Uric acid reduces exercise-induced oxidative stress in healthy adults

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ABSTRACT

Uric acid (UA) possesses free-radical-scavenging properties, and systemic administration is known to increase serum antioxidant capacity. However, it is not known whether this protects against oxidative stress. The effects of raising UA concentration were studied during acute aerobic physical exercise in healthy subjects, as a model of oxidative stress characterized by increased circulating 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) concentrations. Twenty healthy subjects were recruited to a randomized double-blind placebo-controlled crossover study, and underwent systemic administration of 0.5 g of UA in 250 ml of 0.1 % lithium carbonate/4 % dextrose vehicle or vehicle alone as control. Subjects performed high-intensity aerobic exercise for 20 min to induce oxidative stress. Plasma 8-iso-$PGF_{2\alpha}$ concentrations were determined at baseline, after exercise and after recovery for 20 min. A single bout of high-intensity exercise caused a significant increase in plasma 8-iso-$PGF_{2\alpha}$ concentrations from 35.0 ± 4.7 pg/ml to 45.6 ± 6.7 pg/ml ($P < 0.01$). UA administration raised serum urate concentration from 293 ± 16 to 487 ± 16 μmol/l ($P < 0.001$), accompanied by increased serum antioxidant capacity from 1786 ± 39 to 1899 ± 45 μmol/l ($P < 0.01$). UA administration abolished the exercise-induced elevation of plasma 8-iso-$PGF_{2\alpha}$ concentrations. High UA concentrations are associated with increased serum antioxidant capacity and reduced oxidative stress during acute physical exercise in healthy subjects. These findings indicate that the antioxidant properties of UA are of biological importance *in vivo*.

INTRODUCTION

Oxidative stress is characterized by excess free radical activity and is believed to play an important role in the development of atherosclerosis [1]. This has stimulated interest in the possibility that antioxidants could offer protective effects within the cardiovascular system. Until recently, the lack of an adequate biological marker of oxidative stress *in vivo* has limited studies of the efficacy of antioxidant supplementation. A number of oxidative reaction products have been explored, including lipid hydroperoxides, malonyl dialdehyde and prostaglandin F_2-like substances, so called F_2-isoprostanes. F_2-isoprostanes are a family of compounds formed by non-enzymic oxidative modification of arachadonic acid, and result from free radical attack of phospholipids in cell membranes or circulating low-density lipoproteins [2]. They are formed *in situ* in the cell membrane, from which they are cleaved by phospholipase, and circulate in a free form or as a phospholipid-bound ester in a bound/free ratio of approx. 1:2 [3]. Of the F_2-isoprostanes, 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$; also known as $iPF_{2\alpha}$-III) has been shown to be capable of exerting a number of biological effects, such as smooth muscle contraction, vasoconstriction and increased platelet aggregability [4,5]. However, the relevance of these effects remains

Key words: antioxidant, ergometric exercise, isoprostane, oxidative stress, systemic vascular resistance, uric acid.

Abbreviations: ABTS, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid); BP, blood pressure; CI, cardiac index; 8-iso-$PGF_{2\alpha}$, 8-iso-prostaglandin $F_{2\alpha}$; UA, uric acid.

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uncertain, because they are caused by significantly higher 8-iso-$PGF_{2\alpha}$ concentrations than typically found in human plasma. Concentrations of 8-iso-$PGF_{2\alpha}$ are stable in isolated body fluids and quantification in plasma or urine provides a marker of free radical generation *in vivo*. Its concentrations in biological fluids are increased in the presence of major cardiovascular risk factors characterized by oxidative stress, for example hypercholesterolaemia [6], cigarette smoking [7] and diabetes mellitus [8]. Elevated 8-iso-$PGF_{2\alpha}$ concentrations in these conditions support the hypothesis that oxidative modification is an important early step in the development of atherosclerosis. Circulating 8-iso-$PGF_{2\alpha}$ concentrations provide a non-invasive quantitative measure of lipid peroxidation and represent a major advance in assessment of vascular oxidative stress *in vivo*.

Moderately intense physical exercise increases oxygen utilization and causes excess oxygen-derived free radical liberation through mitochondrial lipid peroxidation, neutrophil degranulation and up-regulation of xanthine oxidase activity, which liberates superoxide ($O_2^{\bullet}$) [9]. Intense physical exercise provides a model for studying the effects of acute oxidative stress *in vivo* and has been shown to increase susceptibility of low-density lipoprotein to oxidation and impair endothelial-dependent vasodilatation in the forearm vascular bed [10,11]. Oxidative stress during acute exercise is characterized by elevated circulating 8-iso-$PGF_{2\alpha}$ concentrations, which has provided an opportunity to examine the effects of antioxidant administration on free radical activity *in vivo* [9]. Supplementation with vitamin C or E increases resistance to exercise-induced lipid peroxidation in healthy individuals [12]. However, β-carotene does not reduce oxidative stress during acute physical exercise [13], and combined administration of vitamin C and *N*-acetyl-cysteine paradoxically increase 8-iso-$PGF_{2\alpha}$ concentrations after acute eccentric exercise, despite increased serum antioxidant capacity [14]. Glutathione and ubiquinol supplementation reduce oxidative stress in animal models of acute exercise [15], but their effects have not been fully characterized in humans.

Uric acid (UA) is an abundant aqueous antioxidant that accounts for almost two thirds of all free-radical-scavenging activity in human serum [16]. UA reacts with oxygen-derived free radicals and becomes oxidized in skeletal muscle during high-intensity exercise [17]. Intracellular UA concentrations are rapidly replenished by uptake from plasma after exercise [18]. A previous study [19] found a significant inverse relationship between serum UA concentrations and oxidative stress during acute aerobic exercise. These observations suggest that high UA concentrations could confer protection against free radical activity *in vivo* and indicate that UA may be of biological importance in the setting of acute oxidative stress. The feasibility of systemic UA administration, to increase serum antioxidant capacity, has recently been established in a research setting [20]. The present study aimed to characterize the effects of elevated serum UA concentrations on oxidative stress induced by acute physical exercise, reflected by plasma 8-iso-$PGF_{2\alpha}$ concentrations.

METHODS

Subjects

Twenty healthy men and women were recruited from a community database of healthy subjects held at the Clinical Research Centre of the University of Edinburgh. The protocol was reviewed and approved by the Local Research Ethics Committee, and written informed consent was obtained from each participant. Inclusion criteria were men or women aged 18–45 years. Exclusion criteria were elevated blood pressure (BP; $>160/100$ mmHg), clinical history of joint, kidney or cardiovascular disease, those taking any regular medication or non-prescription medication in the past week, serum creatinine >110 mmol/l, or serum UA >400 mmol/l.

Drugs and reagents

UA and lithium carbonate (Ultrapure preparations; Sigma, Poole, Dorset, U.K.) were reconstituted in sterile dextrose solution (Baxter Healthcare, Thetford, Norfolk, U.K.), filtered (0.22 mm Millex filter; Millipore, Molsheim, France), and prepared on the day of administration. UA (0.5 g) was dissolved in 250 ml of 0.1 % lithium carbonate/4 % dextrose vehicle. This vehicle was chosen because it allows stable dissolution of UA [21] and does not influence serum antioxidant capacity [20].

Protocol

Subjects were enrolled in a randomized two-way double-blind placebo-controlled crossover study. Investigations were performed in a quiet room maintained at 24–26 °C. An 18-standard gauge venous cannula was inserted into a suitable vein in each antecubital fossa, under local anaesthetic using aseptic technique. Subjects remained seated for 20 min to establish baseline haemodynamic conditions, and underwent systemic administration of 0.5 g of UA in 250 ml of vehicle or 250 ml of vehicle alone over 20 min via the non-dominant forearm cannula. Subjects performed lower limb exercise using an upright electronically braked ergometric cycle machine (Ergometry System 380B; Siemens-Elema, Solna, Sweden). Pedalling rate was sustained at 70 ± 10 Hz to maintain a constant workload of 80 W, equivalent to a modestly intense riding speed of 3.8 $m \cdot s^{-1}$ (8.5 miles/h) [22]. Exercise was sustained for 20 min, followed by a 20 min recovery period during which subjects rested while seated. Study visits were performed 1 week apart at the same time of day on each visit.

A venous blood sample (5 ml) was collected in serum gel tubes (Sarstedt, Leicester, U.K.), via the non-infused forearm cannula, for measurement of serum UA and total serum antioxidant capacity at baseline and 20, 40 and 60 min after the start of the infusion. Additional 5 ml samples were collected in potassium/EDTA tubes (Sarstedt) for determination of plasma 8-iso-$PGF_{2\alpha}$ concentrations at baseline and 60 min after the start of infusion. Blood samples were centrifuged at 1000 *g* for 10 min at 4 °C, decanted immediately, and serum and plasma were stored at −40 °C until assays were performed.

Haemodynamic variables were determined at baseline and at 5 min intervals up to 60 min after the start of the infusion. BP was recorded in the dominant arm using a validated oscillometric device (HEM-705CP; Omron, Tokyo, Japan) [23], and the cardiac index (CI) was assessed using transthoracic bioimpedance (NCCOM3-R7; BoMed, Irvine, CA, U.S.A.) [24]. Systemic vascular resistance index was calculated as mean arterial pressure divided by CI.

Serum UA concentration

UA concentration was determined by an automated colorimetric assay (Vitros; Ortho-Clinical Diagnostics, Amersham, Bucks, U.K.).

Serum antioxidant capacity

Antioxidant capacity was measured using the Total Antioxidant Status assay (Randox Laboratories; Crumlin, County Antrim, U.K.). This assay is based on the interaction between a chromogen [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS)] and ferrylmyoglobin, a free radical formed by the reaction of metmyoglobin and H_2O_2, which forms the cation $ABTS^{\bullet+}$, a blue/green chromophore with maximal absorbance at 417, 645, 734 and 815 nm [25]. Antioxidants in added serum scavenge $ABTS^{\bullet+}$ and prevent absorbance to a degree related to the overall serum antioxidant capacity. Absorbance was determined at 600 nm using a Cobas Fara (Roche Diagnostics, Lewes, East Sussex, U.K.), calibrated using 6-hydroxy-2,5,8-tetramethylchroman-2-carboxylic acid (Trolox, a water soluble tocopherol analogue) and expressed as μmol/l of Trolox equivalent. The Total Antioxidant Status assay gives a global measurement of *ex vivo* serum free-radical-scavenging capacity and takes account of summation and interaction between constituent antioxidants [25]. The standard reference range for this assay was 1494–2107 μmol/l and the intra-assay precision was 6.5 %.

Plasma 8-iso-PGF_{2a} concentrations

Solid-phase extraction was performed using an Isosolute C18(EC) 100 mg/3 ml silica-sorbent column (International Sorbent Technology, Hengoed, Mid Glamorgan, U.K.), and 8-iso-$PGF_{2\alpha}$ was eluted with ethylacetate/methanol (99:1, v/v) buffer. Assays were performed on undiluted samples and after 1:2 dilution with buffer, and each concentration was assayed in duplicate. 8-iso-$PGF_{2\alpha}$ concentrations were measured using an ELISA (Cayman Chemical Co., Ann Arbor, MI, U.S.A.). The assay is based on the competition between 8-iso-$PGF_{2\alpha}$ and an acetylcholinesterase–8-iso-$PGF_{2\alpha}$ conjugate for limited amounts of 8-iso-$PGF_{2\alpha}$-specific rabbit IgG bound to 96-well plates [3]. Ellman's reagent [5,5′-dithiobis-(2-nitrobenzoic acid)] contains an acetylcholinesterase substrate, which forms a distinctive yellow reaction product whose absorbance was detected at 412 nm using a Vmax kinetic microplate spectrophotometer (Molecular Devices, Winnersh, U.K.). The extent of absorbance is proportional to the amount of conjugate in each well, which is inversely proportional to free 8-iso-$PGF_{2\alpha}$ concentrations during incubation. Mean values were expressed as a percentage of maximal binding absorbance, and 8-iso-$PGF_{2\alpha}$ content was determined by comparison with absorbance of standard isoprostane concentrations (3.9, 7.8, 15.6, 31.3, 62.5, 125, 250 and 500 pg/ml). The standard reference range was 4.6–65.9 pg/ml and the intra-assay precision was 6.0 %.

Table 1 Baseline characteristics of the study population

Values are means ± S.D.

Characteristic	Value
Number (male)	20 (10)
Age (years)	23 ± 3
Height (m)	1.72 ± 0.34
Weight (kg)	71 ± 5
Body mass index (kg/m^2)	23.0 ± 3.5
Serum creatinine (μmol/l)	72 ± 10
Serum cholesterol (mmol/l)	4.0 ± 2.5
Serum urate (μmol/l)	293 ± 52

Data analysis and statistics

Responses were compared using two-way ANOVA and paired Student's *t* tests where appropriate, and statistical significance was accepted at the 5 % level in all cases.

RESULTS

Baseline characteristics of study subjects are shown in Table 1. As expected, UA administration caused serum concentrations to increase by 194 ± 8 μmol/l ($P < 0.001$) from baseline (Table 2). Administration of vehicle alone did not significantly change circulating UA concentrations from baseline (−9 ± 2 μmol/l). There was a corresponding increase in antioxidant capacity from baseline of 118 ± 18 μmol/l ($P < 0.001$) following UA

Table 2 Serum UA concentration, antioxidant capacity and haemodynamic variables at baseline, after infusion of 0.5 g of UA in 250 ml of vehicle or vehicle alone, after exercise and after recovery

Values are means ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ compared with vehicle alone. SVRI, systemic vasculature resistance index.

	Tretment	Baseline (0 min)	Post-infusion (20 min)	Post-exercise (40 min)	Post-recovery (60 min)
Serum urate (μmol/l)	Vehicle	294 ± 19	286 ± 18	296 ± 19	293 ± 18
	UA	293 ± 16	487 ± 16***	458 ± 15***	429 ± 14***
Antioxidant capacity (μmol/l)	Vehicle	1815 ± 29	1769 ± 46	1817 ± 27	1796 ± 32
	UA	1786 ± 39	1899 ± 45**	1937 ± 44*	1895 ± 47*
Heart rate (beats per min)	Vehicle	75 ± 2	73 ± 2	138 ± 6*	80 ± 2
	UA	76 ± 3	74 ± 2	135 ± 7*	80 ± 3
Systolic BP (mmHg)	Vehicle	93 ± 5	97 ± 6	147 ± 6*	94 ± 4
	UA	94 ± 5	94 ± 5	153 ± 6*	97 ± 5
Diastolic BP (mmHg)	Vehicle	66 ± 2	67 ± 3	101 ± 5*	69 ± 2
	UA	68 ± 2	66 ± 4	103 ± 6*	68 ± 2
Cardiac index (l/min per m^2)	Vehicle	3.9 ± 0.2	3.8 ± 0.2	9.7 ± 0.8*	3.8 ± 0.2
	UA	3.9 ± 0.2	3.7 ± 0.1	9.4 ± 0.6*	4.0 ± 0.2
SVRI	Vehicle	22.0 ± 2.0	23.2 ± 1.7	15.9 ± 2.4*	22.0 ± 1.7
	UA	22.4 ± 1.9	23.6 ± 1.6	14.5 ± 1.2*	23.0 ± 2.0

administration, but no change following vehicle alone (-22 ± 23 μmol/l).

Plasma 8-iso-$PGF_{2\alpha}$ concentrations in the study population were 41.8 ± 3.0 pg/ml at baseline. Intense physical exercise caused a significant increase in plasma 8-iso-$PGF_{2\alpha}$ concentrations from 35.0 ± 4.7 pg/ml to 45.6 ± 6.7 pg/ml immediately after exercise ($P < 0.05$), and 55.7 ± 7.1 pg/ml after recovery ($P < 0.01$) following vehicle administration (Figure 1). However, after systemic administration of UA, there was no significant increase from baseline plasma 8-iso-$PGF_{2\alpha}$ concentrations (37.1 ± 5.4 pg/ml) after exercise (31.6 ± 5.5 pg/ml) or after recovery (37.9 ± 6.6 pg/ml). Plasma 8-iso-$PGF_{2\alpha}$ concentrations after vehicle and UA administration, expressed as a change from baseline, were $+10.6 \pm 5.5$ and -5.5 ± 6.2 pg/ml ($P < 0.05$) respectively, after exercise, and $+20.7 \pm 6.5$ and $+0.8 \pm 7.7$ pg/ml ($P < 0.01$) respectively, after recovery. Two-way ANOVA showed a significant effect of UA administration on isoprostane concentrations ($P = 0.02$) and changes in isoprostane concentrations from baseline ($P < 0.01$) during exercise and recovery.

Administration of neither vehicle nor UA caused any significant changes in resting haemodynamic variables from baseline (Table 2). Intense physical exercise caused significant increases in heart rate ($P < 0.001$), systolic BP ($P < 0.001$), diastolic BP ($P < 0.001$) and CI ($P < 0.005$), and a reduction in systemic vascular resistance index ($P < 0.005$), whereas administration of UA did not influence the haemodynamic responses. No adverse events were reported in any of the subjects.

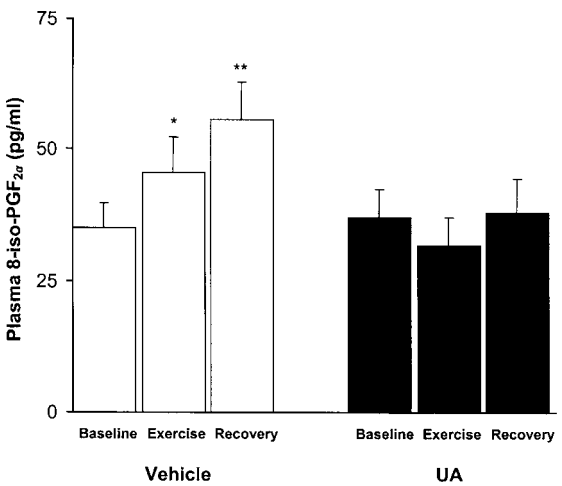

Figure 1 Plasma 8-iso-$PGF_{2\alpha}$ concentrations at baseline, after acute physical exercise and after recovery following administration of 0.5 g of UA in 250 ml of vehicle or vehicle alone

Values are means ± S.E.M. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with baseline.

DISCUSSION

In the present study, the baseline characteristics were consistent with those of a young healthy population free from major cardiovascular risk factors that could potentially exert independent effects on antioxidant capacity and oxidative stress. The chosen exercise intensity and duration caused a significant haemodynamic response in

this group, who were unaccustomed to regular strenuous exercise. Resting plasma free 8-iso-$PGF_{2\alpha}$ concentrations were consistent with those reported previously [26] in healthy subjects. The single bout of acute intense exercise caused oxidative stress in the study population, reflected by increased plasma 8-iso-$PGF_{2\alpha}$ concentrations immediately after exercise and recovery. The magnitude of the rise in plasma isoprostane concentrations was similar to previous observations in healthy subjects and trained athletes after acute exercise [27].

Vehicle administration had no significant effect on circulating UA concentrations or serum antioxidant capacity, as found previously [20]. Administration of UA (0.5 g) achieved a substantial increase in circulating UA concentrations to levels characteristic of the upper limit of the normal reference range (120–420 μmol/l). Raised UA concentrations were associated with increased serum free-radical-scavenging capacity, as indicated by a global antioxidant measurement. Prior administration of UA attenuated the exercise-induced increase in plasma 8-iso-$PGF_{2\alpha}$ concentrations. It is likely that this effect was mediated by the increased ability to counter excess free radical activity, conferred by the antioxidant properties of UA. These findings indicate that high circulating UA concentrations are able to prevent oxidative stress *in vivo* during intense physical exercise and raise the possibility that UA could protect against oxidative stress in other situations. Interestingly, a number of earlier observations suggest that local UA concentrations may be regulated, at least in part, by prevailing redox conditions. For example, oral administration of L-arginine has been found to cause a reduction in systemic oxidative stress, which is accompanied by a fall in circulating UA concentrations [28]. Furthermore, acute ischaemia in several vascular beds, for example the coronary circulation, is associated with oxidative stress and causes an increase in circulating UA concentrations locally [29,30]. Raised UA concentrations could, therefore, provide a physiological mechanism to protect against excess free radical activity.

The lack of effect of vehicle or UA administration on resting BP and systemic vascular resistance indicate that high UA concentrations, at least in the acute setting, do not directly influence vascular tone.

Cardiovascular risk and the development of atherosclerosis are likely to be influenced by exposure to chronic oxidative stress. A limitation of the present study is that only short-term measures of antioxidant capacity and oxidative stress were examined. However, this appears to be a valid approach because there is good correlation between short-term and long-term effects of a variety of cardiovascular risk factors on oxidative stress, for example cigarette smoking [31] and hyperglycaemia [32]. A further potential limitation is that the validity of the Omron HEM-705CP device has not been established in exercising subjects. It is accurate for measuring BP up to 160/100 mmHg in resting subjects and has been validated according to criteria of the British Hypertension Society and Association for the Advancement of Medical Instrumentation [23].

In conclusion, the present study has shown that UA administration temporarily raises circulating UA concentrations, which increases serum antioxidant capacity and reduces exercise-induced oxidative stress in a young healthy population. These findings indicate that the antioxidant properties of UA are of physiological consequence and support the view that UA confers potentially important free-radical-scavenging effects *in vivo*. The implications of raised serum UA concentrations, in the setting of chronic oxidative stress, require further evaluation. Additional research is required to investigate the potential role of high UA concentrations in patients exposed to major cardiovascular risk factors.

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REFERENCES

1 Maxwell, S. R. J. and Lip, G. Y. H. (1997) Free radicals and antioxidants in cardiovascular disease. Br. J. Clin. Pharmacol. **44**, 307–317
2 Morrow, J. D., Hill, K. E., Burk, R. F., Nammour, T. M., Badr, K. F. and Roberts, L. J. (1990) A series of prostaglandin F2-like compounds are produced *in vivo* in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. Proc. Natl. Acad. Sci. U.S.A. **87**, 9383–9387
3 Morrow, J. D., Awad, J. A., Boss, H. J., Blair, I. A. and Roberts, L. J. (1992) Non-cyclooxygenase-derived prostanoids (F_2-isoprostanes) are formed *in situ* on phospholipids. Proc. Natl. Acad. Sci. U.S.A. **89**, 10721–10725
4 Minuz, P., Patrignani, P., Gaino, S. et al. (2002) Increased oxidative stress and platelet activation in patients with hypertension and renovascular disease. Circulation **106**, 2800–2805
5 Patrono, C. and FitzGerald, G. A. (1997) Isoprostanes: potential markers of oxidant stress in atherothrombotic disease. Arterioscler., Thromb., Vasc. Biol. **17**, 2309–2315
6 Reilly, M., Delanty, N., Lawson, J. A. and FitzGerald, G. A. (1996) Modulation of oxidant stress *in vivo* in chronic cigarette smokers. Circulation **94**, 19–25
7 Reilly, M. P., Pratico, D., Delanty, N. et al. (1998) Increased formation of distinct F_2 isoprostanes in hypercholesterolemia. Circulation **98**, 2822–2828
8 Davi, G., Ciabattoni, G., Consoli, A. et al. (1999) *In vivo* formation of 8-iso-prostaglandin $F_{2\alpha}$ and platelet activation in diabetes mellitus. Effects of improved metabolic control and vitamin E supplementation. Circulation **99**, 224–229
9 Ji, L. L. (1999) Antioxidants and oxidative stress in exercise. Proc. Soc. Exp. Biol. Med. **222**, 283–292

10 Wetzstein, C. J., Shern-Brewer, R. A., Santanam, N., Green, N. R., White-Welkley, J. E. and Parthasarathy, S. (1998) Does exercise affect the susceptibility of low density lipoprotein to oxidation? Free Radical Biol. Med. **24**, 679–682
11 Bergholm, R., Makimattila, S., Valkonen, M. et al. (1999) Intense physical training decreases circulating antioxidants and endothelium-dependent vasodilatation *in vivo*. Atherosclerosis **145**, 341–349
12 Rokitzki, L., Logemann, E., Sagredos, A. N., Murphy, M., Wetzel-Roth, W. and Keul, J. (1994) Lipid peroxidation and antioxidative vitamins under extreme endurance stress. Acta Physiol. Scand. **151**, 149–158
13 Sumida, S., Doi, T., Sakurai, M., Yoshioka, Y. and Okamura, K. (1997) Effect of a single bout of exercise and β-carotene supplementation on the urinary excretion of 8-hydroxy-deoxyguanosine in humans. Free Radical Res. **27**, 607–618
14 Childs, A., Jacobs, C., Kaminski, T., Halliwell, B. and Leeuwenburgh, C. (2001) Supplementation with vitamin C and *N*-acetyl-cysteine increases oxidative stress in humans after an acute muscle injury induced by eccentric exercise. Free Radical Biol. Med. **31**, 745–753
15 Sen, C. K., Atalay, M. and Hanninen, O. (1994) Exercise-induced oxidative stress: glutathione supplementation and deficiency. J. Appl. Physiol. **77**, 2177–2187
16 Maxwell, S. R., Thomason, H., Sandler, D. et al. (1997) Antioxidant status in patients with uncomplicated insulin-dependent and non-insulin-dependent diabetes mellitus. Eur. J. Clin. Invest. **27**, 484–490
17 Hellsten, Y., Tullson, P. C., Richter, E. A. and Bangsbo, J. (1997) Oxidation of urate in human skeletal muscle during exercise. Free Radical Biol. Med. **22**, 169–174
18 Hellsten, Y., Svensson, M., Sjodin, B. et al. (2001) Allantoin formation and urate and glutathione exchange in human muscle during submaximal exercise. Free Radical Biol. Med. **31**, 1313–1322
19 Mikami, T., Yoshino, Y. and Ito, T. (2000) Does a relationship exist between the urate pool in the body and lipid peroxidation during exercise. Free Radical Res. **32**, 31–39
20 Waring, W. S., Webb, D. J. and Maxwell, S. R. J. (2001) Systemic uric acid administration increases serum antioxidant capacity in healthy volunteers. J. Cardiovasc. Pharmacol. **38**, 365–371
21 Waring, W. S., Webb, D. J. and Maxwell, S. R. (2002) Lithium carbonate as a potential pharmacological vehicle: intravenous kinetics of single-dose administration in healthy subjects. Eur. J. Clin. Pharmacol. **58**, 431–434
22 Pambianco, G., Wing, R. R. and Robertson, R. (1990) Accuracy and reliability of the Caltrac accelerometer for estimating energy expenditure. Med. Sci. Sports Exercise **22**, 858–862
23 O'Brien, E., Mee, F., Atkins, N. and Thomas, M. (1996) Evaluation of three devices for self-measurement of blood pressure according to the revised British Hypertension Society Protocol: the Omron HEM-705CP, Philips HP5332, and Nissei DS-175. Blood Press. Monit. **1**, 55–61
24 Thomas, S. H. (1992) Impedance cardiography using the Sramek–Bernstein method: accuracy and variability at rest and during exercise. Br. J. Clin. Pharmacol. **34**, 467–476
25 McLemore, J. L., Beeley, P., Thorton, K., Morrisroe, K., Blackwell, W. and Dasgupta, A. (1998) Rapid automated determination of lipid hydroperoxide concentrations and total antioxidant status of serum samples from patients infected with HIV. Am. J. Clin. Pathol. **109**, 268–273
26 Morrow, J. D. and Roberts, L. J. (1999) Mass spectrometric quantification of F_2-isoprostanes in biological fluids and tissues as measure of oxidative stress. Methods Enzymol. **300**, 3–12
27 Mastaloudis, A., Leonard, S. W. and Traber, M. G. (2001) Oxidative stress in athletes during extreme endurance exercise. Free Radical Biol. Med. **31**, 911–922
28 Maxwell, A. J. and Bruinsma, K. A. (2001) Uric acid is closely linked to vascular nitric oxide activity. Evidence for mechanism of association with cardiovascular disease. J. Am. Coll. Cardiol. **38**, 1850–1858
29 Delanty, N., Reilly, M. P., Pratico, D. et al. (1997) 8-epi $PGF_{2\alpha}$ generation during coronary reperfusion. A potential quantitative marker of oxidant stress *in vivo*. Circulation **95**, 2492–2499
30 De Scheerder, I., van de Kraay, A. M., Lamers, J. M., Koster, J. F. and de Jong, J. W. (1991) Myocardial malondialdehyde and uric acid release after short-lasting coronary occlusions during coronary angioplasty: potential mechanisms for free radical generation. Am. J. Cardiol. **68**, 392–395
31 Valkonen, M. M. and Kuusi, T. (2000) Vitamin C prevents the acute atherogenic effects of passive smoking. Free Radical Biol. Med. **28**, 428–436
32 Sampson, M. J., Gopaul, N., Davies, I. R., Hughes, D. A. and Carrier, M. J. (2002) Plasma F_2 isoprostanes: direct evidence of increased free radical damage during acute hyperglycemia in type 2 diabetes. Diabetes Care **25**, 537–541

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