*Free Rad. Res. Comms.*, Vol. 19, No. 3, pp. 191-202
Reprints available directly from the publisher
Photocopying permitted by license only



# CHANGES IN PLASMA ANTIOXIDANT STATUS DURING ECCENTRIC EXERCISE AND THE EFFECT OF VITAMIN SUPPLEMENTATION

S.R.J. MAXWELL*, P. JAKEMAN+, H. THOMASON, C. LEGUEN and G.H.G. THORPE

*Department of Medicine* and Applied Physiology Research Unit+, Birmingham University B15 2TT, and the Wolfson Applied Technology Group, Wolfson Research Laboratories, Queen Elizabeth Hospital, Edgbaston, Birmingham B15 2TH, UK.*

*(Received November 2nd 1992; in revised form January 5th 1993)*

Twenty-four healthy students undertook one hour of box-stepping exercise. Prior to exercise eight had received no medication (Group A), eight received 400 mg of vitamin C daily for three weeks before and one week after exercise (Group C) and eight received 400 mg of vitamin E for the same period (Group E). Groups C and E had significantly higher levels of vitamin C ($p < 0.01$) and vitamin E ($p < 0.01$) respectively than group A at the commencement of exercise. Plasma total antioxidant capacity rose significantly during exercise in all groups (A – $p < 0.05$; C – $p < 0.001$; E – $p < 0.001$). This rise was accounted for by increases in plasma uric acid in all groups. In addition there were significant increases in vitamin C in group C ($p < 0.001$) and vitamin E in group E ($p < 0.05$). There were no significant changes in plasma malondialdehyde following exercise in any group. It is concluded that plasma antioxidant capacity rises in response to one hour of eccentric exercise and that the contribution of individual antioxidants to this change can be influenced by vitamin supplementation. The possible mechanisms of the antioxidant changes during exercise and their implications are discussed.

KEY WORDS: antioxidants, vitamin C, vitamin E, uric acid, malondialdehyde, exercise

ABBREVIATIONS: ANOVA - analysis of variance, AOC - antioxidant capacity, CK - creatine kinase, EDTA - ethylenediaminetetraacetic acid, HPLC - high performance liquid chromatography, NAD - nicotinamide adenine dinucleotide, SD - standard deviation, SE - standard error of the mean, TBARS - thiobarbituric acid-reactive substances, $VO_2$max-maximum oxygen uptake.

## INTRODUCTION

Strenuous or unaccustomed physical exertion often results in damage to the exercising muscle groups with loss of muscle function[1,2], release of muscular enzymes such as creatine kinase (EC 2.7.3.2)[3,4,5], histological evidence of damage[6] and subjective acheing of muscles[7,8]. A number of possible causes for these changes have been suggested including excessive shear stresses within the exercising muscle, build up of toxic metabolites and the exhaustion of intramuscular energy supplies. However, a growing body of literature suggests that exercise-induced production of free radicals and other oxidant species may be implicated in this process[8,9,10].

Exercise may increase oxidative stress within skeletal muscle for several reasons.

Author for Correspondence: Dr. S.R.J. Maxwell, B.Sc. MB.Ch.B. MRCP. Department of Medicine, Queen Elizabeth Hospital, Edgbaston, Birmingham B15 2TH, UK.

During exercise there is increased oxygen uptake and utilization and therefore potential for leakage of radicals from the mitochondrial electron transport system[11,12]. Secondly, vigorously exercising muscles may produce areas of relative ischaemia during contraction which are intermittently reperfused on relaxation allowing production of the superoxide radical[13,14]. Thirdly, the generation of lactate from glycolysis may reduce the levels of NADH and NADPH in the cell and compromise antioxidant enzyme function[15]. Accordingly, a number of studies have suggested that free radical species are released in increased amounts after strenuous exercise and cause oxidative damage to lipids (lipid peroxidation)[10,16,17,18] and proteins[19].

Regular exercise in healthy individuals is not generally held to be dangerous or harmful to the body and is infact encouraged. This raises the question of how, if exercise does generate an excess of oxygen reactive species, the body might defend itself against this threat and whether these defences might be overcome during prolonged strenuous exercise. A variety of antioxidant defence mechanisms exist to protect exercising muscles including intracellular enzymes and a number of low molecular weight radical scavengers. Of the latter the most important are the water-soluble molecules vitamin C (ascorbic acid) and uric acid and lipid-soluble vitamin E (alpha-tocopherol) found in mitochodrial and cell membranes[20,21].

Vitamin E deficiency in animals is associated with increased lipid peroxidation during exercise[22,23]. Furthermore, vitamin E supplementation has been shown to reduce lipid peroxidation[16,18,24] and may reduce efflux of muscle enzymes following strenuous exercise in man[16]. When combined this evidence suggests that vitamin E is both an inhibitor of lipid peroxidation and muscle enzyme release inviting speculation that the processes are linked. The role of vitamin C as an antioxidant in exercise has been less extensively studied. However, vitamin C is known to be a powerful inhibitor of lipid peroxidation in plasma[25] and to function cooperatively with vitamin E[26]. In addition, increased lymphocyte vitamin C content is found in trained individuals[27,28] and in the days following a bout of exercise[29]. Plasma uric acid is known to increase with most forms of exercise[30,31]. Whether the associated increase in antioxidant activity of plasma this causes has any significance in relation to exercise is not known.

Further evidence of the importance of plasma antioxidant status in relation to exercise is its change as one of the biochemical adaptions to training. Regular athletes have a higher erythrocyte vitamin E and lymphocyte vitamin C content[27]. This may represent one of the beneficial effects for those taking regular physical activity who are afforded protection against lipid peroxidation[3,27,32] and also exercise-induced enzyme efflux[33,34]. When combined the foregoing evidence suggests that antioxidant status has an important influence on the response to exercise.

The purpose of this study was to document the acute effects of exercise on plasma antioxidant status – vitamin C, vitamin E, uric acid and total antioxidant (free radical scavenging) capacity – and, secondly, to observe the effect of vitamin supplements on these changes. We chose to use box-stepping as a model for eccentric exercise ie forced lengthening of a muscle as it develops tension. This mode of exercise has previously been shown to produce a reduction in contractile function, increases in enzyme release and other evidence of muscle damage[35,36].

## METHODS

*Subjects*. Twenty-four healthy students, 16 male and 8 female aged 17.9–21.8 years (mean age 19.6 ± 0.3), were recruited into the study and randomly divided into three groups A, C and E. All subjects were non-smokers and maintained their normal physical activity and dietary patterns throughout the study period. Subjects were of normal height (range 173–189 cm : mean 180 ± 1.0 cm) and weight (range 59–83 kg: mean 68.3 ± 1.3 kg). None of the subjects routinely took vitamin supplements.

*Experimental design*. All subjects were habituated to the eccentric exercise test in the week prior to the start of the study. Each undertook an exercise test on the first day of the study (day 0) and were subsequently monitored during 7 days of recovery (days 1 to 7). In contrast to Group A who received no medication prior to the exercise test, Groups C and E had been given 21 days supplementation with vitamin C 400 mg/day and vitamin E (racemic dl-alpha-tocopheryl acetate; Ephenyl, Roche) 400 mg/day respectively. All vitamin supplements were taken with breakfast and continued for the 7 days of the recovery period.

*Exercise Tests*. On the day of exercise testing subjects reported to the laboratory at 7.30am after an overnight fast and prior to ingestion of any vitamin supplements. An intravenous cannula was inserted into an antecubital vein and a resting blood sample taken (time 0 minutes). Sixty minutes of box-stepping exercise then started with further blood samples taken during, exercise (30 and 60 minutes) and recovery (75 and 120 minutes). All subjects returned to the laboratory prior to breakfast on the 7 days following exercise for daily venepuncture.

*Biochemical Measurements*. All blood samples were obtained from an anticubital vein and collected into a potassium EDTA tube before centrifugation and separation of the plasma supernatant. Whole blood was used to determine haematocrit. Total antioxidant capacity was assessed using the newly described enhanced chemiluminescent method of Whitehead *et al*[37]. Briefly, a chemiluminescent reaction is created by mixing the chemiluminescent substrate luminol with an oxidant (perborate) in the presence of an enhancer (paraiodophenol) and the reaction catalysed by the enzyme horseradish peroxidase. This mixture provides a stable light emission for several minutes that is susceptible to quenching by antioxidant solutions for a time period that is proportional to their concentration. The total antioxidant capacity of plasma can then be derived by relating the period of quenching it produces in comparison to that of a standard solution of the water-soluble vitamin E analogue Trolox (Aldrich Chemicals, Poole, UK) of known concentration.

Uric acid was assayed by an automated method based upon the uricase-peroxidase system[38]. Vitamin E was measured in plasma by reversed phase isocratic HPLC after organic extraction[39]. Plasma vitamin C was measured using a reversed phase HPLC technique deproteinization with metaphosphoric acid (modified from Nagy & Degrell[40]). Plasma malondialdehyde levels, an index of lipid peroxidation, were measured using an HPLC method as previously described[41]. Creatine kinase activity was measured using the spectrophotometric method of Szasz *et al*[42]. Plasma ammonia was measured using Sigma Kit No. 170-UV based on the reductive amination of 2-oxoglutarate. Plasma lactate was measured using a fluorimetric method previously described[43].

*Plasma Volume*. Changes in plasma volume during the period of exercise were calculated from the haemoglobin and haematocrit levels in the formula described

TABLE I
Levels of serum antioxidants before 3 weeks of vitamin supplementation compared with the basal levels prior to exercise on day 0 (Mean ± SE; significant differences from Group A = $^{*}p < 0.05$, $^{**}p < 0.01$). (AOC = antioxidant capacity)

| | Group A | Group C | Group E |
|---|---|---|---|
| Vitamin C (micromol/l) | | | |
| pre-vitamins | ---------- | 76.3 ± 7.1 | 79.1 ± 6.5 |
| basal pre-exercise | 53.3 ± 4.4 | 112.5 ± 7.4** | 99.9 ± 16.4** |
| Vitamin E (micromol/l) | | | |
| pre-vitamins | ---------- | 29.8 ± 1.6 | 27.2 ± 2.2 |
| basal pre-exercise | 22.7 ± 1.2 | 23.7 ± 1.3 | 32.7 ± 3.0** |
| Uric Acid (micromol/l) | | | |
| pre-vitamins | ---------- | 281.9 ± 24.6 | 285.5 ± 17.3 |
| basal pre-exercise | 305.1 ± 24.9 | 308.6 ± 28.0 | 287.3 ± 9.7 |
| Total AOC (micromol/l) | | | |
| pre-vitamins | ---------- | 356.1 ± 26.0 | 366.5 ± 20.0 |
| basal pre-exercise | 397.5 ± 27.8 | 400.3 ● 33.5 | 369.0 ± 17.5 |
| Creatine Kinase (IU/l) | | | |
| basal pre-exercise | 121.5 ± 20.4 | 104.3 ± 27.8 | 91.3 ± 15.4 |

by Van Beaumont *et al*[44]. All biochemical parameters were subsequently corrected for changes in plasma volume during the exercise period.

*Statistical Analysis.* Statistical significance of changes in parameters during exercise was tested using analysis of variance (ANOVA) with repeated measure design. Statistical significance has been accepted if $p < 0.05$. All values are given as mean and standard error of the mean (SE) unless otherwise stated.

## RESULTS

### *Response to Vitamin Supplementation*

*Vitamin C.* Vitamin C levels measured in the resting sample prior to exercise testing were as expected significantly higher in Group C than Group A ($p < 0.01$)(Table I). An unexpected finding was the significantly higher plasma vitamin C levels in Group E subjects supplemented only with vitamin E when compared to group A ($p < 0.01$).

*Vitamin E.* Plasma vitamin E levels were significantly higher prior to exercise in Group E when compared to Groups A and C ($p < 0.01$)(Table I).

*Uric Acid.* There were no significant changes in uric acid levels as a result of vitamin supplementation nor were there significant differences between groups A,C and E at commencement of exercise.

*Plasma Antioxidant Capacity.* There was no significant increase in total antioxidant capacity in either Group C or E compared to group A despite supplementation with antioxidant vitamins. This may reflect the fact that these vitamins in molar terms account for less than half of the total antioxidant capacity[20,37].

*Creatine Kinase.* There were no significant differences in creatine kinase activity between groups prior to exercise (Table I).

### *Acute Response to Exercise*

*Antioxidant Status.* Total antioxidant capacity increased significantly during 60 minutes of eccentric exercise in groups A ($p < 0.05$), C ($p < 0.001$) and E

($p < 0.001$). Uric acid increased significantly in groups A ($p < 0.01$) and E ($p < 0.01$) but not in Group C ($p = 0.054$). Vitamin C levels showed a significant increase in Group C ($p < 0.001$) and to a lesser extent in group E ($p < 0.05$). Vitamin E levels rose significantly in group E only ($p < 0.01$) during the 60 minutes of exercise (Table II; Figure 1). To investigate the possibility that the change in vitamin E may have been simply related to changes in lipoprotein content of plasma we determined plasma cholesterol levels before and after 60 minutes of box-stepping exercise in 26 subjects.

TABLE II

The acute effect of 60 minutes of eccentric exercise on antioxidant status and plasma biochemistry (Mean ± SE; significance differences from basal pre-exercise levels within groups $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$). All biochemical analyses have been corrected for changes in plasma volume. (AOC = antioxidant capacity)

| | Group A | Group C | Group E |
|---|---|---|---|
| Vitamin C (micromol/l) | | | |
| basal pre-exercise | 53.3 ± 4.4 | 112.5 ± 7.4 | 99.9 ± 16.4 |
| post-exercise | 59.6 ± 7.5 | 140.4 ± 7.3*** | 110.4 ± 20.0* |
| Vitamin E (micromol/l) | | | |
| basal pre-exercise | 22.7 ± 1.2 | 23.7 ± 1.3 | 32.7 ± 3.0 |
| post-exercise | 22.7 ± 1.3 | 25.4 ± 1.2 | 35.7 ± 4.2** |
| Uric Acid (micromol/l) | | | |
| basal pre-exercise | 305.1 ± 24.9 | 308.6 ± 28.0 | 287.3 ± 9.7 |
| post-exercise | 325.3 ± 26.8** | 329.1 ± 27.2 | 324.9 ± 12.1** |
| Total AOC (micromol/l) | | | |
| basal pre-exercise | 397.5 ± 27.8 | 400.3 ± 33.5 | 369.0 ± 17.5 |
| post-exercise | 435.4 ± 23.7* | 464 ± 33.0*** | 418.5 ± 21.1*** |
| Ammonia (micromol/l) | | | |
| basal pre-exercise | ---------- | 34.7 ± 5.8 | 32.6 ± 3.6 |
| post-exercise | ---------- | 65.5 ± 12.0** | 81.2 ± 11.8** |
| Lactate (mmol/l) | | | |
| basal pre-exercise | 1.10 ± 0.05 | 0.86 ± 0.12 | 0.81 ± 0.08 |
| post-exercise | 2.48 ± 0.50* | 2.48 ± 0.43** | 1.71 ± 0.26** |
| Malondialdehyde (micromot/l) | | | |
| basal pre-exercise | 0.89 ± 0.12 | 0.98 ± 0.15 | 1.26 ± 0.13 |
| post-exercise | 1.23 ± 0.20 | 1.47 ± 0.21 | 2.33 ± 1.37 |

Total cholesterol changed from 3.71 ± 0.14 to 3.82 ± 0.16 millimoles per litre which was statistically non-significant.

*Plasma Ammonia and Lactate.* Plasma ammonia levels rose significantly ($p < 0.01$) with exercise in groups C and E with no significant differences between groups. Plasma lactate rose significantly in all groups during exercise.

*Malondialdehyde.* Small non-significant rises in plasma malondialdehyde levels were seen in all groups during exercise.

### *Recovery from Exercise*

*Antioxidant Status.* All antioxidant levels that had risen significantly with exercise remained elevated during the first 60 minutes of recovery (Figure 1).

*Creatine Kinase.* Creatine kinase levels responded to exercise with a peak on day 1 of recovery in all groups followed by a second smaller peak at day 5 (Group A)

or 6 (Groups C and E) (Figure 2). Although the response to exercise was significant in all groups there were no differences between groups.

## DISCUSSION

The results of this study indicate that one hour of box-stepping eccentric exercise is associated with an acute increase in plasma antioxidant status. Since all biochemical

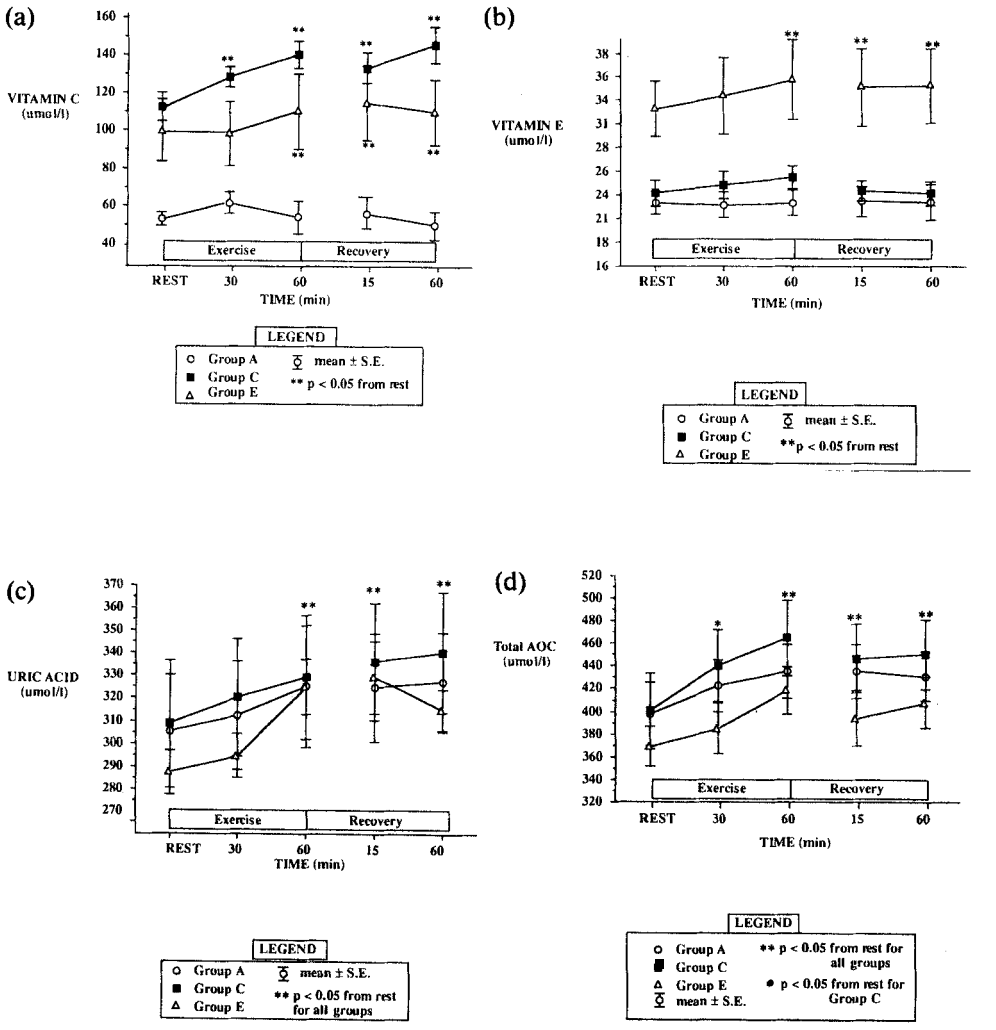


FIGURE 1 Changes in antioxidant levels during exercise and one hour of recovery: (a) vitamic C, (b) vitamin E, (c) uric acid and (d) total antioxidant capacity. All time points illustrated as mean ±SE. Significant changes from resting values within each group are denoted by **.

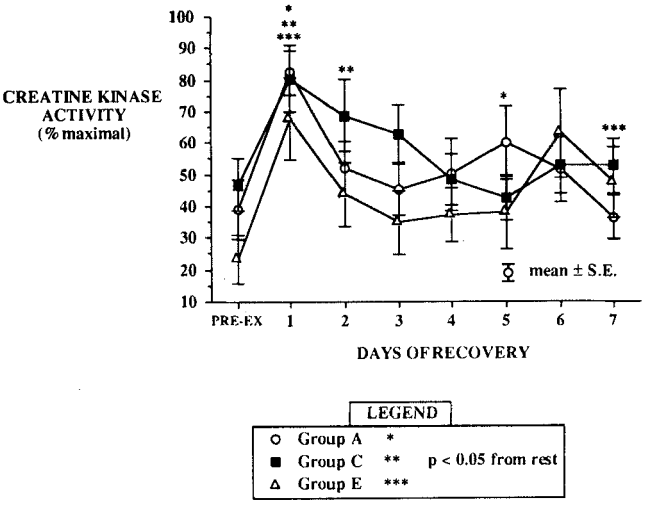


FIGURE 2 Changes in plasma creatine kinase (CK) activity in response to exercise and seven days of recovery. Results for each individual are expressed as % of maximum CK levels. Significant changes from resting values in each group are denoted.

parameters have been corrected for changes in plasma volume we have been able to eliminate the possible influence of exercise-induced fluid losses. Basal vitamin C and vitamin E levels in the control group A were in line with those previously reported[3,27] while vitamin supplements lead to significantly elevated vitamin C and vitamin E levels in groups C and E respectively (Table I). Despite receiving high doses of antioxidant vitamin supplements there were no significant increases in basal total antioxidant capacity in groups C and E compared to group A. This somewhat surprising result can be explained by the relatively small contribution of vitamin C and vitamin E in molar terms to the total radical scavenging capacity of plasma or serum[20,37].

In response to one hour of box-stepping exercise plasma total antioxidant capacity rose significantly in all groups although the rise was more significant in groups C and E ($p < 0.001$) (Table II). When the changes in individual plasma antioxidants are studied differences are noted between the groups. In group A, given no supplements, the only antioxidant to increase was uric acid ($p < 0.01$) with no increase in vitamin C or vitamin E. In group C there were rises in uric acid (n.s.) and vitamin C ($p < 0.001$) while in group E rises in uric acid ($p < 0.01$), vitamin C ($p < 0.05$) and vitamin E ($p < 0.01$) were seen. These data suggest that supplementation of the diet with 400 mg/day of vitamin C or vitamin E provided a ready source of these antioxidants for release during exercise. The interpretation of these data is confused by the finding that vitamin C levels were also significantly higher in group E (given only 400 mg vitamin E/day) than in group A prior to exercise (Table I). The reason for this is not clear but one might speculate that supplementation of one antioxidant (vitamin E) was capable of protecting the other (vitamin C) from oxidation. This effect may have contributed to the significant increases in vitamin C and total antioxidant capacity seen in group E during exercise.

Exercise-induced rises in plasma vitamin C have been described previously[3,29]. In the present study a highly significant rise was seen following vitamin C supplementation when basal plasma vitamin C levels were significantly higher than control (112.5 ± 7.4 v 53.3 ± 4.4 micromol/l; $p < 0.001$). Presumably in this group vitamin supplementation had also provided readily available tissue sources released into the circulation in response to exercise. It has previously been suggested that the adrenal gland may act as the source of stress-related secretion of vitamin C[28]. However other possible sources may include skeletal muscle itself.

Plasma concentrations of vitamin E increased significantly following exercise in group E only ($p < 0.01$). These results are in keeping with previously reported studies where small rises in vitamin E have occurred during exercise[3,45,46]. Since the majority of plasma vitamin E resides in cholesterol-carrying lipoproteins we have considered the possibility that exercise-induced changes in lipoprotein concentrations may have influenced this result. Plasma total cholesterol levels were measured before and after 60 minutes of box-stepping exercise. No significant exercise-related changes in cholesterol were detected (3.71 ± 0.14 to 3.82 ± 0.16 millimoles per litre). Therefore, we believe that this result is due to a mobilization of vitamin E in response to exercise. A possible source of vitamin E during exercise could be adipose tissue stores. These are likely to be replete following dietary supplementation and may be released during exercise-induced lipolysis.

A rise in uric acid was seen in all groups following exercise, a phenomenon that has been well documented previously with various forms of exercise[3,30,31]. Uric acid is a well-recognized antioxidant of the extracellular fluid being present in much greater concentration than the other low molecular weight antioxidants (vitamin C, vitamin E, beta-carotene). For this reason it accounts for approximately half of the radical trapping potential of extracellular fluids[20,37]. How important the role of uric acid might be as a protective antioxidant during exercise is not known. However, its plasma concentration increases with most forms of muscular activity and steady state levels do seem to be lower in those who exercise regularly[27] possibly as a result of altered purine metabolism[47]. Indeed, some authors have found a negative venous-arterial difference for uric acid across exercising skeletal muscle at low intensity[48] indicating uptake or consumption during exercise.

There remains some dispute as to the site of uric acid release. Although uric acid may be produced by xanthine dehydrogenase/oxidase in the skeletal muscle capillary endothelial cells[49] others have argued that the action of this enzyme in sites distant from exercising muscles is more important[50] – the concentration of xanthine dehydrogenase/oxidase seems to be much greater in the liver than skeletal muscle[51]. It is an interesting paradox that this enzyme, which is ultimately responsible for the production of an antioxidant (uric acid) from purine nucleotides may itself, in some circumstances, be a site of free radical formation during exercise[14]. The authors speculate that an important function of uric acid could be to form an antioxidant protection against the oxidative stress imposed in exercising skeletal muscle. This hypothesis necessitates a better understanding of how and where uric acid is generated during exercise and detection of oxidative products of uric acid such as allantoin generated during exercise.

Plasma malondialdehyde has been used extensively as a marker of lipid peroxidation. The HPLC method we have used is more accurate than previous non-specific assays such as thiobarbituric acid reactivity and is likely to be more reliable indicator of lipid peroxidation. While marked changes in antioxidant status of plasma were observed during this study there were no significant increases in plasma malon-

dialdehyde. This might indicate that the mode of exercise used in this study did not cause significant free radical production or oxidative stress, or that any oxidation products remained in the exercising muscle. Alternatively it could be argued that the increases in antioxidant status we observed were sufficient to quench any oxidants that were produced.

The results of previous studies with regard to plasma markers of oxidative stress have been variable. Malondialdehyde levels did not increase with 1 hour of repetitive static exercise[52]. In contrast, two other studies observed exercise-induced increases in thiobarbituric acid-reactive substances (TBARS)[16] and breath pentane exhalation[18] that were prevented by vitamin E supplementation. TBARS and conjugated dienes did not rise in plasma following a half marathon[3] while runners completing an 80.5 kilometre ultramarathon had increased plasma lipid peroxidation[53]. Other investigators found that the appearance of malondialdehyde in plasma was related to exercise intensity, decreasing with exercise up to 70% of maximal oxygen uptake ($VO_2$max) but rising significantly at 100%[54]. Viinkka *et al.*[32] found no increase in plasma peroxides following exhaustive exercise in top athletes undergoing bicycle ergonometry. Resting TBARS are also known to be lower in well-trained individuals[27]. In a subgroup of 14 subjects we assessed the mean intensity of exercise in our box-stepping model as 60 ± 7% of $VO_2$max. One might conclude that the intensity of exercise and the extent of prior training are important variables that explain some of the contradictory findings[3,15,27,50]. Vigorous exercise can certainly result in oxidative damage in some circumstances. The fact that this is often difficult to demonstrate may reflect the efficiency with which the body makes available antioxidants in response to exercise. Indeed, the trained athlete as one adaption to regular exercise develops improved antioxidant status[27].

As expected, eccentric exercise caused a significant increase in plasma creatine kinase activity in the recovery period. Antioxidant vitamin supplementation in the 3 weeks prior to and week after exercise seemed to have little impact on these changes (Figure 2). This result is in keeping with the results of other studies that suggest this parameter is not easily amenable to influence by vitamin supplementation[45,54,55]. This may suggest that either the mechanism of creatine kinase release is not related to oxidant damage or that acute supplementation does not improve antioxidant status in the appropriate muscular compartment. Seemingly the best way to avoid an acute enzyme rise is to be well trained[4].

In summary, one hour of box-stepping eccentric exercise was associated with an increase in plasma total antioxidant capacity that was not explained by changes in plasma volume. The increase in total antioxidant capacity was explained primarily by a rise in plasma uric acid although there were significant rises in vitamin C and vitamin E in those who had previously received vitamin supplements. Small nonsignicant rises in plasma malondialdehyde were observed. Neither antioxidant vitamin attenuated the post-exercise increase in creatine kinase. This study highlights the possible importance of changes in antioxidant status during eccentric exercise. Further studies are required to resolve the antioxidant role of uric acid during exercise.

*Acknowledgements*

The authors acknowledge gratefully the help of Miss E. Grimes and Mr. David Rowbottom in the completion of this project. We are also grateful to the help of Mrs. Judith Evans for the preparation of the study medications.

## *References*


1. D.J. Newham, D.A. Jones, P.M. Clarkson (1987) Repeated high force eccentric exercise: effects on muscle pain and damage. *Journal of Applied Physiology* **63**, 1381–1386.
2. C.T.M. Davies, M.J. White (1981) Muscle weakness following eccentric work in man. *Pflugers Archiv* **393**, 168–171.
3. G.G. Duthie, J.D. Robertson, R.J. Maughan, P.C. Morrice (1990) Blood antioxidant status and erythrocyte lipid peroxidation following distance running. *Archives of Biochemistry and Biophysics* **282**, 78–83.
4. C.B. Ebbeling, P.M. Clarkson (1989) Exercise-induced muscle damage and adaption. *Sports Medicine* **7**, 207–234.
5. D.J. Newham, D.A. Jones, R.H.T. Edwards (1983) Large delayed plasma creatine kinase changes after stepping exercise. *Muscle Nerve* **6**, 380–385.
6. J. Friden (1984) Changes in human skeletal muscle induced by long-term eccentric exercise. *Cell and Tissue Research* **236**, 365–372.
7. R.B. Armstrong (1984) Mechanisms of exercise-induced delayed onset muscle soreness: A brief review. *Medicine ahd Science in Sports and Exercise* **16**, 529–538.
8. R.R. Jenkins (1988) Free radical chemistry: relationship to exercise. *Sports Medicine* **5**, 156–170.
9. B. Sjodin, Y.H. Westing, F.S. Apple (1990) Biochemical mechanisms for oxygen free radical formation during exercise. *Sports Medicine* **10**, 236–254.
10. K.J.A. Davies, A.T. Quintanihla, G.A. Brooks, L. Packer (1982) Free radicals and tissue damage produced by exercise. *Biochemical and Biophysical Research Communications* **107**, 1198.
11. A. Boveris, E. Cadenas, A.O.M. Shoppani (1976) Role of ubiquinone in mitochondrial generation of hydrogen peroxide. *Biochemical Journal* **156**, 435–444.
12. G. Loschen, A. Azzi, L. Flohe (1973) Mitochondrial hydrogen peroxide formation. Relation with energy conservation. *FEBS Letters* **33**, 84–88.
13. W.N. Stainby, W.F. Brechere, D.N. O'Drobnack, J.K. Barclay (1989) Oxidation/reduction state of cytochrome oxidase during repetitive contractions. *Journal of Applied Physiology* **67**, 2158–2162.
14. J.M. McCord 1985 Oxygen-derived free radicals in post-ischaemic tissue injury. *New England Journal of Medicine* **312**, 159–163.
15. R. Lovlin, W. Cottle, I. Pyke, M. Kavanagh, A.N. Belcastro (1987) Are indices of free radical damage related to exercise intensity. *European Journal of Applied Physiolology* **56**, 313–316.
16. S. Sumida, K. Tanaka, H. Kitao, F. Nakadomo (1989) Exercise-induced lipid peroxidation and leakage of enzymes before and after vitamin E supplementation. *International Journal of Biochemistry* **21**, 835.
17. G.G. Corbucci, G. Montanari, M.B. Cooper, D.A. Jones, R.H.T. Edwards (1984) The effect of exertion on mitochondrial oxidative capacity and on some antioxidant mechanisms from marathon runners. *International Journal of Sports Medicine* **5**, 135.
18. C.J. Dillard, R.E. Litov, W.M. Savin, E.E. Dumelin, A.L. Tappel (1978) Effects of exercise, vitamin E and ozone on pulmonary function and lipid peroxidation. *Journal of Applied Physiology* **45**, 927–932.
19. L.L. Ji, F.W. Stratman, H.A. Lardy (1988) Enzymatic down regulation with exercise in rat skeletal muscle. *Archives of Biochemistry and Biophysics* **263**, 137–149.
20. D.D.M. Wayner, G.W. Burton, K.U. Ingold, L.R.C. Barclay, S.J. Locke (1987) The relative contributions of vitamin E, urate, ascorbate and proteins to the total peroxyl radical-trapping antioxidant activity of human blood plasma. *Biochimica et Biophysica Acta* **924**, 408–419.
21. B. Halliwell, J.M.C. Gutteridge (1990) The antioxidants of human extracellular fluids. *Archives of Biochemistry and Biophysics* **280**, 1–8.
22. L. Packer (1984) Vitamin E, physical exercise and tissue damage in animals. Med. Biol. **62**, 105–109.
23. A.T. Quintanihla, L. Packer, J.M.S. Davies, T.L. Racanelli, K.J.A. Davies (1982) Membrane effects of vitamin E deficiency: bioenergetic and surface charge density studies of skeletal muscle and liver mitochondria. *Annals of the New York Academy of Science* **393**, 32–47.
24. A.L. Tappel, C.J. Dillard (1981) 'In vivo' lipid peroxidation measurement via exhaled pentane and protection by vitamin E. *Federation Proceedings* **40**, 174–178.
25. B. Frei, R. Stocker, B.N. Ames (1988) Antioxidant detences and lipid peroxidation in human plasma. *Proceedings of the National Academy of Science* **85**, 9748–9752.
26. A. Bendich, L.J. Machlin, O. Scandurra et al (1986) The antioxidant role of vitamin C. *Free Radicals in Biology and Medicine* **2**, 419–444.
27. J.D. Robertson, R.J. Maughan, G.G. Duthie, P.C. Morrice (1991) Increased blood antioxidant systems of runners in response to training load. *Clinical Science* **80**, 611–618.

28. B. Fishbaine, G. Butterfield (1984) Ascorbic acid status of running and sedentary men. *International Journal of Vitamin and Nutrition Research* **54**, 273.
29. M. Gleeson, J.D. Robertson, R.J. Maughan (1987) Influence of exercise on ascorbic acid status in man. *Clinical Science* **73**, 501–505.
30. Y. Hellsten, B. Ekblom, B. Sjodin (1989) The metabolic relation between hypoxanthine and uric acid in man following maximal short distance running. *Acta Physiologica Scandinavica* **137**, 341–345.
31. H.J. Green, I.G. Fraser (1988) Differential effects of exercise intensity on serum uric acid concentration. *Medicine and Science in Sport and Exercise* **20**, 55–59.
32. L. Viinikka, J. Vuori, D. Ylikorkala (1984) Lipid peroxides, prostacyclin, and thromboxane A2 in runners during acute exercise. *Medicine and Science in Sport and Exercise* **16**, 275–277.
33. B. Ahlborg, J. Brohult (1967) Immediate and delayed metabolic reactions in well-trained subjects after physical exercise. *Acta Medica Scandinavica* **182**, 41–54.
34. F.Q. Nuttall, B. Jones (1968) Creatine kinase and glutamic oxalacetic transaminase activity in serum: kinetics of change with exercise and effect of physical conditioning. *Journal of Laboratory and Clinical Medicine* 71, **847**–854.
35. R.J. Maughan, A.E. Donnelly, M. Gleeson, P.H. Whiting, K.A. Walker (1989) Delayed-onset muscle damage and lipid peroxidation in man after a downhill run. *Muscle Nerve* **12**, 332–336.
36. J.G. Cannon, S.N. Meydani, R.A. Fielding, M.A. Fiatarone, M. Meydani, M Fahrhangmehr, S.F. Orencole, J.B. Blumberg, W.J. Evans (1991) Acute phase response in exercise. II. Associations between vitamin E, cytokines, and muscle proteolysis. *American Journal of Physiology* **260**, R1235–R1240.
37. T.P. Whitehead, G.H.G. Thorpe, S.R.J. Maxwell (1992) An enhanced chemiluminescent assay for antioxidant capacity in biological fluids. *Analytica Chimica Acta* **266**, 265–277.
38. N. Gochman, J.M. Schmitz (1971) Automated determination of uric acid, with use of a uricase-peroxidase system. *Clinical Chemistry* **17**, 1154–1159.
39. D.B. Milne, J. Botnen (1986) Retinol, alpha-tocopherol, lycopene and alpha- and beta-carotene simultaneously determined in plasma by isocratic liquid chromatography. *Clinical Chemistry* **32**, 874–876.
40. E. Nagy, I. Degrell (1989) Determination of ascorbic acid and dehydroascorbic acid in plasma and cerebrospinal fluid by liquid chromatography with electrochemical detection. *Journal of Chromatography and Biomedical Applications* **497**, 276–281
41. C. Largilliere, M.B. Melancon (1988) Free malondialdehyde determination in human plasma by high-performance liquid chromatography. *Analytical Biochemistry* 170, 123–126.
42. G. Szasz, W. Gruber, E. Beret (1976) Creatine kinase in serum. Determination of optimum reaction conditions. *Clinical Chemistry* 22, 650.
43. C. Olsen (1971) An enzymatic fluorimetric micromethod for the determination of acetoacetate, B-hydroxybutyrate, pyruvate and lactate. *Clinica Chimica Acta* 33, 293–300.
44. W. Van Beaumont, J.E. Greenleaf, L. Julios (1972) Disproportionate changes in haematocrit, plasma volume and proteins during exercise and bed rest. *Journal of Applied Physiology* 33, 55–61.
45. J.G. Cannon, S.F. Orencole, R.A. Fielding, M. Meydani et al (1990) Acute phase response in exercise, interaction of age and vitamin E on neutrophils and muscle enzyme release. *American Journal of Physiology* 259, R1214–RI219.
46. J. Pincemail, C. Deby, G. Camus, F. Pimay, R. Bouchez, L. Massaux, R. Goutier (1988) Tocopherol mobilization during intense exercise. *European Journal of Applied Physiology* 57, 189–191.
47. A.W. Williams, D.M. Wilson (1990) Uric acid metabolism in humans. *Seminars in Nephrology* 10, 9–14.
48. Y. Hellsten, G. Ahlborg, M. Jensen-Urstad, B. Sjodin (1988) Indication of in vivo xanthine oxidase activity in human skeletal muscle during exercise. *Acta Physiologica Scandinavica* 134, 159–160.
49. E-D. Jarasch, C. Grund, G. Bruder, H.W. Heid, T.W. Keenan, W.W. Franke (1981) Localization of xanthine oxidase in mammary gland epithelium and capillary endothelium. *Cell* 25, 67–82.
50. K. Sahlin, K. Ekberg, S. Cizinsky (1991) Changes in plasma hypoxanthine and free radical markers during exercise in man. *Acta Physiologica Scandinavica* 142, 257–281.
51. R.W.E. Watts, J.E.M. Watts, J.E. Seegmiller (1965) Xanthine oxidase activity in human tissues and its inhibition by allopurinol (4-hydroxypyrazolo-3-4-d-pyrimidine). *Journal of Laboratory and Clinical Medicine* 66, 688–697.
52. K. Sahlin, S. Cizinsky, M. Warholm, J. Hoberg (1992) Repetitive static muscle contractions in humans–a trigger of metabolic and oxidative stress? *European Journal of Applied Physiology* 64, 228–236.
53. M.M. Kanter, G.R. Lesmes, L.A. Kaminsky, J. La Ham-Saeger, N.D. Nequin (1988) Serum creatine kinase and lactate dehydrogenase changes following an eighty kilometer race. Relationship to lipid peroxidation. *European Journal of Applied Physiology* 57, 60–63.

54. I. Helgheim, O. Hetland, S. Nilsson, F. Ingjer, S. B. Stromme (1979) The effects of vitamin E on serum enzyme levels following heavy exercise. *European Journal Applied Physiology* 40, 283–289.
55. J. A. Warren, R. R. Jenkins, L. Packer, E. H. Witt, R. B. Armstrong (1992) Elevated muscle vitamin E does not attenuate eccentric exercise-induced muscle injury. *Journal of Applied Physiology* 72, 2168–2175.

Accepted by Professor B. Halliwell