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Does a Relationship Exist between the Urate Pool in the Body and Lipid Peroxidation During Exercise?

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In this study, we investigated whether a relationship exists between the levels of urate *in vivo* and lipid peroxidation during exercise. Seven healthy male subjects performed exhaustive cycling exercise under the following three conditions. The levels of urate, thiobarbituric acid reactive substances (TBARS) and allantoin in plasma and urine were examined before exercise and during a 3 h recovery period. (1) Benzbromarone administration experiment: benzbromarone (an uricosuric agent) was administered orally the day before exercise. (2) IMP administration experiment: inosine 5′-monophosphate disodium salt (a precursor of urate) was administered orally the day before exercise. (3) Control experiment: no test substance was administered. The main results obtained were as follows. Plasma urate levels and total peroxyl radical-trapping antioxidant parameter (TRAP) for deproteinized plasma in the resting period significantly decreased depending on the treatment, in the order of IMP > control > benzbromarone. A significant positive correlation was evident between plasma urate levels and TRAP values for deproteinized plasma. The increase in plasma levels of allantoin was observed only in the case of IMP treatment. A significant negative correlation between plasma levels of urate in the resting period and the amounts of urinary TBARS excreted during the recovery period was recognized. These results suggest that the urate level *in vivo* before exercise is a factor influencing lipid peroxidation during exhaustive exercise. Furthermore, these findings support the view that urate may serve as an important free-radical scavenger *in vivo*.

Keywords: Oxidative stress, exercise, urate, TBARS, TRAP, allantoin

INTRODUCTION

Urate has been identified as one of the major antioxidants present in plasma. Ames *et al.*[1] found that urate was as effective an antioxidant *in vitro* as ascorbate. Then, several *in vitro* experiments have demonstrated that urate inhibited some

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forms of oxygen free-radical-induced DNA damage,[2] lipid peroxidation,[3,4] and the oxidation of hemoglobin by nitrite.[5] Two mechanisms contributing to the antioxidant capacity of urate are thought to be as follows: (1) urate directly reacts with free radicals and is oxidized to allantoin, oxonic acid and parabanic acid;[6–8] (2) urate forms urate-Fe^{3+} complexes which serve to inhibit Fe^{3+}-catalyzed ascorbate oxidation without urate being oxidized.[9,10] However, no study has proved that urate is an effective antioxidant *in vivo*.

A number of reports indicate that exhaustive exercise leads to an increase in free-radical generation and lipid peroxidation *in vivo*. Davies *et al.*[11] detected the free radicals generated in muscle and liver during exercise using electron spin resonance. Lovlin *et al.*[12] found that a 26% increase in plasma thiobarbituric acid reactive substances (TBARS) was induced by maximal exercise at 100% of O_2 uptake (100% VO_2max). Sumida *et al.*[13] also found that acute exhaustive exercise resulted in a slight, but significant increase in serum TBARS concentration. Expired pentane and serum TBARS levels have been found to increase as a result of treadmill exercise at 90% VO_2max.[14] The intensity of exercise required to elicit an increase in lipid peroxidation during exercise is nearly 100% VO_2max and submaximal exercise (< 70% VO_2max) does not.[12] It has been reported that vitamin E deficiency increases lipid peroxidation,[15–17] whereas administration of a vitamin E supplement inhibits lipid peroxidation,[14] so that the amounts of antioxidants (vitamin E, vitamin C, etc.) *in vivo* may affect the amount of lipid peroxidation that occurs during exercise.

In the present study, to investigate whether the amount of urate *in vivo* influences lipid peroxidation under conditions of oxidative stress, we made human subjects perform exhaustive exercise as oxidative stress after administration of a uricosuric agent or a urate precursor in order to alter the amount of urate *in vivo*.

MATERIALS AND METHODS

Subjects

Seven healthy male subjects with mean age 28.7 ± 5.5 years, participated in this experiment. Their physical characteristics were as follows: body height 174.3 ± 6.0 cm, body weight 67.7 ± 9.1 kg, body fat percentage 17.4 ± 3.6%. The maximal oxygen uptake per kg body weight of the subjects was 61.6 ± 7.1 ml/min. The subjects were fully informed of the potential risks and discomfort associated with the experiment before giving informed consent to participate. The study was approved by the Ethics Committee of Nippon Medical School.

Experimental Protocol

The subjects performed exercise to exhaustion on a cycle ergometer at one week intervals under the following three experimental conditions. (1) benzbromarone administration experiment (benzbromarone experiment): 100 mg of benzbromarone was orally administered at supper time on the day before exercise and at breakfast on the day of the exercise. Benzbromarone, an agent for treatment of gout and hyperuricemia, facilitates the urinary excretion of urate by inhibiting urate reabsorption in the tubules of the kidney, which leads to a decrease in the urate pool in the body.[18] (2) IMP administration experiment (IMP experiment): 10 g of inosine 5′-monophosphate disodium salt (IMP) was orally administered at supper time on the day before exercise and at breakfast on the day of the exercise. IMP, a precursor of uric acid, administered orally is rapidly absorbed in the intestine, taken into the liver and metabolized to uric acid there, which leads to an increase in the urate pool in the body.[19] (3) control experiment: neither benzbromarone nor IMP was administered.

The subjects were instructed not to perform severe exercise on the day before the experiment,

and food was provided for supper on the day before exercise and breakfast on the day of the exercise. On the day of the experiment, the subjects did a complete bladder emptying and were required to drink 200 ml of distilled water 2 h before the exercise. Urine was collected 1 h before the exercise and the subjects were required to drink 200 ml of distilled water again. Just before the exercise, blood and urine were collected and the subjects performed exercise to exhaustion on a cycle ergometer by an incremental workload method. Immediately after the exercise, blood and urine were collected and the subjects were required to drink 200 ml of distilled water again. Thereafter, the subjects remained at rest for a 3 h recovery period. In the course of recovery from the exercise, blood and urine were collected at 30, 60, 120 and 180 min after exercise. The blood samples were immediately centrifuged, and the plasma and urine was stored at −80°C until analysis.

Analysis

Blood lactate levels were determined using a YSI 1500 Lactate Analyzer (Nitsukaki Bios, Tokyo). Urate levels in plasma and urine were determined using Uric Acid B-Test Wako (Wako Pure Chemical, Tokyo). The levels of lipid peroxidation products in plasma and urine were determined by spectrofluorometry according to the method of Yagi[20] and high performance liquid chromatography (HPLC) according to the method of Kosugi *et al.*,[21] respectively. In both methods, TBARS derived from the sample by heating the sample with thiobarbituric acid (TBA) under acidic conditions were quantitatively determined as lipid peroxidation products, so the level of lipid peroxidation products in plasma and urine were expressed as the amount of TBARS. Plasma allantoin levels were determined by HPLC according to the methods of Grootveld *et al.*[22] and Lagendijk *et al.*[23] Plasma α-tocopherol and ascorbate levels were determined by the method of Milne *et al.*[24] and Otsuka *et al.*,[25] respectively. The total peroxyl radical-trapping antioxidant parameter (TRAP) of deproteinized plasma in the resting period was determined according to the method of Alanko *et al.*[26] Briefly, 0.45 ml of 0.1 M phosphate-buffered saline (pH 7.4), 0.05 ml of 0.5 mg/ml luminol, and 0.02 ml of 120 mM linoleic acid were mixed in a cuvette. The cuvette was placed in a temperature-controlled sample carousel of a Lumi Counter-2500 (Microtec Nition Co., Tokyo) and incubated at 37°C for 5 min. The assay was initiated by adding 0.05 ml of 83 mg/ml 2,2′-azobis(2-amidinopropane)dihydrochloride (ABAP) and chemiluminescence in the cuvette at 37°C was monitored until the values become stable. Then, 0.02 ml of the deproteinized plasma or standard was directly injected into the cuvette, which extinguished the chemiluminescence. The duration of its extinction was measured and the peroxyl radical-trapping capacity was defined by the duration of extinction. The filtrates obtained by passing the plasma through an Amicon Microcon-3 filter (molecular weight cut-off 3000) were used as the samples of deproteinized plasma. A water-soluble tocopherol, Trolox (Aldrich, Tokyo), which is known to trap two radicals per molecule, was used as a standard. The hematocrit (Hct) was measured by a microcapillary method. Changes in plasma volume during exercise were calculated from the hematocrit levels using the formula described by Van Beaumont *et al.*[27] All plasma parameters were subsequently corrected for changes in plasma volume during exercise.

Statistical Analysis

All values are shown as mean ± SEM. The statistical significance of changes in parameter values during exercise and recovery was tested by analysis of variance (ANOVA) with repeated measure design. Statistical significance was accepted if $p < 0.05$.

RESULTS

Urate, allantoin, TBARS, ascorbate, and α-tocopherol levels in plasma and TRAP values for deproteinized plasma are presented in Table I. Plasma urate levels and TRAP values for deproteinized plasma in the resting period decreased depending on the treatment, in the order of IMP experiment > control experiment > benzbromarone experiment, and significant differences were seen among the three treatments ($p < 0.01$). However, comparing the plasma levels of allantoin, TBARS, ascorbate and α-tocopherol in the three experiments, no significant differences were observed. A significant positive correlation was evident between plasma urate levels and TRAP values for deproteinized plasma ($r = 0.988$, $p < 0.01$) (Figure 1).

The mean exercise time was 29.9 ± 0.8 min in the control experiment, 31.6 ± 1.0 min in the benzbromarone experiment, and 31.1 ± 1.2 min in the IMP experiment. The mean maximal heart rate was 194.0 ± 5.9 beats/min in the control experiment, 191.0 ± 5.4 beats/min in the benzbromarone experiment, and 183.3 ± 4.4 beats/min in the IMP experiment. There was no significant difference among the three experiments.

In all experiments, blood lactate levels increased significantly immediately after exercise compared with the resting level, however, there was no significant difference in the levels of blood lactate immediately after exercise (control experiment: rest, 0.9 ± 0.2 mmol/l, postexercise, 8.3 ± 0.5 mmol/l; benzbromarone experiment: rest, 1.0 ± 0.2 mmol/l, postexercise, 8.2 ± 0.4 mmol/l; IMP experiment: rest, 0.7 ± 0.1 mmol/l, postexercise, 7.6 ± 0.3 mmol/l). Furthermore, no significant difference in blood lactate levels among the three experiments was observed throughout the recovery period.

The changes in levels of plasma urate were expressed as the extent of increase or decrease in plasma concentration based on the resting level (Δvalues) because significant differences in the resting levels of plasma urate between the three experiments were shown due to administration of benzbromarone or IMP. Δplasma urate values are shown in Figure 2. In all experiments, a significant

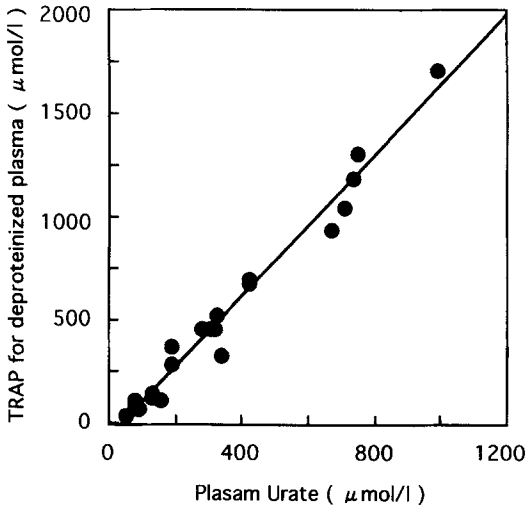

FIGURE 1 Comparison of TRAP for deproteinized plasma in the resting period and plasma urate concentration in the resting period. $n = 21$, $r = 0.988$, $p < 0.001$.

TABLE I Concentration (μmol/l) of urate, allantoin, TBARS, ascorbate, α-tocopherol in plasma and TRAP values for deproteinized plasma in the resting period before exercise

	Control	Benzbromarone	IMP
Urate	$268.4 \pm 21.7^{a,b}$	$115.9 \pm 24.7^{a,c}$	$673.4 \pm 75.7^{b,c}$
Allantoin	12.6 ± 0.8	11.6 ± 1.2	12.7 ± 1.5
TBARS	2.5 ± 0.2	2.4 ± 0.2	2.8 ± 0.1
Ascorbate	55.6 ± 7.5	53.0 ± 6.3	62.2 ± 8.2
α-tocopherol	30.4 ± 5.3	30.1 ± 9.3	27.9 ± 5.5
TRAP in deproteinized plasma	$408.7 \pm 32.6^{a,b}$	$95.2 \pm 13.4^{a,c}$	$1079.5 \pm 137.1^{b,c}$

Values were presented as mean $\pm$ SEM ($n = 7$). $^{a,b,c}p < 0.01$ significant between the same symbols.

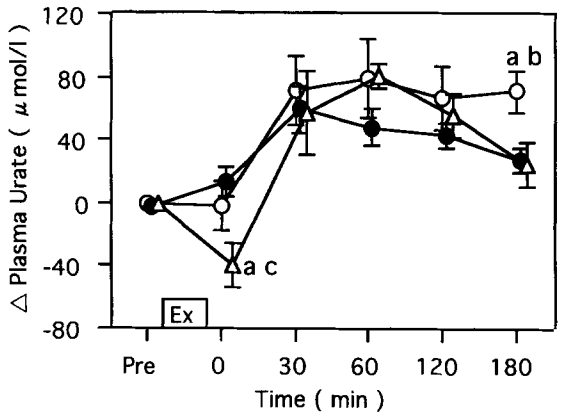

FIGURE 2 Changes in Δplasma urate levels during exercise and 3 h of recovery. Values were presented as mean ± SEM ($n = 7$). a: $p < 0.05$ significantly different between control and IMP. b: $p < 0.05$ significantly different between control and benzbromarone. c: $p < 0.05$ significantly different between benzbromarone and IMP. —○— Control, —●— Benzbromarone, —△— IMP.

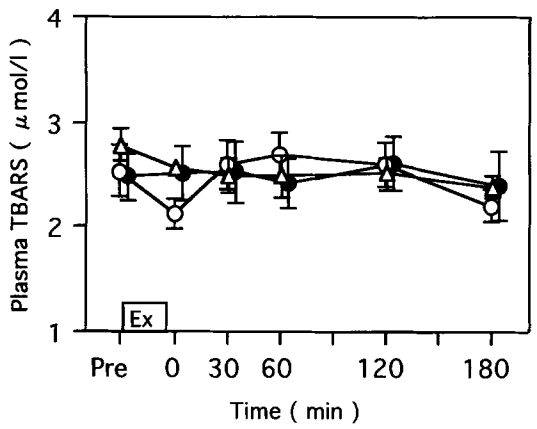

FIGURE 3 Changes in TBARS levels during exercise and 3 h of recovery. Values were presented as mean ± SEM ($n = 7$). —○— Control, —●— Benzbromarone, —△— IMP.

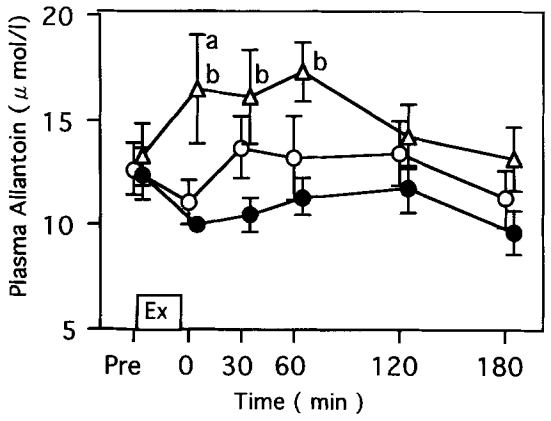

FIGURE 4 Changes in plasma allantoin levels during exercise and 3 h of recovery. Values were presented as mean ± SEM ($n = 7$). a: $p < 0.05$ significantly different between control and IMP. b: $p < 0.05$ significantly different benzbromarone and IMP. —○— Control, —●— Benzbromarone, —△— IMP.

increase was observed 30 min after exercise ($p < 0.01$), and the increase was maintained throughout the recovery period. A significant difference compared to the values in the control experiment was observed in the benzbromarone experiment and the IMP experiment only 3 h after exercise ($p < 0.01$).

In each of the experiments, no significant increase in plasma TBARS values compared with the resting levels was observed during the recovery period (Figure 3) and no significant difference between the three experiments was observed during the recovery period.

Plasma allantoin values in the IMP experiment was significantly higher at 0, 30 and 60 min during the recovery period than that in the control experiment and the benzbromarone experiment ($p < 0.05$) (Figure 4).

Urinary excretion of TBARS, expressed as the cumulative amount, in the IMP experiment was significantly lower than that in either the control experiment or the benzbromarone experiment after 3 h of recovery ($p < 0.01$) (Figure 5). However, no significant difference was observed between the values in the control experiment and in the benzbromarone experiment.

A significant negative correlation between the plasma urate level in the resting period and the cumulative urinary TBARS excretion during 3 h of recovery was observed (Figure 6), however, no significant correlation between the plasma urate level in the resting period and the plasma TBARS level at any time during the recovery period was observed (data not shown).

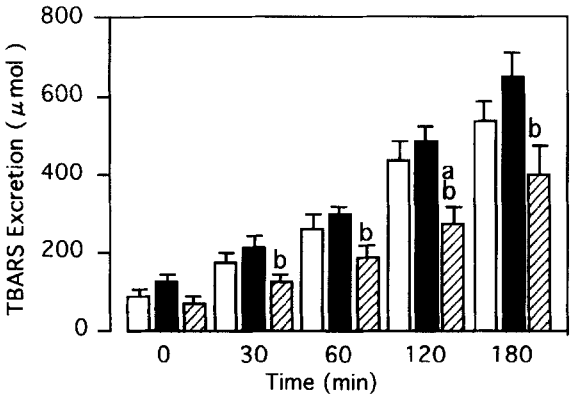

FIGURE 5 Changes in accumulation of urinary TBARS excretion during 3 h of recovery. Values were presented as mean ± SEM ($n = 7$). a: $p < 0.05$ significantly different between control and IMP. b: $p < 0.05$ significantly different benzbromarone and IMP. □ Control, ■ Benzbromarone, ▨ IMP.

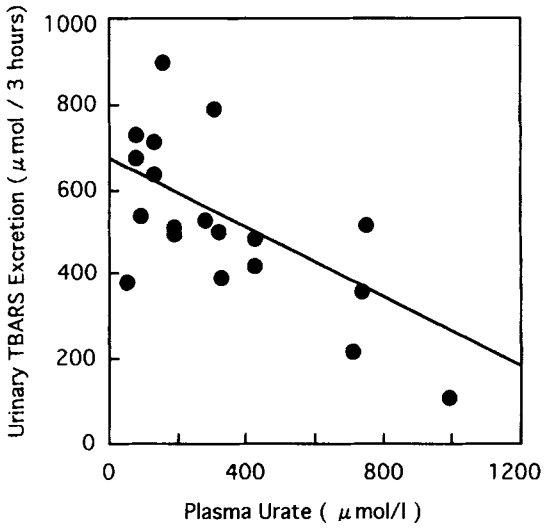

FIGURE 6 Comparison of cumulative urinary TBARS excretion during 3 h of recovery and plasma urate concentration in the resting period. $n = 21$, $r = 0.351$, $p < 0.05$.

DISCUSSION

Lovlin *et al.*[12] have reported that the intensity of exercise may influence the process of lipid peroxidation. Therefore, it was important in the present study that the subjects perform an equal intensity of exercise. No significant difference in the maximal heart rate during exercise, the mean exercise time to exhaustion, or the increase in blood lactate levels after exercise was observed among the three experiments, which suggests that the intensity of exercise for all subjects was approximately equal in the three experiments.

A significant positive correlation between plasma urate levels and the TRAP of deproteinized plasma in the resting period was recognized (Figure 1). Vasankari *et al.*[28] have reported that plasma TRAP values increase after 4 weeks of antioxidant supplementation. On the contrary, Maxwell *et al.*[29] have reported that there is no significant increase in TRAP despite supplementation of the diet with antioxidant vitamins. However, plasma TRAP values have been shown to increase after exercise independent of supplementation of the diet with antioxidant vitamins,[28–30] and this increase may be due to an increase in plasma levels of urate[29] or α-tocopherol.[28,30] In the present study, the plasma α-tocopherol and ascorbate levels was equal in the three experiments, whereas plasma urate levels differed significantly (Table I), so the difference in TRAP values may be due to the difference in urate levels. It seems likely that the antioxidant capacity of the subjects prior to exercise may have differed substantially among the three experimental conditions.

In most of the previous studies examining lipid peroxidation during exercise, serum or plasma TBARS levels were measured as an indicator of *in vivo* oxidation by free radicals. However, the substances which react with TBA are not unique to serum and plasma samples, so it is doubtful that TBARS values really reflect *in vivo* lipid peroxidation.[31] A number of studies in which TBARS were determined during exercise have been reported previously, but the results were inconsistent. Kanter *et al.*[14] reported that expired pentane and serum TBARS increased as a result of treadmill exercise at 90% VO_2max. Lovlin *et al.*[12] found a 26% increase in plasma TBARS induced by maximal exercise at 100% VO_2max. Sumida *et al.*[13] also found that acute exhaustive exercise

resulted in a slight, but significant increase in serum TBARS concentration. On the other hand, Viinikka *et al.*[32] have found that serum lipid peroxide levels did not change during a period of maximal bicycle ergometer exercise. Rokitzki *et al.*[33] reported that the serum TBARS concentration decreased after a marathon.

Urinary TBARS has been also used as an indicator of *in vivo* oxidative stress by free radicals. The TBA reaction of human urine produces the TBARS other than malonaldehyde derivatives,[21,34] so HPLC separation of TBARS is necessary for accurate quantitation of urinary TBARS that appears to reflect lipid peroxidation products *in vivo*.[21] Urinary TBARS measured by the HPLC method used in the present study was mainly due to malonaldehyde derivatives[21] because aldehydes other than malonaldehyde derivatives in the reaction solution are separated by HPLC, so the specificity of the values obtained by the HPLC method is greater in the case of urine than that in the case of serum or plasma.[21] On the other hand, malonaldehyde is derived from not only lipid peroxidation products but also 2-deoxyribose,[34,35] so TBARS does not always reflect lipid peroxidation products. However, several previous studies have suggested that fasting,[17] feeding a vitamin E deficient diet,[15–17] streptozotocin-induced diabetes[36] and exposure to diesel engine exhaust[37] resulted in an increase in the urinary excretion of TBARS. Also, Jenkins *et al.*[38] reported that urinary TBARS excretion increased to 160% after a 2 h of exhaustive treadmill run. These previous observations suggest that induction of oxidative stress *in vivo* may lead to an increase in urinary TBARS excretion and that urinary TBARS excretion may be an indicator which reflects *in vivo* lipid peroxidation to a certain extent.

Lower urinary TBARS excretion in the IMP experiment (Figure 5) and a significant negative correlation between the plasma urate level in the resting period and the cumulative urinary TBARS excretion during 3 h of recovery (Figure 6) suggest that lipid peroxidation during exercise might be inhibited by the urate that increased upon administration of IMP. Thus, urate may play an important role *in vivo* as a radical scavenger.

When urate is oxidized *in vitro* by free radicals, several oxidation products are formed. Among these products, allantoin is the major product.[7] Humans lack uricase, which converts urate to allantoin, but allantoin is found in human body fluids. Grootveld *et al.*[22] reported that the serum urate concentration in rheumatoid patients does not significantly differ from that in healthy persons, whereas the serum allantoin concentration is higher than normal. Ogihara *et al.*[39] also reported that the plasma allantoin concentration is markedly elevated in patients with Wilson's disease. These authors suggest that urate may be non-enzymatically oxidized *in vivo* to allantoin by free radicals and that allantoin may be a useful marker of free-radical reactions taking place *in vivo*.[22,39] Furthermore, exhaustive cycling exercise has been shown to result in a significant increase in allantoin levels in muscle and plasma.[40] In the present study, the resting level of plasma allantoin did not differ among the three experiments in spite of the significant difference in plasma urate levels, and the plasma allantoin levels increased only in the IMP experiment after exercise (Figure 4). Considering that the urate levels increased upon administration of IMP, it seems likely that there may have been more urate available to react with free radicals produced during exercise. That might result in scavenging of more radicals by urate in the IMP experiment, the inhibition of the lipid peroxidation and the decrease in urinary TBARS excretion.

A significant difference in TRAP levels for deproteinized plasma was observed comparing the benzbromarone experiment and the control experiment, but the plasma allantoin levels and urinary TBARS excretion after exercise were not different between these experiments. These findings suggest that the *in vitro* antioxidant capacity of urate was not reflected precisely *in vivo*. The reason seems to be that the antioxidant capacity *in vitro* was measured as the capacity for

scavenging a particular radical in the reaction solution whereas the *in vivo* values reflect the total results for antioxidant reactions in cells or tissues involving several types of radicals. Therefore, further investigation of the relationship between urate levels, antioxidant capacity, and oxidation products in cells or tissue may be necessary.

In summary, the present study provides for the first time evidence indicating that increased urate levels *in vivo* are associated with a decrease in the levels of oxidation products during exhaustive exercise. These findings support the view that urate may serve as an important scavenger *in vivo*.

Acknowledgments

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References

[1] B.N. Ames, R. Cathcart, E. Schwiers and P. Hochstein (1981). Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: a hypothesis. *Proceedings of the National Academy of Sciences USA*, **78**, 6858–6862.

[2] A.M. Cohen, R.E. Aberdroth and P. Hochstein (1984). Inhibition of free radical-induced DNA damage by uric acid. *FEBS Letters*, **174**, 147–150.

[3] J. Meadows, R.C. Smith and J. Reeves (1986). Uric acid protects membranes and linolenic acid from ozone-induced oxidation. *Biochemical and Biophysical Research Communications*, **137**, 536–541.

[4] R.C. Smith and L. Lawing (1983). Antioxidant activity of uric acid and 3-N-ribosyluric acid with unsaturated fatty acids and erythrocyte membranes. *Archives of Biochemistry and Biophysics*, **223**, 166–172.

[5] J. Meadows and R.C. Smith (1986). Uric acid protection of nucleobases from ozone-induced degradation. *Archives of Biochemistry and Biophysics*, **246**, 838–845.

[6] M. Hicks, L.S. Wong and R.O. Day (1993). Identification of products from oxidation of uric acid induced by hydroxyl radicals. *Free Radical Research Communications*, **18**, 337–351.

[7] H. Kaur and B. Halliwell (1990). Action of biologically-relevant oxidizing species upon uric acid. Identification of uric acid oxidation products. *Chemico-Biological Interactions*, **73**, 235–247.

[8] K.R. Maples and R.P. Mason (1988). Free radical metabolite of uric acid. *Journal of Biological Chemistry*, **263**, 1709–1712.

[9] A. Sevanian, K.J. Davies and P. Hochstein (1991). Serum urate as an antioxidant for ascorbic acid (Review). *American Journal of Clinical Nutrition*, **54**, 1129S–1134S.

[10] K.J. Davies, A. Sevanian, S.F. Muakkassah-Kelly and P. Hochstein (1986). Uric acid–iron ion complexes. A new aspect of the antioxidant functions of uric acid. *Biochemical Journal*, **235**, 747–754.

[11] K.J. Davies, A.T. Quintanilha, G.A. Brooks and L. Packer (1982). Free radicals and tissue damage produced by exercise. *Proceedings of the National Academy of Sciences USA*, **107**, 1198–1205.

[12] R. Lovlin, W. Cottle, I. Pyke, M. Kavanagh and A.N. Bekcastro (1987). Are indices of free radical damage related to exercise intensity. *European Journal of Applied Physiology and Occupational Physiology*, **56**, 313–316.

[13] S. Sumida, K. Kiyohi, H. Kitao and F. Nakadomo (1989). Exercise-induced lipid peroxidation and leakage of enzymes before and after vitamin E supplementation. *International Journal of Biochemistry*, **8**, 835-838.

[14] M.M. Kanter, L.A. Nolte and J.O. Holloszy (1993). Effects of an antioxidant vitamin mixture on lipid peroxidation at rest and postexercise. *Journal of Applied Physiology*, **74**, 965–969.

[15] H.S. Lee, D.W. Shoeman and A.S. Csallany (1992). Urinary response to *in vivo* lipid peroxidation induced by vitamin E deficiency. *Lipids*, **27**, 124–128.

[16] H.H. Draper, L. Polensek, M. Hadley and L.G. McGirr (1984). Urinary malondialdehyde as an indicator of lipid peroxidation in the diet and in the tissues. *Lipids*, **19**, 836–843.

[17] S.N. Dhanakoti and H.H. Draper (1987). Response of urinary malondialdehyde to factors that stimulate lipid peroxidation *in vivo*. *Lipids*, **22**, 643–646.

[18] A.K. Jain, J.R. Ryan, F.G. McMahon and R.J. Noveck (1974). Effect of single oral doses of benzbromarone on serum and urinary uric acid. *Arthritis and Rheumatism*, **17**, 149–157.

[19] A. Griebsch and N. Zollner (1974). Effect of ribomononucleotides given orally on uric acid production in man. *Advances in Experimental Medicine and Biology*, **41**, 443–449.

[20] K. Yagi (1984). Assay for blood plasma or serum. *Methods in Enzymology*, **105**, 328–331.

[21] H. Kosugi, T. Kojima and K. Kikugawa (1993). Characteristics of the thiobarbituric acid reactivity of human urine as a possible consequence of lipid peroxidation. *Lipids*, **28**, 337–343.

[22] M. Grootveld and B. Halliwell (1987). Measurement of allantoin and uric acid in human body fluids. A potential index of free-radical reactions *in vivo*? *Biochemical Journal*, **243**, 803–808.

[23] J. Lagendijk, J.B. Ubbink and W.J.H. Vermaak (1995). The determination of allantoin, a possible indicator of oxidant status, in human plasma. *Journal of Chromatographic Science*, **33**, 186–193.

[24] D.B. Milne and 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.

[25] M. Otsuka, T. Kurata and N. Arakawa (1986). Antiscorbutic effect of dehydro-L-ascorbic acid in vitamin C-deficient guinea pigs. *Journal of Nutritional Science and Vitaminology*, **32**, 183–190.

[26] J. Alanko, A. Riutta, I. Mucha, H. Vapaatalo and T. Metsa-Ketela (1993). Modulation of arachidonic acid metabolism by phenols: relation to positions of hydroxyl

groups and peroxyl radical scavenging properties. *Free Radical Biology and Medicine*, **14**, 19–25.
[27] W. Van Beaumont, J.E. Greenleaf and L. Juhos (1972). Disproportional changes in hematocrit, plasma volume, and proteins during exercise and bed rest. *Journal of Applied Physiology*, **33**, 55–61.
[28] T.J. Vasankari, U.M. Kujala, T.M. Vasankari, T. Vuorimaa and M. Ahotupa (1997). Increased serum and low-density-lipoprotein antioxidant potential after antioxidant supplementation in endurance athletes. *American Journal of Clinical Nutrition*, **65**, 1052–1056.
[29] S.R. Maxwell, P. Jakeman, H. Thomason, C. Leguen and G.H. Thorpe (1993). Changes in plasma antioxidant status during eccentric exercise and the effect of vitamin supplementation. *Free Radical Research Communications*, **19**, 191–202.
[30] T.J. Vasankari, U.M. Kujala, T.M. Vasankari, T. Vuorimaa and M. Ahotupa (1997). Effects of acute prolonged exercise on serum and LDL oxidation and antioxidant defences. *Free Radical Biology and Medicine*, **22**, 509–513.
[31] T. Kojima, K. Kikugawa and H. Kosugi (1990). Is the thiobarbituric acid-reactivity of blood plasma specific to lipid peroxidation? *Chemical and Pharmaceutical Bulletin*, **38**, 3414–3418.
[32] L. Viinikka, J. Vuori and O. Ylikorkala (1984). Lipid peroxides, prostacyclin, and thromboxane A2 in runners during acute exercise. *Medicine and Science in Sports and Exercise*, **16**, 275–277.
[33] L. Rokitzki, E. Logemann, A.N. Sagredos, M. Murphy, W. Wetzel-Roth and J. Keul (1994). Lipid peroxidation and antioxidative vitamins under extreme endurance stress. *Acta Physiologica Scandinavica*, **151**, 149–158.
[34] J. Gutteridge and T. Tickner (1978). The characterisation of thiobarbituric acid reactivity in human plasma and urine. *Analytical Biochemistry*, **91**, 250–257.
[35] B. Halliwell and J. Gutteridge (1981). Formation of thiobarbituric-acid-reactive substance from deoxyribose in the presence of iron salts: the role of superoxide and hydroxyl radicals. *FEBS Letters*, **128**, 347–352.
[36] D.D. Gallaher, A.S. Csallany, D.W. Shoeman and J.M. Olson (1993). Diabetes increases excretion of urinary malonaldehyde conjugates in rats. *Lipids*, **28**, 663–666.
[37] H. Seto, T. Suzuki, T. Ohkubo and T. Kanoh (1990). Increased urinary excretion of 2-thiobarbituric acid reactants in rats exposed to diesel engine exhaust. *Bulletin of Environmental Contamination and Toxicology*, **45**, 582–589.
[38] R.R. Jenkins, K. Krause and L.S. Schofield (1993). Influence of exercise on clearance of oxidant stress products and loosely bound iron. *Medicine and Science in Sports and Exercise*, **25**, 213–217.
[39] H. Ogihara, T. Ogihara, M. Miki, H. Yasuda and M. Mino (1995). Plasma copper and antioxidant status in Wilson's disease. *Pediatric Research*, **37**, 219–226.
[40] Y. Hellsten, P.C. Tullson, E.A. Richter and J. Bangsbo (1997). Oxidation of urate in human skeletal muscle during exercise. *Free Radical Biology and Medicine*, **22**, 169–174.