respectively. Cigarette smoking and/or Vit E supplementation did not influence plasma lipid peroxidation or the antioxidant status at rest. Exercise caused significant haemoconcentration in all groups. When the post-exercise concentrations were adjusted for haemoconcentration, a significant elevation in La concentrations due to exercise was observed in all groups. Similarly, there were significant elevations in the adjusted WBC counts in all groups except the Vit E supplemented controls. The MDA concentrations on the other hand, when adjusted for haemoconcentration, did not exhibit any difference due to exercise. Exercise did not affect the GPx and CER activities either, while causing a SOD activity loss in all groups except the Vit E supplemented non-smokers. Serum Vit E concentrations diminished significantly in all groups after exercise. Post-exercise plasma MDA and blood antioxidant concentrations were not altered by smoking. The results would suggest that plasma volume changes should always be taken into account when assessing post-exercise plasma concentrations and that smoking and exercise do not have an additional collective effect on plasma lipid peroxidation and the dose of Vit E administered was insufficient to maintain the serum concentrations after exercise.

Key words Free radical · Exhausting exercise · Vitamin E · Smoking · Antioxidant

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Introduction

It has been shown that oxygen free radicals cause lipid peroxidation and produce damage in many biological tissues. Generation of these toxic substances may be induced both by environmental factors such as chemicals, pollutants and irradiation, and physiologically, for example under conditions of physical exercise (Vasankari et al. 1995). Cigarette smoke, as a pollutant, has been demonstrated to contain a variety of xenobiotics, some of which are known to be oxidant or free radicals that can directly and indirectly initiate and propagate the process of lipid peroxidation (Hoshino et al. 1990). Thus, it is believed that smokers encounter a sustained free radical load. It has been shown that cigarette smoking caused an increase in blood and serum malodialdehyde (MDA) content. Despite many studies that have shown increased lipid peroxidation due to cigarette smoking (Kalra et al. 1991; Eiserich et al. 1995), there have also been reports indicating no significant difference in plasma lipid peroxidation between smokers and non-smokers (Harats et al. 1989; Duthie et al. 1991).

Physical exercise is another factor that has been suggested to be responsible for the increase in free radical-mediated reactions due to elevation in oxygen consumption and modified reduced nicotinamide-adenine dinucleotide: nicotinamide-adenine dinucleotide phosphate ratio due to lactate production being affected (Lovlin et al. 1987; Alessio and Goldfarb 1988; Tiidus and Houston 1994). However, there have been discrepancies among the studies investigating the peroxidative

effects of physical exercise. This has been attributed partly to the various methods used to evaluate lipid peroxidation and partly to the exercise model or characteristics of the subjects chosen in these studies (Dekkers et al. 1996).

In addition to the studies investigating the free radical generating effects of these factors, there have been a number of studies showing that free radical generating factors such as smoking and physical exercise cause favourable changes in plasma antioxidants (Camus et al. 1990; Eiserich et al. 1995), and antioxidant enzyme activities (Dekkers et al. 1996), and various human and animal studies have shown that dietary supplementation with antioxidant vitamins has favourable effects on lipid peroxidation due to exercise (Simon-Schnass and Pabst 1988; Sumida et al. 1989; Kanter et al. 1993; Meydani et al. 1993; Dekkers et al. 1996) or smoking (Eiserich et al. 1995; Brown et al. 1997).

The extent of lipid peroxidation is generally considered to be determined by the balance between oxidants (including free radicals) and antioxidants. It has been found that the antioxidants are consumed at a constant rate during the inhibition period, and when all of the antioxidants, especially ascorbate (Frei et al. 1989), are used up, the inhibition period is over and oxidation proceeds as rapidly as that in the absence of an antioxidant (Niki 1987). Therefore, to decrease lipid peroxidation, the oxidants have to be decreased and/or the antioxidants to be increased. Thus it has been suggested that for smokers and for people who undertake strenuous physical activity, the administration of additional antioxidants could be means of reducing their total body lipid peroxidation (Hoshino et al. 1990).

Vitamin E (Vit E) is known as a potent, lipid soluble, chain breaking type of antioxidant and it has been suggested that it is the only significant lipid soluble, chain breaking antioxidant present in human body cells (Niki 1987). The few human studies conducted to date have indicated that Vit E supplementation reduces oxidative stress and lipid peroxidation induced by exercise, and suggest that Vit E requirements may increase with exercise (Simon-Schnass and Pabst 1988; Meydani et al. 1993), and with cigarette smoking (Eiserich et al. 1995). This physiologically existing blood antioxidant has been shown to be relatively safe even when given in large doses (3200 mg $\cdot$ day^{-1}); (Bendich and Machlin 1988). In this study we investigated the collective effects of exercise and smoking on plasma lipid peroxidation and the possible recovery effect of Vit E as an antioxidant, and used plasma MDA, erythrocyte superoxide dismutase (SOD) and glutathione peroxidase (GPx), serum Vit E and ceruloplasmin (CER) concentrations to evaluate oxidation and antioxidant status.

Methods

Subjects and experimental groups

A group of 36 healthy, sedentary, male students volunteered for the study. The physical characteristics of the subjects, who were aged between 18 and 24, showed normal body mass for height, had no unusual dietary habits, and none of above were taking any kind of medication (Table 1). After being informed about the study and test procedures, and the possible risks and discomfort that might ensue they gave their written informed consent to participate; the volunteers were classified as smokers (those who had smoked at least ten cigarettes a day for at least 1 year) and non-smokers (those never having smoked). The non-smokers were randomly divided into two groups, control (C) and Vit E administered non-smokers (E); and the smokers were divided into two groups, smokers (S) and Vit E administered smokers (SE). The subjects in the C (n = 9) and S (n = 8) groups performed only the exercise protocol, while the subjects in E (n = 11) and the SE (n = 8) groups, also took 400-mg α-tocopherol (Bilim Turkey) each day for 28 days, after their blood samples for basal Vit E concentrations had been collected. They performed the exercise protocol on the day following the day they had taken the last dose of Vit E.

Exercise protocol

The subjects were instructed to abstain from strenuous physical activity the day before and on the day of the tests. Following a light breakfast 3 h before the tests, they arrived at the laboratory and skinfolds were measured at four sites, namely triceps, biceps, suprailiac and subscapular, using skinfold calipers (Holtain Ltd., England), and body fat percentages were calculated using the equation suggested by Wommersly and Durnin (1974). After resting for 20 min lying on a bed, resting blood samples (pre-ex) were taken.

Following the rest, to evaluate maximal oxygen consumption ($\dot{V}O_{2\ max}$), the subjects cycled on an ergometer (Monark 814E, Sweden) until they became exhausted in 7–10 min. They warmed-up for 5 min at 75 Watt and then 3 min was allowed for some stretching exercises. The exercise intensity during the maximal test was increased every 3 min from an initial 120 W. Because a greater

Table 1 Characteristics of subject groups, controls (C, n = 9), smokers (S, n = 8), vitamin E supplemented non-smokers (E, n = 11) and vitamin E supplemented smokers (SE, n = 8)

	C		S		E		SE	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (years)	18.9	0.6	20.1	1.6	20.2	2.0	19.5	1.4
Height (cm)	175	6	176	8	175	9	173	4
Body mass (Kg)	74.6	9.5	68.4	5.4	67.6	11.4	68.1	6.1
Maximal O_2 uptake ($ml \cdot kg^{-1} \cdot min^{-1}$)	38.9	4.4	36.3	4.9	34.0	7.0	36.9	4.1
Body fat (%)	17.7	5.3	12.5	4.8	13.0	4.6	13.8	2.4

No significant difference among four groups by ANOVA

cardiorespiratory and blood/muscle lactic acid (La) response has been reported for cycling at 60 rpm compared to higher pedalling rates during constant-power exercise (Carnevale and Gaesser 1991), the subjects were instructed to maintain the pedalling rate as close as possible to 60 rpm. When the subjects reached the rate of 60 rpm, data recording was started. Exhaustion was defined as the point at which the subject could no longer maintain the appropriate pedalling speed of 60 rpm. If the pedalling rate fell to 55 rpm, the subjects were given verbal encouragement to pedal faster. The actual pedalling rate was then 60 (SD 3) rpm for the tests. During the maximal test, oxygen consumption and carbon dioxide production were continuously measured breath-by-breath using a metabolic analyser of Sensor Medics 2900C (USA).

Following the maximal test, the subjects sat on the Cybex 6000 (Lumex Division, New York) computer controlled isokinetic dynamometer which had been calibrated before every test. The subjects were positioned sitting with the backrest at 90° and were instructed to grip the sides of the seat during the test. The thigh, pelvis and trunk were stabilized with straps. An adjustable lever arm was attached to the leg by a padded cuff just proximal to the lateral malleolus. The axis of rotation of the dynamometer arm was positioned just lateral to the lateral femoral epicondyle. Gravity correction to torque at 45° (0° = straight leg) were calculated by the computer software.

There was a 3-min interval between the maximal test and the exhausting isokinetic test. The subjects were familiarized with the test procedures by performing five consecutive submaximal warm-up trials. Thereafter, they performed three periods of 20 concentric-eccentric combined maximal contractions using the dominant knee extensors at an angular velocity of $60° \cdot s^{-1}$, a 1-min rest being allowed in between. The knee moved through a whole range of motion between 10° and 90° (0° = straight leg) during the isokinetic exercise. During the tests, the subjects were verbally encouraged to produce maximal efforts. Following the isokinetic exercise, the subjects sat on the dynamometer for 6 min and then post-exercise (post-ex) blood samples were taken. The 6th min was chosen because thiobarbituric acid reactive substances (TBARS) has been stated to reach a peak value in an exercised muscle group at the 6th min (Winrow et al. 1993).

Collection and treatment of blood samples

Pre- and post-ex blood samples were kept on ice and in the dark until assayed. Haematocrit (Hct), was measured on the day of the experiment, as were haemoglobin (Hb), leucocyte (white blood cell, WBC) counts, and La concentration. Whole blood samples separated for SOD and GPx measurements were kept at +4°C, plasma separated for MDA measurements and sera separated for Vit E and CER measurements were kept at −40°C until studied a week later.

Biochemical analyses

The Hct and Hb concentrations, and WBC counts were determined using Abbott Cell-Dyn (USA).

As an index of plasma lipid peroxidation, plasma MDA concentrations determined by measuring the thiobarbutiric acid reactive substances according to the spectrophotometric method of Kamal et al. (1989), using 1,1,3,3-tetraethoxypropane (Fluka, Switzerland) as the external standard, and expressed as nanomoles per millilitre.

To evaluate blood antioxidant status, erythrocyte SOD and GPx contents were evaluated using test kits (Randox, UK), and expressed as units per gram Hb. Serum CER and Vit E concentrations were measured using the methods described by Schosinsky et al. (1974) and Varley et al. (1976), and expressed as units per litre and milligrams per decilitre, respectively.

Plasma La concentrations were determined using commercial kits (BioMerieux, France) and expressed as millimoles per litre.

Plasma volume changes (ΔPV) were calculated using the equation suggested by van Beaumont et al. (1981) and expressed as %ΔPV:

$$\%\ \Delta PV = 100\left[\frac{Hb_{pre}(100 - Hct_{post})}{Hb_{post}(100 - Hct_{pre})}\right] - 100$$

In evaluating the results all plasma or serum post-ex values other than Hb and Hct were adjusted by the following equation, derived according to the suggestions of van Beaumont et al. (1981), to adjust for haemoconcentration, and used in addition to the uncorrected post-ex values:

$$post - ex_{corrected} = post - ex\left[\frac{Hb_{pre}(100 - Hct_{post})}{Hb_{post}(100 - Hct_{pre})}\right]$$

Statistics

Statistical evaluations were made using Student's *t*-tests for paired observations to compare pre- and post-ex values and one-way analyses of variance (ANOVA) and Scheffé's test for multiple comparison among means were used to compare intergroups differences. Statistical significance was accepted at $P < 0.05$.

Results

The physical characteristics of the subjects in the four groups were statistically alike (Table 1), and as shown in Table 2, Hct and concentrations were no different among the groups before exercise. Both these parameters were significantly increased compared to their pre-ex values after the exercise. Using these changes, ΔPV due to exercise was found to be reduced by 7.3%–11.7% with no significant differences among the groups (Table 2).

Pre-ex values of WBC counts and La concentrations were the same in all groups, but according to the results, these parameters were significantly increased after the exercise (Table 2). The cause of this increase was not merely haemoconcentration, since, except for the WBC counts in group E, the adjusted post-ex values were still significantly greater than the pre-ex values.

Pre-ex serum Vit E concentrations were similar in S and C, whereas they were significantly increased in E and SE groups (Fig. 1). Basal Vit E concentrations were measured as 1.52 (SD 0.12) $mg \cdot dl^{-1}$ and 1.53 (SD 0.16) $mg \cdot dl^{-1}$ and 1.53 (SD 0.16) $mg \cdot dl^{-1}$ in E and SE, respectively. Vit E supplementation significantly increased Vit E concentrations in both S [2.22 (SD 0.32) $mg \cdot dl^{-1}$; 31%] and C [2.11 (SD 0.20) $mg \cdot dl^{-1}$, 28%] compared to the respective basal concentrations ($P < 0.0001$). Serum Vit E concentrations were decreased in all the groups after the exercise, but the decrements were statistically significant only in C and SE. Nevertheless, when the post-ex values were adjusted according to ΔPV, significant Vit E reductions from the pre-ex values (C: 11.6%; S:13.1%; E: 10.9% and SE: 18%) were observed in all four groups (Fig. 1).

Pre-ex concentrations of plasma MDA were statistically the same in all groups (Fig. 1). Post-ex values were significantly increased in C, S and SE, whereas they remained unchanged in E (Fig. 1). However, when post-ex plasma MDA concentrations were adjusted for Hct and Hb, the significance disappeared.

Table 2 Pre-(*pre-ex*) and post-exercise (*post-ex*), and post-ex corrected (*post-ex$_C$*) values of haematocrit (*Hct*), haemoglobin (*Hb*), leucocyte (white blood cell, *WBC*) count, lactic acid (*La*) concentrations, and % plasma volume changes (%ΔPV) in controls (*C*, $n = 9$), smokers (*S*, $n = 8$), vitamin E supplemented non-smokers (*E*, $n = 11$) and vitamin E supplemented smokers (*SE*, $n = 8$)

		C		S		E		SE	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Hct (%)	Pre-ex	45.5	2.6	44.4	3.6	46.2	3.2	46.4	2.9
	Post-ex	48.3	2.7***	47.4	3.6***	48.6	3.3***	49.1	2.7***
Hb · (g-dl^{-1})	Pre-ex	15.4	0.8	15.1	1.5	15.2	1.2	16.1	1.3
	Post-ex	15.8	0.7**	15.9	1.2*	16.0	1.2*	17.4	1.6**
%ΔPV		−7.3		−10.6		−9.1		−11.7	
WBC	Pre-ex	6.3	1.1	6.9	1.1	6.4	1.4	7.2	1.2
(×10^3 · µl^{-1})	Post-ex	8.8	3.0**	9.1	2.3**	7.7	1.5**	9.7	2.0***
	Post-ex$_C$.	8.2	3.0*	8.1	2.0**	7.0	1.6	8.6	1.9**
La	Pre-ex	2.8	0.3	2.7	0.3	3.2	0.8	3.1	0.3
(mmol · l^{-1})	Post-ex	9.4	1.9***	9.2	1.2***	9.6	1.7***	9.8	1.8***
	Post-ex$_C$.	8.8	1.9***	8.2	1.0***	8.7	1.7***	8.7	1.6***

*, **, *** $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively, compared to *pre-ex* value
No significant difference among four groups by ANOVA

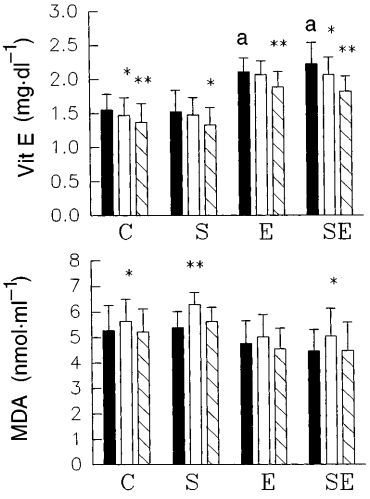

Fig. 1 Pre-exercise (*filled bars*), post-exercise (*unfilled bars*) and post-exercise corrected (*dashed bars*) values of vitamin E (*Vit E*) and malondialdehyde (*MDA*) in controls (*C*, $n = 9$), smokers (*S*, $n = 8$), *Vit E* supplemented non smokers (*E*, $n = 11$) and *Vit E* supplemented smokers (*SE*, $n = 8$). Figures represent mean and SD. * $P < 0.05$ and ** $P < 0.01$, compared to pre-exercise value, student's paired-*t* test, [a] $P < 0.001$, compared to C and S groups, ANOVA

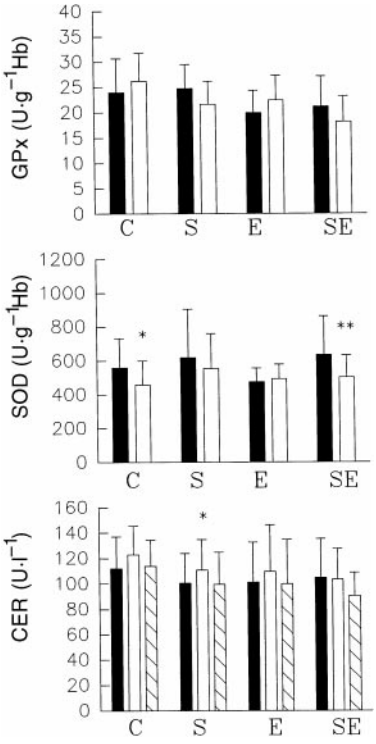

Fig. 2 Pre-exercise (*filled bars*), post-exercise (*unfilled bars*) and post-exercise corrected (*dashed bars*) values of glutathione peroxidase (*GPx*), superoxide dismutase (*SOD*) and ceruloplasmin (*CER*) in controls (*C*, $n = 9$), smokers (*S*, $n = 8$), vitamin E supplemented non-smokers (*E*, $n = 11$) and vitamin *E* supplemented smokers (*SE*, $n = 8$). Figures represent mean and SD. *Hb* Haemoglobin * $P < 0.05$ and ** $P < 0.01$, compared to pre-exercise value, student's paired-*t* test

Erythrocyte SOD and GPx contents are given in Fig. 2. These antioxidant enzyme contents were statistically no different in all four groups before the exercise, and GPx content remained unchanged after the exercise, while SOD contents were significantly decreased only in C and SE.

Pre-ex concentrations of CER were statistically alike (Fig. 2). Post-ex values were found to be increased in C, S, and E and unchanged in SE. The increases were statistically significant only in the smokers. When the post-ex values were corrected for haemoconcentration, however, CER concentrations were unchanged compared to the pre-ex values.

Discussion

Several studies have investigated the oxidative effects of exercise and smoking individually. However, there has

not been much work on the combined effects of these factors. We, therefore, organized the groups in the study to investigate the individual and combined effects of exercise and smoking on the same experimental model. Various exercise models have been used in studies investigating the effects of physical work. Exhausting exercise has been reported to lead to oxidative damage and favorable changes in antioxidant status (Dekkers et al. 1996). Therefore, an incremental exercise model consisting of both eccentric and concentric contractions until exhaustion, following maximal cycling exercise, was designed to be performed by smokers in the present study.

Alpha-tocopherol has been widely studied antioxidant, and its effect has been investigated in various doses. However, there have been conflicting reports about its behavior in exercise and smoking (Morrow et al. 1995; Dekkers et al. 1996). In this study, we planned to investigate the effect of this antioxidant when given at the recommended daily dose and therefore designed the supplementation as 400 mg $\cdot$ day^{-1}.

According to the pre and post-ex Hct and Hb results, the present model of exercise caused significant ΔPV (Table 2). When %ΔPV of the groups were compared, it was clear that being a smoker and/or taking α-tocopherol supplementation did not influence the haemoconcentration caused by exercise.

The WBC counts, and La concentrations were increased after exercise, and it would not be right to attribute these changes to haemoconcentration since the significances of the increases persisted even when the results were adjusted for haemoconcentration (Table 2). There have been studies showing that a short period of exercise caused WBC demargination leading to elevated WBC counts in the peripheral blood (Ferry et al. 1990). Also, in vitro and in vivo studies have suggested that radical oxygen species (ROS) are implicated in such WBC margination (Shingu and Nobunaga 1984). In the present work, elevated post-ex WBC counts in C, S and SE can be explained by this mechanism, and the antioxidant effect of α-tocopherol, that scavenges ROS and may prevent WBC demargination in turn, would seem to be responsible for the unchanged WBC counts in the Vit E supplemented non-smokers.

That the WBC counts were no different between smokers and non-smokers, agrees with the results of Kalra et al. (1991). Although there have been conflicting results reporting increased total WBC count in smokers (Bridges et al. 1985), these differences in the findings might have been due to the consumption of a large number of cigarettes for prolonged periods in those studies.

Elevated La concentrations due to exercise in the present study, agree with the results reported by several studies (Lovlin et al. 1987; Maxwell et al. 1993). According to Stanley et al. (1985), the more intense the exercise, the more lactate is produced and taken up by the working muscle.

Although SOD content was not influenced by either smoking or Vit E supplementation, its content tended to decrease after exercise in all groups except E. The changes in content were statistically significant in C and SE. Since the only group not exhibiting a post-ex decrease in SOD activity was E (Fig. 1), we would suggest that Vit E administration prevented activity loss in the non-smokers, but this dose of α-tocopherol was not adequate to protect SOD activity in the smokers.

On the other hand, GPx content did not exhibit the expected loss after exercise. When the post-ex contents were compared to the pre-ex values, no significant changes were observed. Erythrocyte GPx content was not affected by cigarette smoking, either. According to our results, pre-ex erythrocyte GPx contents in E and SE were 16% reduced from C and S, respectively. However, these reductions were not statistically significant, indicating that the present dose of α-tocopherol did not have a significant influence on erythrocyte GPx content.

Similarly, CER concentrations were not affected by smoking and/or Vit E supplementation, in the present study. Although Duthie et al. (1991) have reported greater CER concentrations in smokers, the mean age of the subjects in their study (48 $\pm$ 3 years) might be a reason for this difference. When corrected post-ex CER concentrations were compared with the pre-ex levels, it was concluded that CER concentrations was unchanged by the present exhausting exercise model in both the smokers and non-smokers.

The effect of Vit E supplementation on oxidative changes caused by exercise or smoking has been widely studied (Helgheim et al. 1979; Simon-Schness and Pabst 1988; Sumida et al. 1989; Maxwell et al. 1993). Oral Vit E administration has been reported to elevate significantly plasma concentrations of this vitamin (Duthie et al. 1991; Baker et al. 1996; Dimitrov et al. 1996). In the present work, there was no significant difference between the Vit E concentrations of the smokers and non-smokers before supplementation, and after 28 days of α-tocopherol (400 mg $\cdot$ day^{-1}) administration, serum concentrations of the vitamin were significantly increased in both groups ($P < 0.001$). This is in agreement with the results of Duthie et al. (1991) and Morrow et al. (1995).

There have been reports on the effect of exercise on Vit E concentrations While Pincemail et al. (1988) and Meydani et al. (1993) have reported increased Vit E concentrations during or shortly after exercise, many investigations on human and animal models have shown decreased serum and muscle Vit E concentrations (Bowles et al. 1991). The decrease in muscle Vit E concentration in animals during prolonged exercise has been attributed to the generation of free radicals and lipid peroxidation (Bowles et al. 1991). Meydani et al. (1993), who have found increased Vit E concentrations after exercise, have stated that, when their results were adjusted for the haemoconcentration effect after exercise, the significance was lost. Therefore, they have suggested that the decrease in plasma volume and haemoconcen-

tration after eccentric exercise might be a major contributions factor in such observations and thus previous observations of exercise induced changes in the plasma concentrations of antioxidants need to be re-evaluated. In the present experimental model, a significant depletion in adjusted serum α-tocopherol concentrations was observed in all groups, due to physical exercise (Fig. 1). The decrements, being more pronounced when evaluated using the adjusted values would suggest that our results are in accordance with the comment of Meydani et al. (1993) and that the increased Vit E demand of exercising muscle is supplied from the plasma.

Numerous studies have demonstrated that antioxidant vitamin supplementations can be beneficial in lowering markers indicative of oxidant stress and lipid peroxidation (Packer 1986; Kanter et al. 1993; Brown et al. 1997). However, an almost equal number of studies have failed to show protective effects of antioxidants in preventing tissue damage and lipid peroxidation (Helgheim et al. 1979). Similarly, our results showed that Vit E supplementation did not change the pre-ex concentrations of the plasma lipid peroxidation marker MDA (Fig. 1). Our results also showed that plasma MDA concentrations were different in the smokers compared to non-smokers. These results agree with those of Harats et al. (1989), while they conflict with those of Kalra et al. (1991). The TBARS method has been suggested as the cause of these discrepancies, not being an accurate indicator of lipid peroxidation in biological samples (Gutteridge and Halliwell 1990).

In contrast to the reports of increased post-ex plasma lipid peroxidation in exercise tests on cycle ergometers or treadmills in the present study the adjusted post-ex MDA values indicated no changes following exercise in any of the groups. In those studies that have shown significant MDA elevations after exercise, the shift in plasma volume and osmolarity either had not been taken into consideration (Lovlin et al. 1987; Laaksonen et al. 1996) or considered but, a simpler method, using only Hct percentages was used for the adjusting of post-ex values (Sumida et al. 1989). Thus, we would suggest that in evaluation it would be more accurate to use adjusted post-ex plasma values, and in so doing, our results indicated that the type of exercise used in this study did not cause significant MDA production.

In conclusion, the results of this study would indicate that:

1. Neither 400 mg · day^{-1} Vit E supplementation nor cigarette smoking affects MDA, SOD, GPx and CER concentrations at rest.

2. A significant water shift from the blood plasma compartment to muscle tissue occurs with exhausting exercise. Thus, plasma parameters should be adjusted for haemoconcentration.

3. MDA concentrations when adjusted as suggested does not exhibit post-ex elevations, therefore, previous reports about exercise-induced oxidative damage assessed by plasma MDA elevations should be re-evaluated. Also, smoking and/or Vit E supplementation do not have any influence on post-ex plasma MDA concentrations.

4. Vit E is significantly depleted by exhausting exercise, and Vit E supplementation in a recommended daily dose helps to prevent WBC demargination and SOD content decrements in non-smokers.

5. Further studies should be conducted better to discuss the relationship between blood free radical generation and muscle free radical production by evaluating indicators of muscle membrane damage and more reliable plasma lipid peroxidation markers.

References

Alessio HM, Goldfarb AH (1988) Lipid peroxidation and scavenger enzymes during exercise: adaptive response to training. J Appl Physiol 64:1333–1336

Baker H, DeAngelis B, Baker E, Khalil M, Frank O (1996) Human plasma patterns during 14 days ingestion of Vitamin E, beta-Carotene, ascorbic acid, and their various combinations. J Am College Nutr 14:159–163

Bendich A, Machlin LJ (1988) Safety of oral intake of vitamin E. Am J Clin Nutr 48:612–619

Bowles DK, Torgan CE, Ebner S, Kehrer JP, lvy JL, Starnes JW (1991) Effects of acute, subacute maximal exercise on skeletal muscle vitamin E. Free Red Res Commun 14:139–143

Bridges RB, Wyatt RJ, Rehm SR (1985) Effect of smocking on peripheral blood leukocytes and serum antiproteases. Eur J Respir Dis 139 [Suppl 66]:24–33

Brown KM, Morrice PC, Duthie GG (1997) Erythrocyte vitamin E and plasma ascorbate concentrations in relation to erythrocyte peroxidation in smokers and nonsmokers: dose response to vitamin E supplementation. Am J Clin Nutr 65:496–502

Camus G, Pincemail J, Roesgen A, Dreezen E, Sluse FE, Deby C (1990) Tocopherol mobilization during dynamic exercise after beta-adrenergic blockade. Arch Int Physiol Biochim 98:121–126

Carnevale TJ, Gaesser GA (1991) Effects of pedalling speed on the power-duration relationship for high-intensity exercise. Med Sci Sports Exerc 234:242–246

Dekkers JC, Van Doornen LJP, Kemper HCG (1996) The role of antioxidant vitamins and enzymes in the prevention of exercise-induced muscle damage. Sports Med 21:213–238

Dimitrov NV, Meyer-Leece C, McMillan J, Gilliland D, Perloff M, Malone W (1996) Plasma α-tocopherol concentrations after supplementation with water-and-fat-soluble vitamin E. Am J Clin Nutr 64:329–335

Duthie GG, Arthur JR, James WPT (1991) Effects of smoking and vitamin E on blood antioxidant status. Am J Clin Nutr 53:1061S–1063S

Eiserich J, van der Vliet A, Handelman GJ, Halliwell B, Cross CE (1995) Dietary antioxidants and cigarette smoke-induced biomolecular damage: a complex interaction. Am J Clin Nutr 62S:l490S–1500S

Ferry A, Picard F, Duvallet A, Weill B, Rien M (1990) Changes in blood leukocyte populations induced by acute maximal and chronic submaximal exercise. Eur J Appl Physiol 59:435–442

Frei B, England L, Ames BN (1989) Ascorbate is an outstanding antioxidant in human blood plasma. Proc Natl Acad Sci 86:6377–6381

Gutteridge JMC, Halliwell B (1990) The measurement and mechanism of lipid peroxidation in biological systems. Trends Biochem Sci 15:129–135

Harats D, Ben-Naim M, Dabach Y, Hollander G, Stein O, Stein O, Stein Y (1989) Cigarette smoking renders LDL susceptible to peroxidative modification and enhanced metabolism by macrophages. Atherosclerosis 79:245–252

Helgheim I, Hetland Ø, Nilsson S, Ingjer F, Strømme SB (1979) The effects of vitamin E on serum enzyme level following heavy exercise. Eur J Appl Physiol 40:283–289

Hoshino E, Shariff R, Van Gossum A, Allard JP, Pichard C, Kurian R, Jeejeebhoy K (1990) Vitamin E suppresses increased lipid peroxidation in cigarette smokers. J Parenter Enteral Nutr 14:300–305

Kalra J, Chaudhary AK, Prasad K (1991) Increased production of oxygen free radicals in cigarette smokers. Int J Exp Pathol 72:1–7

Kamal A, Gomaa A, Khafif M, Hammad A (1989) Plasma lipid peroxides among workers exposed to silica or asbestos dust. Environ Res 49:173–180

Kanter MM, Nolte LA, Holloszy JO (1993) Effects of an antioxidant vitamin mixture on lipid pereoxidation at rest and post-exercise. J Appl Physiol 74:965–969

Laaksonen DE, Atalay M, Niskanen L, Uusitupa M, Hänninen O, Sen CK (1996) Increased resting and exercise-induced oxidative stress in young IDDM men. Diabetes Care 19:596–574

Lovlin R, Cottle W, Pyke I, Kavanagh M, Belcastro AN (1987) Are indices of free radical damage related to exercise intensity. Eur J Appl Physiol 56:3l3–316

Maxwell SRJ, Jakeman P, Thomason H, Leguen C, Thorpe GHG (1993) changes in plasma antioxidant status during eccentric exercise and the effect of vitamin supplementation. Free Rad Res Comms 19:l91–202

Meydani M, Evans WJ, Handelman G, Biddle L, Fielding RA, Meydani SN, Burrill J, Fiatarone MA, Blumberg JB, Cannon JG (1993) Protective effect of vitamin E on exercise-induced oxidative damage in young and older adults. Am J Physiol 264:R992–R998

Morrow JD, Frei B, Longmire AW, Gaziano M, Lynch SM, Shyr Y, Strauss WE, Oates JA, Roberts LJ (1995) Increase in circulating products of lipid peroxidation (F_2-Isoprostanes) in smokers. N Eng J Med 332:1198–1203

Niki E (1987) Antioxidants in relation to lipid peroxidation. Chem Phys Lipids 44:227–253

Packer L (1986) Oxygen radicals and antioxidants in endurance training. In: Benzi G, Packer L, Siliprandi (eds) Biochemical aspects of physical exercise. Elsevier Science, New York, pp 73–92

Pincemail J, Camus DCG, Pirnay F, Bouchez R, Massaux L, Goutier R (1988) Tocopherol mobilization during intensive exercise. Eur J Appl Physiol 57:189–191

Schosinsky KH, Lehmann HP, Beeler M (1974) Measurement of Ceruloplasmin from its oxidase activity in serum by use of o-dianisidine dihydrochloride. Clin Chem 20:1556–1563

Shingu M, Nobunaga M (1984) Chemotactic activity in human serum from the fifth component of complement by hydrogen peroxide. Am J Pathol 117:201–206

Simon-Schnass, I, Pabst H (1988) Influence of vitamin E on physical performance Int J Vitam Nutr Res 58:49–54

Stanley WC, Gertz EW, Wisnski JA, Morris DL, Neese RN, Brooks GA (1995) Systemic lactate kinetics during graded exercise in man. Arm J Physiol 249:1E 595–607

Sumida S, Tanaka K, Kitao H, Nakadomo F (1989) Exercise-induced lipid peroxidation and leakage of enzymes before and after vitamin E supplementation. Int J Biochem 21:835–838

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

Van Beaumont W, Underkofler S, Van Beaumont S (1981) Erythrocyte volume, plasma volume, and acid-base change in exercise and heart dehydration. J Appl Physiol Respir Environ Exerci Physiol 50:1255–1262

Varley H, Gowenlock AH, Bell M (1976) The tocopherols. In: Varley H (ed) Practical clinical biochemistry, vol. 2. Hormones, vitamins, drugs and poisons. Heinmann Medical, London, pp 222–223

Vasankari T, Kujula U, Heinonen O, Kapanen J, Ahotupa M (1995) Measurement of serum lipid peroxidation during exercise using three different methods: diene conjugation, thiobarbituric acid reactive material and fluorescent chromolipids. Clin Chim Acta 234:63–69

Winrow VR, Winyard PG, Morris CJ (1993) Free radicals in inflammation: second messengers and mediators of tissue destruction. Br Med Bull 49:506–522

Wommersly J, Dumin IUGA (1974) Body fat assessed from total body density and its estimation from skin fold thickness: measurement on 481 men and women aged from 16–72 years. Br J Nutr 32:77–79