ELSEVIER

Influence of one bout of vigorous exercise on ascorbic acid in plasma and oxidative damage to DNA in blood cells and muscle in untrained rats

Keizo Umegaki,* Pang Daohua,*,§ Ayako Sugisawa,* Michiyo Kimura,† and Mitsuru Higuchi†

**Divisions of Applied Food Research and †Health Promotion, National Institute of Health and Nutrition, Shinjuku-ku, Tokyo, Japan and §Department of Nutrition and Food Hygienics, Jining Medical College, Jining, China*

We investigated the influence of a single exhaustive bout of downhill running on oxidative damage to DNA and changes of antioxidant vitamin concentrations in rats. Plasma vitamin E levels were unchanged up to 48 hr postexercise. However, plasma ascorbic acid (AA) levels increased after the exercise, then decreased thereafter. This increase corresponded to a marked decrease in AA concentration in the adrenal glands. The activity of hepatic l-gulono-γ-lactone oxidase, which catalyzes AA synthesis, was unaltered after the exercise. The weight of the adrenal glands was significantly increased 24 hr postexercise. These results indicate that the change in the plasma AA concentration after vigorous exercise was due mainly to the release of AA from the adrenal glands. The plasma creatine phosphokinase (CPK) activity and white blood cell (WBC) count increased 3 to 6 hr postexercise. Over this same period, a marker of oxidative DNA damage, 8-hydroxydeoxyguanosine in DNA, increased in the WBC, but not in the foreleg muscle. Lipid peroxide and vitamin E levels were also unchanged in the foreleg muscle. There was a positive correlation between CPK activity in the plasma and DNA damage in the WBC, suggesting that the DNA damage in the WBC was closely related with muscle damage due to exercise.

Keywords: 8-hydroxydeoxyguanosine; DNA damage; stress; ascorbic acid; white blood cells

Introduction

Oxidative damage of biomolecules such as DNA and lipids has been implicated in the modification of aging and degenerative diseases.[1] During intensive exercise, uptake and consumption of oxygen greatly increase, resulting in the production of oxygen radicals.[2] The increase in oxygen consumption has been shown to be closely related to oxidative damage of DNA in humans.[3] These findings raise the possibility that exercise induces oxidative damage of biomolecules in the body, thereby modifying the incidence of degenerative diseases and aging. However, epidemiologic studies suggest that physical activity is associated with decreased incidence of certain types of cancers.[4,5]

Induction of oxidative damage in the body seems to be the result of an oxidative stress that exceeds the antioxidant capacity, which is composed of antioxidants and antioxidative enzymes. Ascorbic acid (AA) is known to be the most effective water-soluble antioxidant.[6] AA regenerates vitamin E, an important lipid soluble antioxidant, from the vitamin E radical.[7] Several studies have been conducted to examine exercise-induced oxidative stress and the change in plasma AA.[8–11] These studies reported that the concentration of plasma AA was not decreased, but rather was increased, after exercise. The distribution of AA differs

This work was supported by Meiji Life Foundation and the Human Science Foundation in Japan.
Address correspondence to Dr. Keizo Umegaki, Division of Applied Food Research, The National Institute of Health and Nutrition, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8636, Japan.
Received February 1, 2000; accepted June 6, 2000.

655 Avenue of the Americas, New York, NY 10010
0955-2863/00/$–see front matter
PII S0955-2863(00)00096-6

markedly throughout the body: The concentration is approximately 700 times higher in the adrenal glands than in the plasma.[12] Stress induces the release of AA, along with cathecholamine, from the adrenal glands into the bloodstream.[13] Increase in plasma AA concentrations have been positively correlated with increases in plasma cortisol in both rats and humans.[8,14] Although these findings suggest that the change in AA concentration in plasma after exercise is a reflection of the change in AA in the adrenal glands, this relationship has not been adequately investigated.

Some studies have observed exercise-induced DNA damage,[15–17] but others have failed to detect such damage.[18–20] Among those studies that did observe exercise-induced DNA damage, most involved vigorous exercise conditions accompanied by muscle damage. In contrast, the studies that failed to detect DNA damage were conducted using moderate exercise conditions and/or trained subjects who may have acquired elevated antioxidant capacity through training.[21,22] Generally, exercise-induced DNA damage is thought to be due to increased uptake of oxygen in the body. However, it is also possible that muscle damage and the subsequent inflammation are involved in exercise-induced DNA damage, particularly in the case of blood cells. In fact, exercise-induced DNA damage in blood cells has been shown to be closely related to an increase in plasma creatine phosphokinase (CPK) activity,[15] a marker of muscle damage. It has also been reported that intensive exercise induces an increase in plasma myeloperoxidase levels, a marker of neutrophil activation in vivo.[23] When the neutrophils are activated, they release reactive oxygen species (ROS) that damage the neutrophils themselves, in addition to damaging other cells or tissues.[24,25] On the other hand, influx of oxygen into active muscle is increased approximately 20-fold during exercise, and muscle is susceptible to oxidative stress.[26] However, little is known about DNA damage in muscle due to exercise.

It is well known that more muscle damage is induced by the decreased oxygen uptake following downhill running than by that following level running.[27] In addition, a single bout of exercise induces more muscle damage than do repeated daily bouts.[28] Thus, in this study, untrained rats were subjected to a single bout of intensive downhill running in order to examine (1) the changes in the AA concentration in plasma and in the adrenal glands following exercise and (2) the changes in the levels of 8-hydroxydeoxyguanosine (8-OHdG) in white blood cells (WBC) and muscle and the relationship between these changes and the level of plasma CPK activity.

Materials and methods

Chemicals

L-gulono-γ-lactone and acid phosphatase were purchased from Sigma Chemical Co. (St. Louis, MO USA). P1 Nuclease was obtained from Seikagaku Kogyo (Tokyo, Japan). The DNA extraction kit and CPK assay kit were obtained from Wako Pure Chemical Ind. (Osaka, Japan).

Experimental animals

Male Wistar rats (aged 6 weeks), purchased from Japan Clea (Tokyo, Japan), were housed individually in stainless steel wire-bottomed cages in a room with a constant temperature of 23 ± 1°C under a 12-hr light (2:00 PM to 2:00 AM)/dark cycle. The rats were fed a commercial rodent chow (CE2; Japan Clea) throughout the experiment and were acclimatized to the environment for 1 week before the exercise test. The exercise rats performed one bout of exhaustive treadmill running (down a 17-degree slope) at 9:00 AM. The running speed was set at 20 m/min for 5 min, then at 24 m/min for 90 min.

Rats were divided into a control (sedentary) and two or three exercise groups of five to seven rats each. The rats were anesthetized with pentobarbital and sacrificed at a predetermined time. Blood was taken from the abdominal aorta, and organs were removed and weighed. The blood was immediately centrifuged at 5,000 × g for 3 min and the plasma was prepared. For the analysis of AA, 40 μL of the plasma was immediately mixed with 200 μL of 6% metaphosphoric acid and stored at −80°C for several days until analyzed.[29]

All procedures were in accordance with the National Institute of Health and Nutrition guidelines for the care and use of laboratory animals.

Analytical methods

Tissues were homogenized with 5 volumes of distilled water. The tissue homogenate was immediately mixed with 5 volumes of 6% metaphosphoric acid for the analysis of AA and with 10 volumes of 0.15% butylated hydroxytoluene for the analysis of vitamin E (α-tocopherol), respectively. In case of the dehydroascorbic acid (DHAA), samples was treated with dithiothreitol to reduce DHAA to AA as described elsewhere.[29] AA and vitamin E were analyzed by high performance liquid chromatography (HPLC) with an electrochemical detector (ECD) according to the method described previously.[29,30] L-gulono-γ-lactone oxidase (EC1.1.3.8) activity was analyzed principally according to the method of Kito et al.[31] Briefly, rat liver microsomes were incubated with 50 mM sodium citrate, 1.7 mM dithiothreitol, and 2.5 mM l-gulono-γ-lactone in 50 mM potassium phosphate buffer, pH 7.0, at 37°C for 30 min. The enzymatic reaction was stopped by the addition of a one-sixth volume of 30% metaphosphoric acid, and the reactants were centrifuged at 10,000 × g for 10 min at 4°C. The supernatant was injected to HPLC with ECD for the analysis of AA.

Analysis of 8-OHdG in WBC and muscle was performed as follows. DNA was extracted using a DNA extraction kit (DNA Extraction WB kit No. 293-50501, Wako Pure Chemical Ind.). The isolated DNA was digested by P1 nuclease and acid phosphatase according to the method of Yamaguchi et al.[32] Content of 8-OHdG and deoxyguanosine (dG) in the deoxynucleotides mixture were simultaneously analyzed using HPLC (Shimadzu LC10AD, Shimadzu Co., Kyoto, Japan) with ECD (Coulochem II, ESA, MA) equipped analytical cells (detector 1, 180 mV; detector 2, 380 mV) and ultraviolet detector (Shimadzu SPD-10A, at 280 nm). The separating conditions were as follows: column Beckman Ultrasphere ODS (Beckman Instruments Inc., CA USA) (4.6 × 250 mm); column temperature, 23°C; mobile phase, 10 mM NaH_2PO_4 containing 8% methanol; flow rate, 1 mL/min. The level of 8-OHdG in DNA was expressed as numbers of 8-OHdG per 10^5 dG.

CPK activity in plasma was determined using diagnostic kits. Tissue homogenate was mixed with an equal volume of 1% sodium dodecyl sulphate. Protein levels in the tissue homogenate were determined using a BCA protein assay kit (No. 23225, Pierce, Rockford, IL USA). Lipid peroxide levels were determined by the thiobarbituric acid method[33] and expressed as thiobarbituric react-

Table 1 Influence of one bout of exercise on weights of body, liver, and adrenal glands, various blood parameters, and L-gulono-γ-lactone oxidase activities in liver (experiment 1)

	Control (sedentary)	Exercise		
		2 hr after	24 hr after	48 hr after
Body weight (BW) (g)	171 ± 2.4	169 ± 6.2	169 ± 4.2	174 ± 3.6
Liver (BW %)	4.75 ± 0.12	4.61 ± 0.10	4.96 ± 0.11	5.03 ± 0.07
Adrenal glands (BW % × 10^2)	1.98 ± 0.11	2.06 ± 0.15	2.39 ± 0.09[a]	2.26 ± 0.06
Hematocrit (%)	36.6 ± 0.5	37.6 ± 1.2	37.9 ± 1.9	35.4 ± 0.8
Red blood cells (× 10^5/mL)	494 ± 11	510 ± 10	507 ± 23	474 ± 13
White blood cells (× 10^5/mL)	45.3 ± 5.3	70.6 ± 6.3[a]	48.0 ± 5.1	43.4 ± 2.0
CPK activity in plasma (U/L)	100 ± 9.5	342 ± 79[a]	128 ± 22	89 ± 12
Vitamin E in plasma (μM)	14.6 ± 0.68	13.1 ± 0.55	14.8 ± 0.34	13.9 ± 0.67
L-gulono-γ-lactone oxidase activities in liver (nmol/mg protein/min)	4.51 ± 0.17	4.11 ± 0.28	3.99 ± 0.08	3.77 ± 0.23

Values are mean ± SE for 5 to 7 rats.
[a]Significance versus control ($P < 0.05$).
CPK— creatine phosphokinase.

ing substances. Blood cell count was measured by a coulter counter (Toa Iyou Densi, Kobe, Japan).

Statistical analyses

The data are presented as means with standard error (SEM) for the individual groups. Statistical analyses of the data for the groups were carried out using analysis of variance (ANOVA) followed by a post-hoc test of Fisher's Protected Least Significant Difference. All statistical analyses were performed using the computer program Stat View 4.5 (Abacus Concepts, Inc., Berkeley, CA USA).

Results

In experiment 1, rats performed one bout of a downhill running trial for 90 min and then were sacrificed at 2, 24, or 48 hr postexercise. Body weight and relative liver weight were unaffected by exercise, but the weight of the adrenal glands tended to be higher in the exercised rats, and increased significantly 24 hr postexercise (*Table 1*). Hematocrit was unchanged by the exercise, but CPK activity in the plasma was higher in the exercised rats 2 hr postexercise (*Table 1*). Plasma vitamin E concentration did not differ significantly between the exercised and control rats. However, in the exercised rats, the concentration of AA in plasma was significantly higher at 2 hr and tended to be lower at 24 hr and 48 hr than that in control rats (*Figure 1*). The exercise-induced change in hepatic AA was similar to that of plasma AA. In contrast, the change in AA in the adrenal glands, where AA concentration was 10 times higher than in the liver, was inversely related to the change in plasma AA. The concentrations of AA in the plasma and adrenal glands showed a significant negative correlation ($n = 21$, $r = -0.461$, $P < 0.05$). In addition, the change in the whole amount of AA in the adrenal glands due to exercise corresponded negatively to the actual changes in

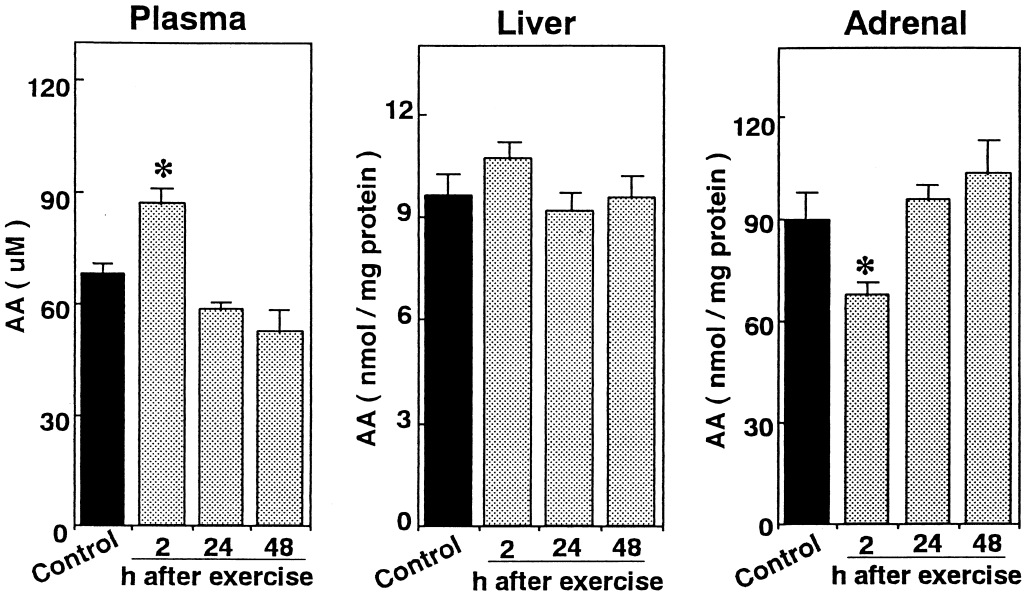

Figure 1 Concentration of ascorbic acid (AA) in plasma, liver, and adrenal glands in rats subjected to 90 min of running and in control rats (experiment 1). Untrained rats were divided into a control (sedentary) and three exercise groups. Rats in the exercise groups were subjected to 90 min of downhill running and then sacrificed at 2, 24, or 48 hr postexercise. *Significance versus control level ($P < 0.05$). Each column and vertical bar indicate the mean ± SE for five to seven rats.

Table 2 Influence of adrenal vitamin C on plasma ascorbic acid (AA) due to exercise (experiment 1)

	Changes over control levels		
	2 hr after	24 hr after	48 hr after
Change in whole amount of AA in adrenal glands (nmol)	−148 ± 26	+139 ± 44	+151 ± 57
Estimated change in plasma AA (nmol/mL)[1]	+18 ± 3	−17 ± 5	−17 ± 7
Actual change in plasma AA (nmol/mL)	+19 ± 4	−11 ± 3	−16 ± 7

Values are mean ± SE for five to seven rats.
[1]Estimated change in plasma AA was calculated according to the following formula: Estimated change in plasma AA (nmol/mL) = $-(AG) \times (CPB)^{-1}$ where AG is the change in whole amount of AA in adrenal glands; CPB is the calculated plasma volume, body weight (kg) × 80 (mL/kg) × (1-hematocrit(%) × 100^{-1}). (The value of 80 mL/kg is from *Biochemist's Hand Book* (1961).

the concentrations of AA in the plasma (*Table 2*). To evaluate the influence of AA biosynthesis on the change in the plasma AA, the activity of l-gulono-γ-lactone oxidase, which catalyzes the last step of AA synthesis, was measured in the liver (*Table 1*). However, the activity of this enzyme was somewhat lower in the exercised rats, and no correlation was observed between this activity and AA concentration in the liver.

To reconfirm the changes of AA in the plasma and adrenal glands after exercise, rats were sacrificed immediately after or 2 hr postexercise. Similar to the result shown in *Table 2*, AA concentration in the plasma was significantly increased, and the whole amount of AA in the adrenal glands was significantly decreased in the exercised rats compared with the control (sedentary) rats. The AA concentration (nmol/mL) in the plasma versus the whole amount of AA (nmol) in the adrenal glands in five rats (mean ± SE) were 62 ± 1.2 versus 451 ± 22 for control, 82 ± 6.6 versus 222 ± 18 for immediately after exercise, and 73 ± 3.7 versus 292 ± 26 for 2 hr postexercise. The presence of DHAA (oxidized form of AA) was less than 5% of total AA (DHAA + AA) in both the plasma and adrenal glands and did not differ among the exercised and control rats.

In experiment 2, rats were subjected to the same downhill running trial as in experiment 1, then were sacrificed 3 hr and 6 hr postexercise to examine the changes of plasma CPK activity and the oxidative damages to DNA in the WBC and foreleg muscle. In the exercised rats, the plasma CPK activity and the circulating WBC count were higher than were those in the control rats (*Figure 2a and 2b*). The level of 8-OHdG in DNA from the WBC was also higher in the exercised rats at 3 hr (*Figure 2c*). When the relationship between plasma CPK activity and 8-OHdG in WBC DNA was examined using these data, a significant positive correlation was noted between them (*Figure 3*). In contrast, 8-OHdG levels in DNA from muscle did not differ between the exercised and control rats (*Figure 2d*). Similarly, the levels of lipid peroxide and vitamin E in muscle were comparable between the exercised and control rats (*Table 3*).

Discussion

In untrained humans and animals, one bout of intensive exercise may cause muscle damage, followed by activation of neutrophils in response to inflammation. The activated neutrophils produce ROS, such as superoxide and hydrogen peroxide, which damage the neighboring cells as well as the neutrophils themselves.[24,25] Both the presence and activation of neutrophils have been demonstrated after intensive exercise.[34,35] Oh-ishi et al.[23] have reported that superoxide production by neutrophils is increased after intensive exercise in untrained but not in trained rats. These findings may indicate that intensive exercise induces oxidative DNA damage in muscle and blood cells not by increased uptake of oxygen, but by muscle damage. We considered that one bout of downhill running in untrained rats, which causes more muscle damage with less oxygen uptake, would be a good model to examine this hypothesis. Therefore, we subjected untrained rats to a single, intensive downhill exercise trial, then examined the changes of oxidative DNA damage in their blood cells and muscle and the changes of AA in their plasma.

As shown in the Results, we detected DNA damage as an increase in 8-OHdG in DNA from WBC. We also observed a postexercise increase in the circulating WBC. Although we did not examine the subpopulation of circulating WBC, it has already been reported that intensive exercise increases WBC count in the circulation and that neutrophils constitute the main component of this increase.[36,37] Interestingly, the increase in 8-OHdG in DNA from the WBC was positively correlated with the increase in plasma CPK activities after the exercise trial (*Figure 3*). In our previous study,[11] we also observed a significant correlation ($n = 16$, $r = 0.77$, $P < 0.001$) between plasma CPK and DNA damage, as evaluated by a micronucleus assay using lymphocytes obtained from untrained and trained human subjects. A similar correlation between CPK and DNA damage in WBC has been detected by single-cell gel assay in humans after intensive exercise.[15] Furthermore, a positive correlation between plasma CPK activity and superoxide release from neutrophils has been reported in humans who performed acute exercise.[38] These facts indicate that intensive exercise-induced DNA damage is not related to increased oxygen uptake into the body, but rather to the muscle damage and neutrophil activation in response to inflammation. It is well known that 8-OHdG in DNA is efficiently repaired.[39] The present decrease in 8-OHdG in WBC DNA 6 hr postexercise may have been related to the DNA repair.

Exercise increases the susceptibility of muscle to both oxidative and mechanical damage. For example, oxygen influx and consumption in muscle have been shown to be enhanced 20-fold during exercise.[26] In the present study, however, we did not detect DNA damage in muscle. The lack of change in lipid peroxide and vitamin E levels in muscle due to exercise was consistent with the absence of oxidative DNA damage in muscle. These findings suggest that intensive exercise-induced oxidative DNA damage is more prone to occur in blood cells than in muscle. If the ROS are mainly produced in neutrophils, then interaction involving them will be higher in blood cells than in other tissues. A marked increase in 8-OHdG in neutrophil DNA

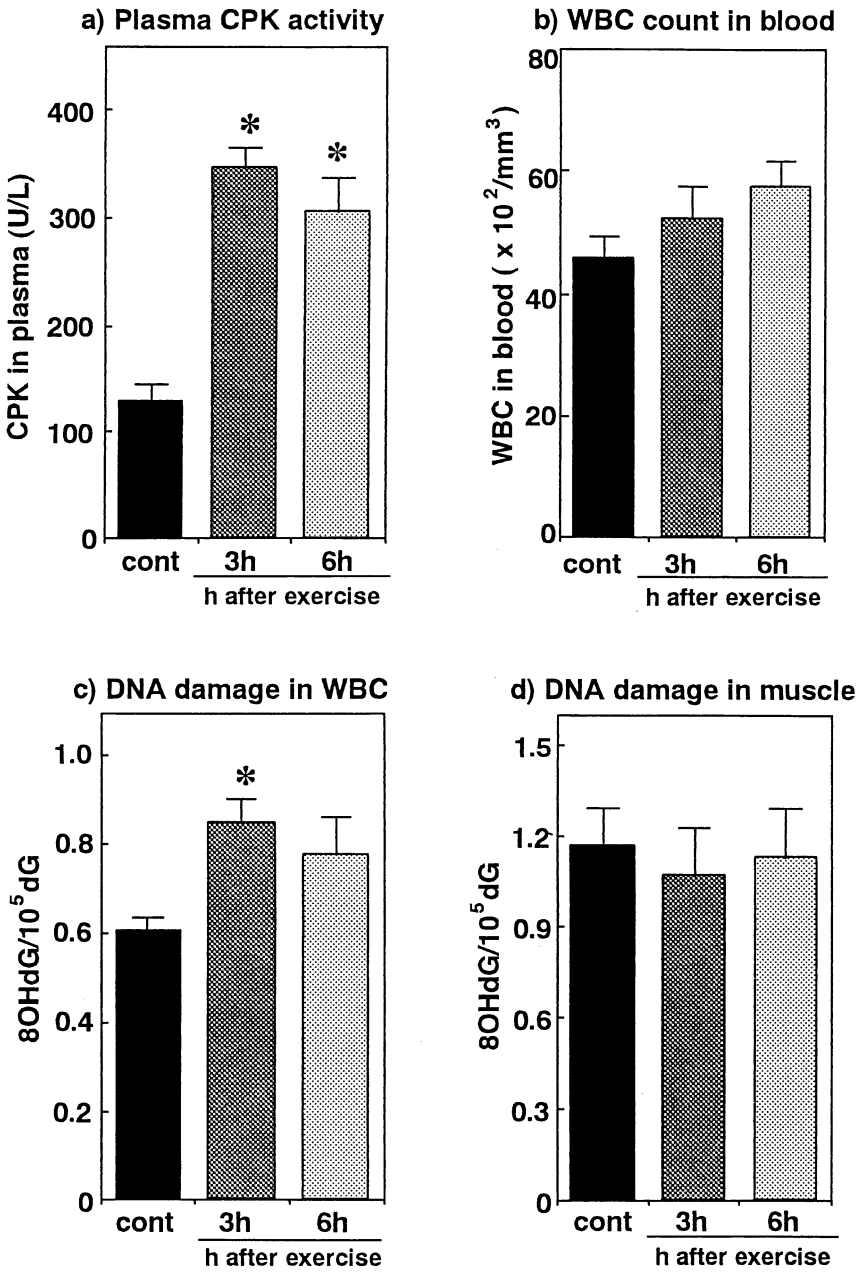

Figure 2 Plasma creatine phosphokinase (CPK) activity, circulating white blood cell (WBC) counts, and DNA damage in WBC and muscle in rats subjected to 90 min of running and in control rats (experiment 2). Untrained rats were divided into a control (sedentary) and three exercise groups. Rats in the exercise groups were subjected to 90 min of downhill running and then sacrificed at 3 hr or 6 hr postexercise. *Significance versus control level ($P < 0.05$). Each column and vertical bar indicate the mean ± SE for five to seven rats.

has been detected following neutrophil activation.[25] These findings may explain why DNA damage was only detected in blood cells in this study. We could not detect oxidative damage in muscle, but detected increase in plasma CPK after the intensive exercise. This fact suggests that mechanical damage in the muscle is more important than oxidative damage after the single bout of intensive downhill running. The mechanical damage in muscle could activate inflammatory reaction in the body by releasing cytokines and activating neutrophils.[40] The ROS released from the activated neutrophils would damage neighboring blood cells and neutrophils themselves, resulting in the positive correlation between the levels of 8-OHdG in WBC and CPK activity in plasma. Further detailed study will be needed to demonstrate this hypothesis.

Generally, vigorous exercise induces two types of stress to the body: oxidative stress and physical and/or psychological stress. Dietary AA requirements are known to be increased during periods of physical and/or psychological stress, such as during exposure to intense heat or cold.[41] In this study, we investigated changes in plasma AA following vigorous exercise in rats. As shown in *Figure 1*, plasma AA concentration was significantly increased after exercise, then decreased thereafter. The change in the plasma AA concentration was negatively correlated with that in the adrenal glands. The concentration of AA in the adrenal glands of sedentary rats is approximately 700 times higher than that in the plasma.[12] The estimated change in the plasma AA concentration following exercise was quite consistent with the change in the whole amount of AA in the

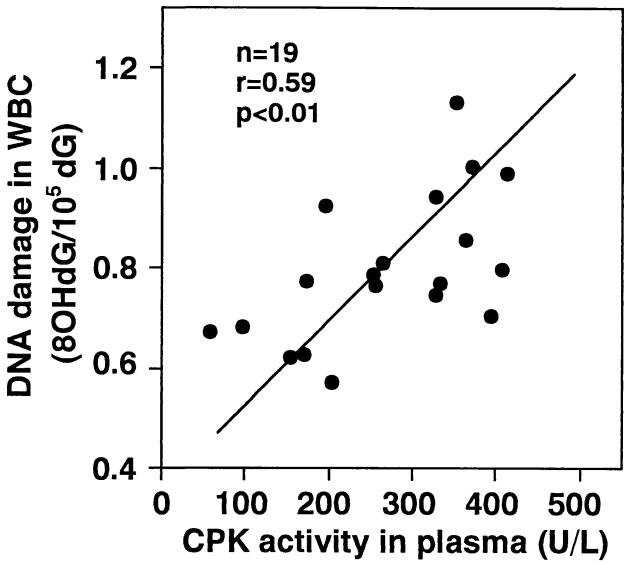

Figure 3 Correlation between plasma creatine phosphokinase (CPK) activity and DNA damage in white blood cells (WBC; experiment 2). This correlation was examined using the data from *Figure 2*. Each point represents one rat.

adrenal glands, as shown in *Table 2*. In addition, an increase in the weight of the adrenal glands, which is an indicator of stress, was detected after exercise (*Table 1*). Similar decreases in AA concentration and increases in the weight of adrenal glands have been reported following exercise in guinea pigs and rats.[14,42] Unlike humans, rats can synthesize AA in the liver.[43] However, in this study, the activity of hepatic l-gulono-γ-lactone oxidase, which is involved in AA synthesis, neither increased following the exercise trial nor correlated with the concentration of AA in the liver. Further, similar increases in plasma AA concentration after exercise have been reported in humans.[8–10,21] Consistent with our present findings, Gleeson et al.[8] found that plasma AA concentration was increased immediately after a 21 km race in male subjects and that this increase was significantly correlated to an increase in plasma cortisol. AA is required for the synthesis of norepinephrine from dopamine in the adrenal glands.[44] It has been shown that when stress is induced, AA is released from the adrenal glands into the blood concomitant with cathecolamine and cortisol[13,40] and that uptake of AA from the blood into the adrenal glands is reduced in the presence of adrenocorticotrophic hormone or cortisol.[45,46] In this study, no significant change in plasma vitamin E was detected due to exercise. These facts suggest that the major changes in plasma AA concentration after the intensive exercise trial were due to physical stress, which is known to influence both release and uptake of AA in the adrenal glands.

Concentration of AA in plasma is approximately 60 μM, whereas that in WBC is in mM. In this study, we did not evaluate AA in WBC, but Gleeson et al. [8] showed that AA in WBC is also increased after exercise as well as in plasma. The increases of AA concentration, especially in WBC, may influence the oxidative DNA damage in WBC after exercise. Further studies focussing on these issues are needed to clarify the mechanism of the oxidative DNA damage in WBC after a single bout of intensive exercise.

Table 3 Influence of one bout of downhill running on lipid peroxide and vitamin E in muscle (experiment 2)

	Control (sedentary)	Exercise	
		3 hr after	6 hr after
Vitamin E (nmol/g tissue)	16.4 ± 1.1	15.7 ± 0.7	18.1 ± 1.2
Lipid peroxides (TBARS) (nmol/g tissue)	40.1 ± 1.9	43.2 ± 3.2	43.0 ± 2.7

Values are mean ± SE for six to seven rats.
TBARS—thiobarubituric acid reacting substances.

References

1 Ames, B.N., Shigenaga, M.K., and Hagen, T.M. (1993). Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. USA* **90,** 7915–7922
2 Ji, L.L. (1995). Oxidative stress during exercise: Implication of antioxidant nutrients. *Free Radic. Biol. Med.* **18,** 1079–1086
3 Loft, S., Astrup, A., Buemann, B., and Poulsen, H.E. (1994). Oxidative DNA damage correlates with oxygen consumption in humans. *FASEB J.* **8,** 534–537
4 Sternfild, B. (1992) Cancer and the protective effect of physical activity: The epidemiological evidence. *Med. Sci. Sports Exerc.* **24,** 1195–1209
5 Blair, S.N., Kohl, H.W.D., Paffenbarger, R.S., Jr. Clark, D.G., Cooper, K.H., and Gibbons, L.W. (1989). Physical fitness and all-cause mortality. A prospective study of healthy men and women. *JAMA* **262,** 2395–2401
6 Frei, B. (1991). Ascorbic acid protects lipids in human plasma and low-density lipoprotein against oxidative damage. *Am. J. Clin. Nutr.* **54,** 1113S–1118S
7 Packer, J.E., Slater, T.F., and Willson, R.L. (1979). Direct observation of a free radical interaction between vitamin E and vitamin C. *Nature* **278,** 737–738
8 Gleeson, M., Robertson, J.D., and Maughan, R.J. (1987). Influence of exercise on ascorbic acid status in man. *Clin. Sci.* **73,** 501–505
9 Duthie, G.G., Robertson, J.D., Maughan, R.J., and Morrice, P.C. (1990). Blood antioxidant status and erythrocyte lipid peroxidation following distance running. *Arch. Biochem. Biophys.* **282,** 78–83
10 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. *J. Appl. Physiol.* **75,** 566–572
11 Umegaki, K., Higuchi, M., Inoue, K., and Esashi, T. (1998). Influence of one bout of intensive running on lymphocyte micronucleus frequencies in endurance-trained and untrained men. *Int. J. Sports Med.* **19,** 581–585
12 Behrens, W.A. and Madere, R. (1987). A highly sensitive high-performance liquid chromatography method for the estimation of ascorbic and dehydroascorbic acid in tissues, biological fluids, and foods. *Anal. Biochem.* **165,** 102–107
13 Daniels, A.J., Dean, G., Viveros, O.H., and Diliberto, E.J., Jr. (1983). Secretion of newly taken up ascorbic acid by adrenomedullary chromaffin cells originates from a compartment different from the catecholamine storage vesicle. *Mol. Pharmacol.* **23,** 437–444
14 Akamatsu, A., Whan, Y.W., Yamada, K., and Hosoya, N. (1986). The effect of high environmental temperature and exercise on the metabolism of ascorbic acid in rats. *Vitamins* (in Japanese) **60,** 199–204
15 Hartmann, A., Plappert, U., Raddatz, K., Grunert-Fuchs, M., and Speit, G. (1994). Does physical activity induce DNA damage? *Mutagenesis* **9,** 269–272
16 Poulsen, H.E., Loft, S., and Vistisen, K. (1996). Extreme exercise and oxidative DNA modification. *J. Sports Sci.* **14,** 343–346
17 Schiffl, C., Zieres, C., and Zankl, H. (1997). Exhaustive physical

exercise increases frequency of micronuclei. *Mutat. Res.* **389,** 243–246
18 Nakajima, M., Takeuchi, T., Takeshita, T., and Morimoto, K. (1996). 8-Hydroxydeoxyguanosine in human leukocyte DNA and daily health practice factors: Effects of individual alcohol sensitivity. *Environ. Health Perspect.* **104,** 1336–1338
19 Pilger, A., Germadnik, D., Formanek, D., Zwick, H., Winkler, N., and Rudiger, H.W. (1997). Habitual long-distance running does not enhance urinary excretion of 8- hydroxydeoxyguanosine. *Eur. J. Appl. Physiol.* **75,** 467–469
20 Sumida, S., Doi, T., Sakurai, M., Yoshioka, Y., and Okamura, K. (1997). Effect of a single bout of exercise and beta-carotene supplementation on the urinary excretion of 8-hydroxy-deoxyguanosine in humans. *Free Radic. Res.* **27,** 607–618
21 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
22 Niess, A.M., Hartmann, A., Grunert-Fuchs, M., Poch, B., and Speit, G. (1996). DNA damage after exhaustive treadmill running in trained and untrained men. *Int. J. Sports Med.* **17,** 397–403
23 Oh-ishi, S., Kizaki, T., Ookawara, T., Sakurai, T., Izawa, T., Nagata, N., and Ohno, H. (1997). Endurance training improves the resistance of rat diaphragm to exercise-induced oxidative stress. *Am. J. Respir. Crit. Care Med.* **156,** 1579–1585
24 Shacter, E., Beecham, E.J., Covey, J.M., Kohn, K.W., and Potter, M. (1988). Activated neutrophils induce prolonged DNA damage in neighboring cells. *Carcinogenesis* **9,** 2297–2304
25 Takeuchi, T., Nakajima, M., and Morimoto, K. (1994). Establishment of a human system that generates O2- and induces 8-hydroxydeoxyguanosine, typical of oxidative DNA damage, by a tumor promotor. *Cancer Res.* **54,** 5837–5840
26 Sen, C.K. (1995). Oxidants and antioxidants in exercise. *J. Appl. Physiol.* **79,** 675–686
27 Armstrong, R.B., Ogilvie, R.W., and Schwane, J.A. (1983). Eccentric exercise-induced injury to rat skeletal muscle. *J. Appl. Physiol.* **54,** 80–93
28 Smith, H.K., Plyley, M.J., Rodgers, C.D., and McKee, N.H. (1997). Skeletal muscle damage in the rat hindlimb following single or repeated daily bouts of downhill exercise. *Int. J. Sports Med.* **18,** 94–100
29 Umegaki, K., Yoshimura, M., Nishimuta, M., and Esashi, T. (1999). A practical method for determination of vitamin C in plasma by high-performance liquid chromatography with an electrochemical detector. *J. Jpn. Soc. Nutr. Food Sci.* (in Japanese) **52,** 107–111
30 Umegaki, K., Aoki, S., and Esashi, T. (1995). Whole body X-ray irradiation to mice decreases ascorbic acid concentration in bone marrow: Comparison between ascorbic acid and vitamin E. *Free Radic. Biol. Med.* **19,** 493–497
31 Kito, M., Ohishi, N., and Yagi, K. (1991). Micro-determination of L-gulono-gamma-lactone oxidase activity. *Biochem. Int.* **24,** 131–135
32 Yamaguchi, R., Hirano, T., Asami, S., Chung, M.H., Sugita, A., and Kasai, H. (1996). Increased 8-hydroxyguanine levels in DNA and its repair activity in rat kidney after administration of a renal carcinogen, ferric nitrilotriacetate. *Carcinogenesis* **17,** 2419–2422
33 Kikugawa, K., Kojima, T., Yamaki, S., and Kosugi, H. (1992). Interpretation of the thiobarbituric acid reactivity of rat liver and brain homogenates in the presence of ferric ion and ethylenediaminetetraacetic acid. *Anal. Biochem.* **202,** 249–255
34 Belcastro, A.N., Arthur, G.D., Albisser, T.A., and Raj, D.A. (1996). Heart, liver, and skeletal muscle myeloperoxidase activity during exercise. *J. Appl. Physiol.* **80,** 1331–1335
35 Wetzstein, C.J., Shern-Brewer, R.A., Santanam, N., Green, N.R., White-Welkley, J.E., and Parthasarathy, S. (1998). Does acute exercise affect the susceptibility of low density lipoprotein to oxidation? *Free Radic. Biol. Med.* **24,** 679–682
36 Pizza, F.X., Mitchell, J.B., Davis, B.H., Starling, R.D., Holtz, R.W., and Bigelow, N. (1995). Exercise-induced muscle damage: Effect on circulating leukocyte and lymphocyte subsets. *Med. Sci. Sports Exerc.* **27,** 363–370
37 Margaritis, I., Tessier, F., Richard, M.J., and Marconnet, P. (1997). No evidence of oxidative stress after a triathlon race in highly trained competitors. *Int. J. Sports Med.* **18,** 186–190
38 Cannon, J.G., Orencole, S.F., Fielding, R.A., Meydani, M., Meydani, S.N., Fiatarone, M.A., Blumberg, J.B., and Evans, W.J. (1990). Acute phase response in exercise: Interaction of age and vitamin E on neutrophils and muscle enzyme release. *Am. J. Physiol.* **259,** R1214–R1219
39 Asami, S., Hirano, T., Yamaguchi, R., Tomioka, Y., Itoh, H., and Kasai, H. (1996). Increase of a type of oxidative DNA damage, 8-hydroxyguanine, and its repair activity in human leukocytes by cigarette smoking. *Cancer Res.* **56,** 2546–2549
40 Pedersen, B.K., Bruunsgaard, H., Klokker, M., Kappel, M., MacLean, D.A., Nielsen, H.B., Rohde, T., Ullum, H., and Zacho, M. (1997). Exercise-induced immunomodulation—possible roles of neuroendocrine and metabolic factors. *Int. J. Sports Med.* **18,** S2–S7
41 Burri, B.J. and Jacob, R.A. (1997). Human metabolism and requirement for vitamin C. In *Vitamin C in Health and Disease* (L. Paker and J. Fuchs eds.), pp. 341–366, Marcel Dekker, Inc., New York, NY, USA
42 Hughes, R.E., Jones, P.R., Williams, R.S., and Weight, P.F. (1971). Effect of prolonged swimming on the distribution of ascorbic acid and cholesterol in the tissues of the guinea-pig. *Life Sci. [I]* **10,** 661–668
43 Banhegyi, G., Braun, L., Csala, M., Puskas, F., and Mandl, J. (1997). Ascorbate metabolism and its regulation in animals. *Free Radic. Biol. Med.* **23,** 793–803
44 Herman, H.H., Wimalasena, K., Fowler, L.C., Beard, C.A., and May, S.W. (1988). Demonstration of the ascorbate dependence of membrane-bound dopamine beta-monooxygenase in adrenal chromaffin granule ghosts. *J. Biol. Chem.* **263,** 666–672
45 Levine, M.A. and Pollard, H.B. (1983). Hydrocortisone inhibition of ascorbic acid transport by chromaffin cells. *FEBS Lett.* **158,** 134–138
46 Garcia, R. and Municio, A.M. (1990). Effect of Escherichia coli endotoxin on ascorbic acid transport in isolated adrenocortical cells. *Proc. Soc. Exp. Biol. Med.* **193,** 280–284