Free Rad. Res., Vol. 27, pp. 607–618
Reprints available directly from the publisher
Photocopying permitted by license only

Effect of a Single Bout of Exercise and β-Carotene Supplementation on the Urinary Excretion of 8-Hydroxy-deoxyguanosine in Humans

SATOSHI SUMIDA[a,*], TATSUYA DOI[b], MASAO SAKURAI[b], YASUYUKI YOSHIOKA[b] and KOJI OKAMURA[b]

[a]*Laboratory of Exercise Physiology and Biochemistry, Osaka Gakuin University, 2-36-1 Kishibe-minami, Suita, Osaka 564; and*
[b]*Saga Research Institute, Otsuka Pharmaceutical Co., Ltd. Higashisefuri-son, Kanzaki-gun, Saga 842-01, Japan*

Accepted by Prof. B. Halliwell

(Received 10 July 1997)

We investigated the effects of acute exhaustive exercise and β-carotene supplementation on urinary 8-hydroxy-deoxyguanosine (8-OHdG) excretion in healthy nonsmoking men. Fourteen untrained male (19–22 years old) volunteers participated in a double blind design. The subjects were randomly assigned to either the β-carotene or placebo supplement group. Eight subjects were given 30 mg of β-carotene per day for 1 month, while six subjects were given a placebo for the same period. All subjects performed incremental exercise to exhaustion on a bicycle ergometer both before and after the 1-month β-carotene supplementation period. The blood lactate and pyruvate concentrations significantly increased immediately after exercise in both groups. The baseline plasma β-carotene concentration was significantly 17-fold higher after β-carotene supplementation. The plasma β-carotene decreased immediately after both trials of exercise, suggesting that β-carotene may contribute to the protection of the increasing oxidative stress during exercise. Both plasma hypoxanthine and xanthine increased immediately after exercise before and after supplementation. This thus suggests that both trials of exercise might enhance the oxidative stress. The 24-h urinary excretion of 8-OHdG was unchanged for 3 days after exercise before and after supplementation in both groups. However, the baseline urinary excretion of 8-OHdG before exercise tended to be lower after β-carotene supplementation. These results thus suggest that a single bout of incremental exercise does not induce the oxidative DNA damage, while β-carotene supplementation may attenuate it.

Keywords: Exercise, β-Carotene, Urine, 8-Hydroxy-deoxyguanosine, DNA damage

INTRODUCTION

8-Hydroxy-deoxyguanosine (8-OHdG) has been recognized to be a biomarker of oxidative DNA damage by oxygen radicals generated endogenously.[1,2] The excretion of 8-OHdG in urine reflects the integrated rate of oxidative DNA damage and the repair of DNA in the whole body.[2–4] The urinary 8-OHdG may thus result from oxidation of DNA and oxidation of deoxyguanosinetriphosphate (dGTP) in the cellular nucleotide pool.[5] Since oxidized dGTP is a mutagenic substrate for DNA synthesis, the formation of 8-OHdG occurs in DNA.[6] Oxygen radicals are

* Corresponding author. Tel.: +81-6-381-8434. Fax: +81-6-382-4363. E-mail: sumida@center.osaka-gu.ac.jp.

generated through the normal oxygen metabolism. An increase in oxygen consumption usually results in the formation of oxygen radicals from mitochondrial electron transport chains.[7] A previous study reported a close association between oxidative DNA damage as assessed by the urinary excretion of 8-oxo-7,8-dihydro-2′-deoxyguanosine and oxygen consumption in humans.[8]

Exercise-induced oxidative stress is involved in the generation of oxygen radicals by increasing the efflux of oxygen through the mitochondria during exercise as well as the activation of xanthine oxidase or the activation of neutrophils.[9–12] Hypoxanthine and xanthine are substrates of xanthine oxidase. The increase in these substrates after intensive exercise is associated with an increase in oxidative stress. Numerous studies have demonstrated that exercise induced oxidative damage to the cellular molecules by the production of oxygen radicals.[13–15] However, it is still not clear whether acute exercise induces oxidative damage to DNA. Several studies have shown that the ratio of urinary 8-OHdG to creatinine excretion increased in runners after a marathon race[16] as well as in runners and swimmers after a single bout of normal exercise.[17] In contrast, no changes were observed in the excretion of urinary 8-hydroxyguanosine, as a biomarker for oxidative damage to RNA, for 3 days after 90 minutes of moderate exercise.[18]

β-Carotene, an antioxidant which has been shown to be an efficient quencher of singlet oxygen, plays an important role in protecting against the oxidation of cell components by oxygen radicals.[19] In an early epidemiologic study, the dietary intake of β-carotene was found to reduce the risk for cancer.[20] Recently, however, epidemiological studies have reported that a high dietary intake of β-carotene did not decrease the incidence of lung cancer in cigarette smokers.[21–23] The protective effect of antioxidant vitamin supplements containing β-carotene against exercise-induced lipid peroxidation as estimated by exhaled pentane and thiobarbituric acid reactive substances has also been demonstrated in human serum.[24] However, it has also been reported that the urinary excretion of 8-hydroxyguanosine, an oxidatively damaged RNA base in humans, was not affected by 1-month supplementation with vitamin E, vitamin C or β-carotene.[25]

The main purpose of this study was thus to determine if acute exhaustive exercise would influence urinary 8-OHdG as an indicator of oxidative DNA damage and if the supplementation of β-carotene the would influence the effect of a single bout of exhaustive exercise on this indicator. The subjects underwent a single bout of exhaustive exercise both before and after double-blind supplementation for 1 month. The excretion of urinary 8-OHdG 1 day before and 1–3 days after exercise was determined.

MATERIALS AND METHODS

Human Subjects

Fourteen nonsmoking male university students (19–22 years old) volunteered to participate in this study. All subjects gave their informed consent. The procedures for this study conformed to the guidelines of the Declaration of Helsinki for research on human subjects. The subjects were in good health, and took no medications, vitamins or minerals. In addition, none had participated in any specific exercise training for a period of 1–3 yrs. The subject characteristics before exercise and their performance during exercise are shown in Table I.

Experimental Design

The subjects were randomly assigned by a double blind design to either a β-carotene supplemented group or a placebo group. The β-carotene supplemented subjects consumed three capsules (10 mg of β-carotene in one capsule) per day (Nihon

TABLE I Characteristics of the subjects and their performance during maximal exercise before and after the 1-month β-carotene supplementation

	n	Age (years)	Body weight (kg)	BMI (kg/m^2)	VO_2 peak (ml/kg/min)	HR peak (beats/min)	Endurance Time (min)
Exercise 1							
β-Carotene	8	19.9 ± 0.4	58.1 ± 2.9	20.7 ± 1.2	38.9 ± 2.2	175.4 ± 3.8	27.7 ± 1.0
Placebo	6	19.8 ± 0.3	61.9 ± 3.8	20.8 ± 0.9	37.5 ± 2.2	183.0 ± 5.0	28.5 ± 1.8
Exercise 2							
β-Carotene	8	19.9 ± 0.4	58.0 ± 2.7	20.7 ± 1.1	43.1 ± 2.1*	185.5 ± 2.5	29.4 ± 1.2*
Placebo	6	19.8 ± 0.3	61.3 ± 4.1	20.8 ± 0.9	43.5 ± 1.6*	191.0 ± 3.1	30.8 ± 2.1*

Values are the means ± SE. n, number of subjects; BMI, Body mass index; VO_2 peak, peak of O_2 consumption; HR peak, peak of heart rate; Exercise 1, the exercise trial before β-carotene supplementation; Exercise 2, the exercise trial after β-carotene supplementation. * Significantly different from Exercise 1 in the same group. ($P < 0.05$).

Roche, Inc., Tokyo Japan). The placebo subjects consumed 30 mg of lactose per day. The capsules were taken for 33 days from the day of the first exercise trial (Exercise 1) to day 3 after the second exercise trial (Exercise 2). The subjects were all asked to maintain their normal eating habits during the study period, and were also instructed to refrain from exercise for 2 days before the exercise trial. On the day of the exercise test, the subjects arrived in the laboratory after a 12-h overnight fast. Blood samples were drawn from the antecubital vein at rest in a sitting position for 15–20 min, as well as immediately, at 24, 48, and 72 h after exercise into heparinized tubes. After centrifugation, the plasma was immediately stored at –80°C. For the lactate and pyruvate measurements, perchloric acid was added to the blood immediately. After centrifugation, an aliquot of the supernatant was stored at –80°C.

The subjects performed incremental exercise until exhaustion using an electrically-braked bicycle ergometer (Combi 232 C, Tokyo, Japan) at a load increasing every 3 min by 20 W and 60 rpm after a warm-up which consisted of 4 min at 20 W. The oxygen uptake was analyzed using an Aeromonitor AE-280 S (Minato Medical Science, Osaka, Japan). This system consisted of a microcomputer, a hot-wire respiratory flowmeter, and oxygen and carbon dioxide gas analyzers. Peak O_2 consumption (VO_2 peak) was defined as the highest VO_2. The heart rate (HR) was recorded continuously during exercise by an electrocardiogram recorder (Bioview E, NEC, Osaka, Japan). The Borg scale of perceived exertion was used both during exercise and immediately after the completion of exercise. The subjects repeated the exhaustive exercise trial after 30 days of either β-carotene or placebo supplementation.

Urine Collection

The 24-h urine samples were collected on the day before exercise, and on days 1 (the day of exercise), 2 and 3 after exercise. The urine samples were collected separately in plastic bottles. The volumes of the urine samples were measured and aliquots were stored at –80°C.

Measurement of Lactate and Pyruvate in the Blood

To 1 ml of blood, 1 ml of 0.8 N perchloric acid was added immediately after blood sampling. After centrifugation at 3,000 rpm for 10 min, the supernatant was stored at –80°C until the lactate and pyruvate assays. The blood lactate and pyru-

vate concentrations were measured using an enzymatic assay kit (Determiner LA, Determiner PA, Kyowa Medex, Tokyo, Japan).

Measurement of β-Carotene in the Plasma

The all-*trans*-β-carotene concentration in the plasma was measured by a high-performance liquid chromatography (HPLC) with UV detection as described by Miller and Yang.[26]

Measurement of 8-OHdG in the Urine

The concentration of urinary 8-OHdG was determined by a modification of the method of Loft *et al.*[2] The amount of 8-OHdG was quantitatively determined using the standard described by Kasai and Nishimura.[1] The measurement were carried out using HPLC (L-6200 pump, AS-2000 automatic injector, L-5030 column oven, Hitachi, Tokyo, Japan). Equipment was connected with a UV detector (L-4250, Hitachi, Tokyo, Japan) and an electrochemical detector (ECD; ECD-100, Eicom, Kyoto, Japan). Briefly, the aliquots of a 24 hour urine sample were mixed and centrifuged. One milliliter of urine supernatant was added to 1 ml of 1 M Tris-HCl buffer pH 7.9. After filtration through a 0.45 μm pore-size nylon filter (Ekicrodisc Acro LC, German Science, Tokyo, Japan), a 50 μl aliquot was injected into the HPLC-ECD. The automatic six-port valve switched the effluent from the LiChrospher 100 RP-18 column (4.0 mm I.D. × 250 mm, 4.0 mm I.D. × 4 mm as a guard column, Merck, Darmstadt, Germany) to the ion exchange resin 2619F packed column (4.0 mm I.D. × 10 mm, Hitachi, Tokyo, Japan), and a LiChrospher 100 RP-18 column (4.0 mm I.D. × 75 mm) with 0.01 M borate buffer pH 7.9/acetonitrile/methanol (96:2.5:1.5, v/v) containing EDTA (5 μg/ml) at a flow rate of 1.0 ml/min. The retained effluent was eluted further on a LiChrospher 100 RP-18 column (4.0 mm I.D. × 250 mm, Merck, Darmstadt, Germany) with 0.1 M phosphate buffer pH 2.2/acetonitrile/methanol (96:2.5:1.5, v/v) containing EDTA (5 μg/ml) at a flow rate of 1.0 ml/min. The column temperature was kept at 30°C. The eluant containing 8-OHdG was detected by an electrochemical detector at a potential of 750 mV. A chromato-integrator (D-2500, Hitachi, Tokyo, Japan) was used for peak integration.

Measurement of Hypoxanthine, Xanthine, Uric Acid in the Plasma

The hypoxanthine, xanthine and uric acid concentrations in the plasma were measured by a modification of the HPLC methods described by Wung and Howell.[27] Briefly, 50 μl of 0.6 mM allopurinol solution containing 12 mM EDTA was added to 500 μl of plasma as an internal standard. After adding 50 μl of 4.8 M perchloric acid, the mixture was vigorously mixed. After centrifugation at 12,000 rpm for 3 min, 55 μl of 4.0 M potassium hydroxide and 1.0 M potassium phosphate solution was added to 400 μl of the supernatant. After mixing, an aliquot of 50 μl was injected into the HPLC. The HPLC procedure was then carried out using a L-6200 pump, AS-2000 autoinjector, and L-4500 photodiode array detector (Hitachi, Tokyo, Japan). The detector was set at 268 nm for xanthine and uric acid analysis, and at 250 nm for the hypoxanthine analysis. A TSKgel ODS-80TM (4.6 mm I.D. × 250 mm, Tosoh, Tokyo, Japan) column was used with a mobile phase composed of 10 mM potassium phosphate at a flow rate of 1.2 ml/min.

Measurement of Creatinine in the Urine

The creatinine concentration in the samples was measured using a commercially available kit (Creatinine-HR test, Wako, Osaka, Japan) based on the Jaffe reaction.

Statistical Analysis

The values are expressed as the means ± SE. The values in the plasma samples are adjusted

for the percent change in the plasma volume after exercise.[28] The significance of the differences between the means was analyzed by two-way repeated-measures analysis of variance or one-way repeated-measures analysis of variance and a Bonferroni post hoc test. The Student's paired t test was used to evaluate the differences of the mean blood lactate, pyruvate, characteristics of subjects and performance during exercise. The lowest level of significance was set at $P < 0.05$ for all statistical tests.

RESULTS

As shown in Table I, there was no significant difference in the body weight and the body mass index between the Exercise 1 and Exercise 2. The VO_2 peak and endurance time of exercise were significantly ($P < 0.05$) higher for the Exercise 2 in both groups. The subjects did not exercise-trained during the 30 day period between the Exercise 1 and 2. The increase in the endurance performance observed in the Exercise 2 may be due to the experience of the bicycle ergometer exercise test. The blood lactate and pyruvate concentrations significantly (blood lactate: $P < 0.001$, blood pyruvate: $P < 0.001$ and $P < 0.05$) increased immediately after both exercise trials in both groups (Table II). The rate of the increase in the blood lactate after exercise tended to be slightly higher in the Exercise 2. The blood pyruvate concentrations also were similar immediately after exercise in both groups.

The baseline plasma β-carotene concentration was significantly higher after β-carotene supplementation; 6.23 ± 0.66 vs. 0.36 ± 0.06 μmol/l ($P < 0.001$) (Fig. 1). The plasma β-carotene concentrations in the β-carotene supplemented group were significantly higher 48 h ($P < 0.01$) and 72 h ($P < 0.01$) after the Exercise 1 than the baseline values. These significant differences could be due to the fact that the supplementation of β-carotene was started on the day of exercise. The plasma β-carotene concentrations decreased significantly ($P < 0.01$ and $P < 0.05$) immediately after exercise of the Exercise 2 in both groups, and the Exercise 1 in the placebo group.

The urinary 8-OHdG to creatinine excretion ratio is presented in Figure 2. There was no significant difference in the urinary excretion of creatinine among all 24-h samples. The urinary excretion of 8-OHdG was unchanged for 3 days after both exercise trials in β-carotene supplemented and placebo groups. In β-carotene supplemented group compared with placebo group, the excretion of urinary 8-OHdG was lower, but no significant differences were found before and after supplementation. Moreover, there was no significant difference in the baseline excretion of urinary 8-OHdG between before and after supplementation in β-carotene supplemented group, although the baseline urinary excretion of

TABLE II Blood lactate and pyruvate concentrations at rest and immediately after exercise

	n	Exercise 1		Exercise 2	
		Rest	Immediately After	Rest	Immediately After
Blood lactate (mM)					
β-Carotene	8	0.91 ± 0.09	7.69 ± 0.56§	0.91 ± 0.06	8.84 ± 0.65§
Placebo	6	1.39 ± 0.33	6.92 ± 0.70§	1.27 ± 0.17	8.07 ± 0.52§
Blood pyruvate (μM)					
β-Carotene	8	56.2 ± 5.0	175.9 ± 10.9§	54.1 ± 3.80	163.1 ± 12.6§
Placebo	6	75.5 ± 12.2	171.7 ± 13.6§	77.2 ± 22.2	150.3 ± 5.80*

Values are the means ± SE. n, number of subjects. Significantly different from rest in the same supplement group, § $P < 0.001$, * $P < 0.05$.

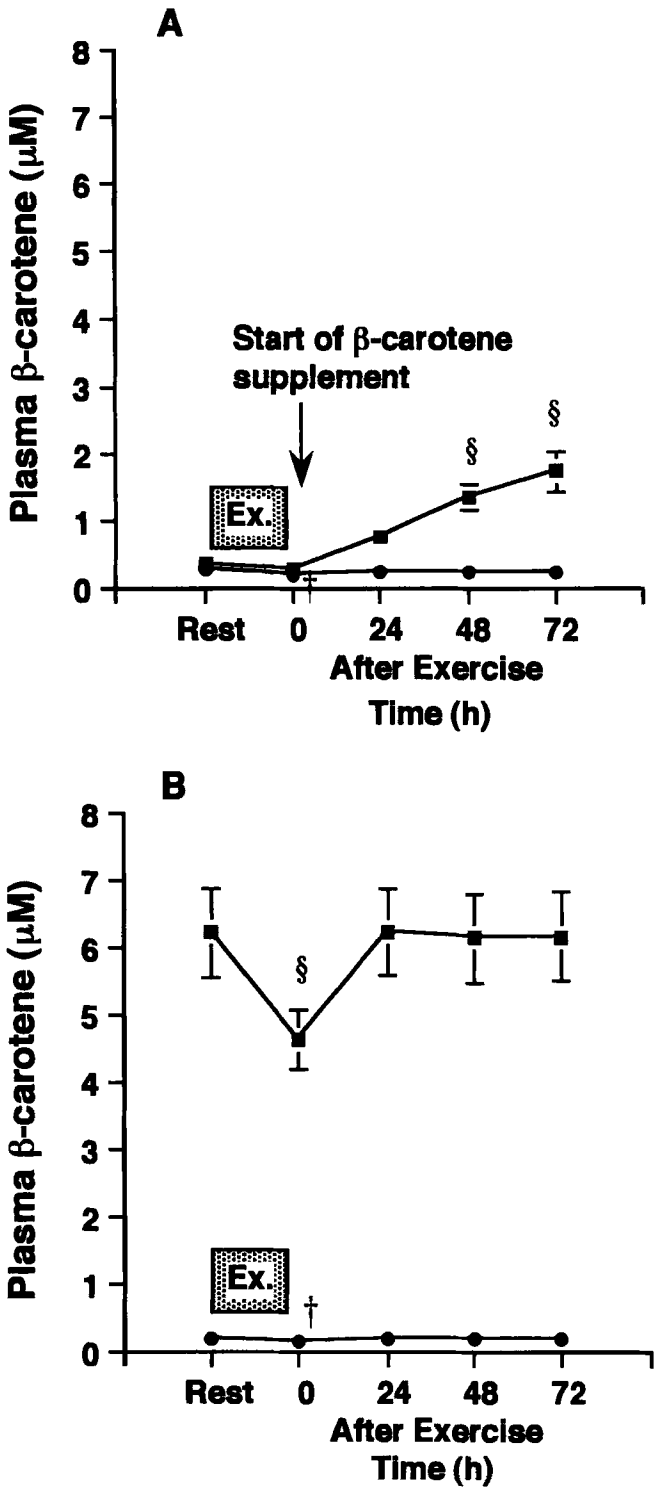

FIGURE 1 Changes in the plasma β-carotene concentration before and after exercise pre- and post-supplementation. The values are the means ± SE. **A:** pre-supplementation, **B:** post-supplementation. The β-carotene supplemented group (■, n = 8), The placebo group (●, n = 6). Ex.: incremental exercise to exhaustion. † $P < 0.01$, § $P < 0.001$ compared with the values at rest in the same supplement group.

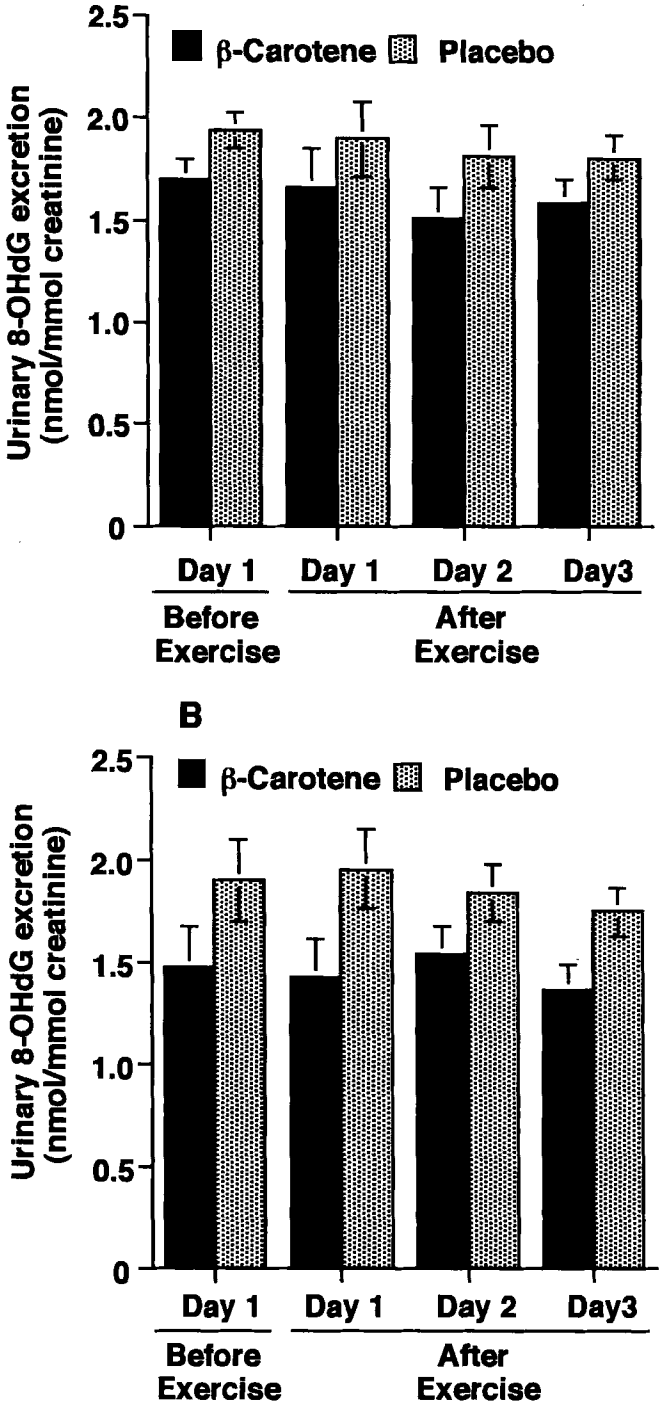

FIGURE 2 The urinary excretion of 8-OHdG from 1 day before exercise to day 3 postexercise for pre- and post-supplementation in both the β-carotene supplemented group and the placebo group. The values are the means ± SE. **A:** pre-supplementation, **B:** post-supplementation.

TABLE III Plasma hypoxanthine, xanthine and uric acid concentrations at rest and after maximal exercise before and after the 1-month β-carotene supplementation.

		Exercise 1					Exercise 2			
		Rest	After Exercise			Rest	After Exercise			
	n		0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
Hypoxanthine (μM)										
β-Carotene	8	1.64 ± 0.14	6.26 ± 0.85§	1.50 ± 0.23	1.40 ± 0.18	1.42 ± 0.23	1.35 ± 0.17	9.00 ± 1.93§	1.42 ± 0.23	1.82 ± 0.54	1.62 ± 0.14
Placebo	6	1.54 ± 0.20	6.19 ± 1.41†	1.45 ± 0.19	1.31 ± 0.34	1.95 ± 0.53	1.83 ± 0.18	7.46 ± 1.56§	2.26 ± 0.35	2.54 ± 0.56	2.86 ± 1.01
Xanthine (μM)										
β-Carotene	8	0.76 ± 0.03	1.03 ± 0.15*	0.72 ± 0.06	0.69 ± 0.07	0.74 ± 0.06	0.71 ± 0.06	1.19 ± 0.16†	0.67 ± 0.05	0.65 ± 0.07	0.70 ± 0.05
Placebo	6	0.80 ± 0.12	1.21 ± 0.32	0.66 ± 0.09	0.87 ± 0.20	0.73 ± 0.12	0.74 ± 0.09	1.43 ± 0.33	0.93 ± 0.10	0.84 ± 0.10	0.83 ± 0.11
Uric acid (μM)										
β-Carotene	8	290.1 ± 31.1	245.2 ± 23.4	320.3 ± 28.5	315.4 ± 37.0	309.9 ± 15.7	313.8 ± 18.5	211.7 ± 20.9*	317.7 ± 33.1	348.4 ± 28.6	302.7 ± 31.0
Placebo	6	274.6 ± 29.1	244.9 ± 30.9	322.0 ± 42.7	294.1 ± 36.1	278.4 ± 23.4	291.8 ± 36.1	215.7 ± 29.3	313.6 ± 32.6	304.6 ± 18.7	303.5 ± 37.6

Values are the means ± SE. n, number of subjects. Significantly different from rest in the same supplement group of Exercise 1 and 2, respectively, §$P < 0.001$, †$P < 0.01$, *$P < 0.05$.

8-OHdG tended to be slightly lower after supplementation.

The plasma hypoxanthine, xanthine and uric acid concentrations are presented in Table III. The plasma hypoxanthine concentrations in both groups increased significantly ($P < 0.001$ and $P < 0.01$) immediately after both exercise trials. In addition, the plasma xanthine concentrations in the β-carotene supplemented group also increased significantly ($P < 0.01$ and $P < 0.05$) immediately after both exercise trials, while this increase was not significant in the placebo group. No significant changes in the plasma uric acid concentrations were observed immediately after exercise, except for the β-carotene supplemented group after the Exercise 2. At 24 h and 48 h post-exercise, the plasma uric acid concentrations in both groups were slightly higher, but not significantly, compared with preexercise. The peak of the plasma uric acid concentrations after exercise was delayed compared with the peaks of the plasma hypoxanthine and xanthine concentrations.

DISCUSSION

The 8-OHdG is formed on DNA strands and is excreted into urine as the repair product of DNA damage.[4] The urinary excretion of 8-OHdG has been used as a biomarker to estimate the degree of *in vivo* oxidative damage to cellular DNA or to the nucleotide DNA precursor pool.[29,30] It has been reported that the increased excretion of 8-OHdG in the urine of humans was caused by oxygen radicals generated by radiation, carcinogens, smoking and endogenuous oxidation processes such as cancer and other degenerative diseases.[2,4,31–33]

The higher metabolic rates with higher oxygen consumption increased the urinary excretion of 8-OHdG.[2,3] Loft *et al.*[8] also reported a positive correlation between the 24-h urinary excretion of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) and oxygen consumption in healthy women. It has been shown that the strenuous exercise induces oxidative damage to lipids, proteins and other molecules.[14,15,34] Exercise might enhance the generation of oxygen radicals in the mitochondrial electron transport chains, the activation of oxidases such as xanthine oxidase and peroxidase, the metabolism of leukocytes such as neutrophils, monocytes and macrophage, and the autoxidation of catecholamines.[13]

We found an increase in the plasma hypoxanthine and xanthine concentrations immediately after exercise. In addition, the plasma uric acid concentrations also tended to be higher at 24 h or 48 h after exercise before and after supplementation in both groups. Hellsten[35] suggested that, following exercise, muscle is a major source of blood hypoxanthine whereas the liver appears to be a main source of blood uric acid. The majority of hypoxanthine in blood is taken up by the liver and the oxidation of hypoxanthine to uric acid occurs by xanthine oxidase. Therefore, we expect that the acute exercise performed in this study enhanced the generation of oxygen radicals by xanthine oxidase. However, it is still not clear as to whether or not maximal exercise with an increasing plasma hypoxanthine in fact causes oxidative DNA damage.

Several oxygen radicals attack both nuclear and mitochondrial DNA,[36] and oxidative DNA damage was found to correlate with an increased metabolic rate.[3] In a single cell gel electrophoresis assay of DNA damage due to exercise, it has been reported that a significant increase in DNA migration among human white blood cells was found 6, 24, and 48 h after strenuous physical activity.[37]

In the present study, the urinary excretion of 8-OHdG did not change significantly during the 3 day period after acute exercise to exhaustion either pre- or post-supplementation in the β-carotene supplemented and placebo groups, while the mean urinary 8-OHdG excretions in the 3 day postexercise period tended to be slightly lower compared with the 1 day preexercise. These findings correlate with those from other

studies in which no change in 8-hydroxyguanosine, a marker of oxidative RNA damage, in the human urine was found after 90 min of exercise at 65% of maximal O_2 uptake.[18,25] In contrast to our results, however, it has been reported that the ratio of urinary 8-OHdG excretion to creatinine excretion increased slightly after a marathon race[16] as well as after swimming or running.[17] In these findings, the urine samples for 8-OHdG measurement were partially collected for 15 min or 10 h after the end of exercise. These urinary collection periods were short.[38] Since the 24-h excretion of 8-OHdG is suitable to estimate the total oxidative damage to DNA in the whole body, our results were thus multiplied by the 24-h urine volume and expressed per mmol creatinine. In this study, no significant changes in the urinary 8-OHdG and urinary creatinine excretion per 24-h after exercise were found. These findings suggest that acute strenuous exercise might not induce oxidative damage to DNA and cellular DNA precursors during the 3 days after exercise. The duration and intensity of exercise performed in this study might be shorter and lower, respectively. We have already reported that the urinary 8-OHdG excretion significantly increased during an 8-day training camp which included 30 km of running per day.[39]

We have no explanation for the higher urinary excretion of 8-OHdG observed before the Exercise 1 in the placebo group, as there was no significant difference in the body composition between the β-carotene supplemented and placebo groups.

β-Carotene is the most prominent antioxidant in carotenoids and protects against oxidative damage mediated by oxygen radicals, especially singlet oxygen radicals.[19] An epidemiologic study has shown that the intake of β-carotene reduced the incidence of cancer in humans.[20] Recently, however, the protective effect of β-carotene supplementation against lung cancer in smokers has been questioned.[21,22] In this study, the plasma β-carotene concentration after a 1-month supplementation of β-carotene (30 mg of β-carotene/day) was found to be significantly higher than that before supplementation. The baseline urinary excretion of 8-OHdG before exercise tended to be lower after β-carotene supplementation. In the both exercise trials, the decrease in the plasma β-carotene concentrations observed immediately after exercise may be associated with the quenching oxygen radicals induced during intensive exercise. However, these findings are insufficient to explain the protective effects of β-carotene against oxidative damage to DNA and the cellular DNA precursor under resting or exercising conditions.

In conclusion, our results show that acute strenuous exercise does not induce oxidative DNA damage as assessed by urinary 8-OHdG excretion in nonsmokers, and 30 mg/day of β-carotene supplementation for 1 month tended to attenuate the oxidative DNA damage. To further evaluate oxidative DNA damage by exercise, more studies will be required for understanding the intensity, duration and type of exercise or the balance between the rate of damage to the cellular nucleotide pool and DNA and the rate of repair.

Acknowledgments

This study was supported by a Grant-in-Aid for General Scientific Research (06454632) from The Japanese Ministry of Education, Science and Culture and a Research Grant from Osaka Gakuin University.

References

[1] Kasai, H. and Nishimura, S. (1984). Hydroxylation of deoxyguanosine at C-8 position by ascorbic acid and other reducing agents. *Nucleic Acids Research*, **12**, 2137–2145.

[2] Loft, S., Vistisen, K., Ewertz, M., Tjønneland, A., Overvad, K. and Poulsen, H. E. (1992). Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index. *Carcinogenesis*, **13**, 2241–2247.

[3] Adelman, R., Saul, R. L. and Ames, B. N. (1988). Oxidative damage to DNA: Relation to species metabolic rate and life span. *Proceedings of the National Academy of Sciences of the United States of America*, **85**, 2706–2708.

[4] Shigenaga, M. K., Gimeno, C. J. and Ames, B. N. (1989). Urinary 8-hydroxy- 2'-deoxyguanosine as a biological marker of *in vivo* oxidative DNA damage. *Proceedings of the National Academy of Sciences of the United States of America*, **86**, 9697–9701.

[5] Tajiri, T., Maki, H. and Sekiguchi, M. (1995). Functional cooperation of MutT, MutM and MutY proteins in preventing mutations caused by spontaneous oxidation of guanine nucleotide in Escherichia coli. *Mutation Research*, **336**, 257–267.

[6] Hayakawa, H., Taketomi, A., Sakumi, K., Kuwano, M. and Sekiguchi, M. (1995). Generation and elimination of 8-oxo-7,8-dihydro-2'-deoxyguanosine 5'-triphosphate, a mutagenic substrate for DNA synthesis, in human cells. *Biochemistry*, **34**, 89–95.

[7] Chance, B., Sies, H. and Boveris, A. (1979). Hydroperoxide metabolism in mammalian organs. *Physiological Reviews*, **59**, 527–605.

[8] Loft, S., Astrup, A., Buemann, B. and Poulsen, H. E. (1994). Oxidative DNA damage correlates with oxygen consumption in humans. *The FASEB Journal*, **8**, 534–537.

[9] Davies, K. J. A., Quintanilha, A. T., Brooks, G. A. and Packer, L. (1982). Free radicals and tissue damage produced by exercise. *Biochemical and Biophysical Research Communications*, **107**, 1198–1205.

[10] Lovlin, R., Cottl, W., Pike, I., Kavanaugh, M. and Belcastro, A. N. (1987). Are indices of free radical damage related to exercise intensity? *European Journal of Applied Physiology and Occupational Physiology*, **56**, 313–316.

[11] Pincemail, J., Camus, G., Roesgen, A., Dreezen, E., Bertrand, Y., Lismonde, M., Deby-Dupont, G. and Deby, C. (1990). Exercise induces pentane production and neutrophil activation in humans: effect of propranol. *European Journal of Applied Physiology and Occupational Physiology*, **61**, 319–322.

[12] Suzuki, K., Sato, H., Kikuchi, T., Abe, T., Nakaji, S., Sugawara, K., Totsuka, M., Sato, K. and Yamaya, K. (1996). Capacity of circulating neutrophils to produce reactive oxygen species after exhaustive exercise. *Journal of Applied Physiology*, **81**, 1213–1222.

[13] Bendich, A. (1991). Exercise and free radicals: effects of antioxidant vitamins. *Medicine and Sport Science*, **32**, 59–78.

[14] Maughan, R. J., Donnely, A. E., Gleeson, M., Whiting, P. H., Walker, K. A. and Clough, P. J. (1989). Delayed onset muscle damage and lipid peroxisation in man after a downhill run. *Muscle and Nerve*, **12**, 332–336.

[15] Sumida, S., Tanaka, K., Kitao, H. and Nakadomo, F. (1989). Exercise-induced lipid peroxidation and leakage of enzymes before and after vitamin E supplementation. *The International Journal of Biochemistry*, **21**, 835–838.

[16] Alessio, H. M. (1993). Exercise-induced oxidative stress. *Medicine and Science in Sports and Exercise*, **25**, 218–224.

[17] Inoue, T., Mu, Z., Sumikawa, K., Adachi, K. and Okochi, T. (1993). Effect of physical exercise on the content of 8-hydroxydeoxyguanosine in nuclear DNA prepared from human lymphocytes. *Japanese Journal of Cancer Research*, **84**, 720–725.

[18] Viguie, C. A., Frei, B., Shigenaga, M. K., Ames, B. N., Packer, L. and Brooks, G. A. (1993). Antioxidant status and indexes of oxidative stress during consecutive days of exercise. *Journal of Applied Physiology*, **75**, 566–572.

[19] Sies, H. and Stahl, W. (1995). Vitamin E and C, β-carotene, and other carotenoids as antioxidants. *American Journal of Clinical Nutrition*, **62**(suppl), 1315S–1321S.

[20] van Poppel, G. and Goldbohm, R. A. (1995). Epidemiologic evidence for β-carotene and cancer prevention. *American Journal of Clinical Nutrition*, **62**(suppl), 1393S–1402S.

[21] Albanes, D., Heinonen, O. P., Huttunen, J. K., Taylor, P. R., Virtamo, J., Edwards, B. K., Haapakoski, J., Rautalahti, M., Hartman, A. M., Palmgren, J. and Greenwald, P. (1995). Effects of α-tocopherol and β-carotene supplements on cancer incidence in the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study. *American Journal of Clinical Nutrition*, **62**(suppl), 1427S–1430S.

[22] Hennekens, C. H., Buring, J. E., Manson, J. E., Stampfer, M., Rosner, B., Cook, N. R., Belanger, C., Lamotte, F., Gaziano, J. M., Ridker, P. M., Willett, W. and Peto, R. (1996). Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. *New England Journal of Medicine*, **334**, 1145–1149.

[23] The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group (1994). The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. *New England Journal of Medicine*, **330**, 1029–1035.

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

[25] Witt, E. H., Reznick, A. Z., Viguie, C. A., Starke-Reed, P. and Packer, L. (1992). Exercise, oxidative damage and effects of antioxidant manipulation. *Journal of Nutrition*, **122**, 766–773.

[26] Miller, K. W. and Yang, C. S. (1985). An isocratic high-performance liquid chromatography method for the simultaneous analysis of plasma retinol, α-tocopherol, and various carotenoids. *Analytical Biochemistry*, **145**, 21–26.

[27] Wung, W. E. and Howell, S. B. (1980). Simultaneous liquid chromatography of 5-fluorouracil, uridine, hypoxanthine, xanthine, uric acid, allopurinol, and oxipurinol in plasma. *Clinical Chemistry*, **26**, 1704–1708.

[28] Costill, D. L. and Fink, W. J. (1974). Plasma volume changes following exercise and thermal dehydration. *Journal of Applied Physiology*, **37**, 521–525.

[29] Mo, J.-Y., Maki, H. and Sekiguchi, M. (1992). Hydrolytic elimination of a mutagenic nucleotide, 8-oxodGTP, by human 18-kilodalton protein: Sanitization of nucleotide pool. *Proceedings of the National Academy of Sciences of the United States of America*, **89**, 11021–11025.

[30] Sakumi, K., Furuichi, M., Tsuzuki, T., Kakuma, T., Kawabata, S., Maki, H. and Sekiguchi, M. (1993). Cloning and expression of cDNA for a human enzyme that hydrolyzes 8-oxo-dGTP, a mutagenic substrate for DNA synthesis. *Journal of Biological Chemistry*, **268**, 23524–23530.

[31] Brown, R. K., Mcburney, A., Lunec, J. and Kelly, F. J. (1995). Oxidative damage to DNA in patients with cystic fibrosis. *Free Radical Biology and Medicine*, **18**, 801–806.

[32] Lagorio, S., Tagesson, C., Forastiere, F., Iavarone, I., Axelson, O. and Carere, A. (1994). Exposure to benzene and urinary concentrations of 8-hydroxydeoxyguanosine, a biological marker of oxidative damage to DNA. *Occupational and Environmental Medicine*, **51**, 739–743.

[33] Tagesson, C., Kallberg, M., Klintenberg, C. and

Starkhammar, H. (1995). Determination of urinary 8-hydroxydeoxyguanosine by automated coupled-column high performance liquid chromatography: a powerful technique for assaying *in vivo* oxidative DNA damage in cancer patients. *European Journal of Cancer*, **31A**, 934–940.

[34] Sen, C., Atalay, M. and Hänninen, O. (1994). Exercise-induced oxidative stress: glutathione supplementation and deficiency. *Journal of Applied Physiology*, **77**, 2177–2187.

[35] Hellsten, Y. (1994). Xanthine dehydrogenase and purine metabolism in man-with special reference to exercise. *Acta Physiologica Scandinavica*, **151** (Suppl 621), 1–73.

[36] Richter, C., Park, J.-W. and Ames, B. N. (1988). Normal oxidative damage to mitochondrial and nuclear DNA is extensive. *Proceedings of the National Academy of Sciences of the United States of America*, **85**, 6465–6467.

[37] Hartmann, A., Plappert, U., Raddatz, K., Grünert-Fuchs, M. and Speit, G. (1994). Does physical activity induce DNA damage? *Mutagenesis*, **9**, 269–272.

[38] Loft, S. and Poulsen, H. E. (1996). Cancer risk and oxidative DNA damage in man. *Journal of Molecular Medicine*, **74**, 297–312.

[39] Okamura, K., Doi, T., Hamada, K., Sakurai, M., Yoshioka, Y., Mitsuzono, R., Migita, T., Sumida, S. and Sugawa-Katayama, Y. (1997). Effect of repeated exercise on urinary 8-hydroxy-deoxyguanosine excretion in humans. *Free Radical Research*, **26**, 507–514.