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Reduction of 8-Hydroxyguanine in Human Leukocyte DNA by Physical Exercise

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We investigated the effect of physical exercise on the level of 8-hydroxyguanine (8-OH-Gua), a form of oxidative DNA damage, and its repair activity in human peripheral leukocytes. Whole blood samples were collected by venipuncture from 21 healthy male volunteers (10 trained athletes and 13 untrained men), aged 19–50 years, both before and after physical exercise. Trained athletes showed a lower level of 8-OH-Gua ($2.4 \pm 0.5/10^6$ Gua, $p = 0.0032$) before exercise when compared to that of untrained men (6.2 ± 3.5). The mean levels of 8-OH-Gua of untrained subjects decreased significantly ($p = 0.0057$) from $6.2 \pm 3.5/10^6$ Gua (mean $\pm$ SD/10^6 Gua) to $3.3 \pm 1.4/10^6$ Gua after physical exercise. On the other hand, the mean levels of repair activity of untrained subjects significantly increased after exercise ($p = 0.0093$) from 0.037 ± 0.024 (mean DNA cleavage ratio $\pm$ SD) to 0.056 ± 0.036. In the trained athletes 8-OH-Gua level and its repair activity were not changed before and after the exercise. We also observed inter-individual differences in 8-OH-Gua levels and its repair activities. These results suggest that physical exercise causes both rapid and long-range reduction of oxidative DNA damage in human leukocytes, with individually different efficiencies.

Keywords: Oxygen radical, 8-hydroxyguanine, DNA repair, physical exercise

INTRODUCTION

It has been reported that, in the course of physical exercise, increased oxygen consumption is accompanied by an increase in the production of reactive oxygen species.[1] Therefore, physical exercise is predicted to cause oxidative DNA damage. However, a wide variety of animal and human studies have shown that physical exercise contributes to a reduction in cancer risk.[2–5] Is it plausible that physical exercise reduces cancer risk? We recently observed that forced exercise enhances oxidative DNA damage, but spontaneous exercise reduces it, in rat organs.[6] In this study, we investigated the effect of physical exercise on oxidative DNA damage in human

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peripheral leukocytes, using 8-OH-Gua and its repair activity as markers.

MATERIALS AND METHODS

Exercise Program and Sampling

Twenty-three male volunteers (19–50 yr) performed exercise requiring maximal oxygen uptake on a cycle ergometer, after passing a physical examination. Maximal oxygen uptake was measured by a symptom limited ramp cycle ergometer method with a gas analyzer and a turbine transducer to detect breath by breath tidal volume, O_2 consumption, and expired CO_2 content. Subjects were healthy, of normal weight-for-height, had usual dietary habits, and were non-smokers. Ten volunteers were moderately trained athletes, and the other volunteers (untrained subjects) performed occasional sports activities. We collected whole blood samples by venipuncture from these volunteers, both before and 5 min after exercise requiring maximal oxygen consumption.

Determination of 8-OH-Gua in Leukocyte DNA

The 8-OH-Gua level in the DNA was determined according to Nakae *et al.*[7] with some modifications. Briefly the samples were homogenized in lysis buffer with a potter-type homogenizer, and the nuclear DNA in the homogenate was extracted using the DNA Extractor WB Kit (Wako, Japan). The extracted nuclear DNA was digested with nuclease P1 and acid phosphatase (37°C for 30 min), and injected onto a HPLC column (BECKMAN, Ultrasphere-ODS, 5 μm 4.6 × 250 nm) equipped with an ECD (ESA Coulochem II, detector 1: 0.15 V, detector 2: 0.30 V, guard cell: 0.35 V).

Measurement of 8-OH-Gua Repair Activity

Fifteen ml of the collected blood samples were mixed with a Ficoll-Paque solution to separate the blood leukocyte fractions. The crude extracts (10 μg total protein) of leukocyte fractions from blood samples were incubated with 0.05 pmol of the ds-DNA substrate (5′-^{32}p GGATC-CAGCG*ATCCATACGTAT-3′, G*: 8-OH-Gua). After 2 ethanol precipitations, 10 μl of the sample were applied to a 20% denaturing polyacrylamide gel for electrophoresis. After electrophoresis, the autoradiograms were processed and the radioactivity was analyzed using a Bioimage analyzer system (Fujix BAS 2000).

RESULTS AND DISCUSSION

Figure 1 summarizes the effect of the endurance exercise by cycle ergometer on the 8-OH-Gua level in human leukocyte DNA from 21 donors. Trained athletes showed a lower level of 8-OH-Gua (mean ± SD, $2.4 \pm 0.5/10^6$ Gua, $p = 0.0032$) before exercise when compared to that of untrained men ($6.3 \pm 3.6/10^6$ Gua). Five minutes after exercise, 8-OH-Gua level of the untrained subjects decreased to $3.3 \pm 1.4/10^6$ Gua. The decrease was statistically significant ($p = 0.0057$).

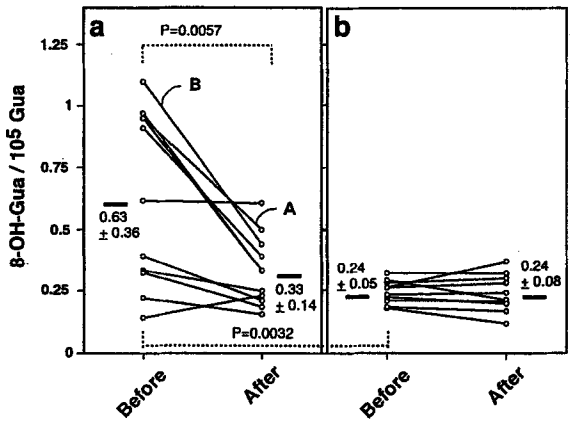

FIGURE 1 Effect of physical exercise on the level of 8-OH-Gua in human leukocyte DNA. (a) untrained men; (b) trained athletes. The 8-OH-Gua level was calculated as the number per 10^5 Gua. Mean values ± SD are shown. The significance of the differences between the data of before and after the exercise was evaluated by a paired *t*-test and that between the data of untrained and trained men before the exercise was by an unpaired *t*-test.

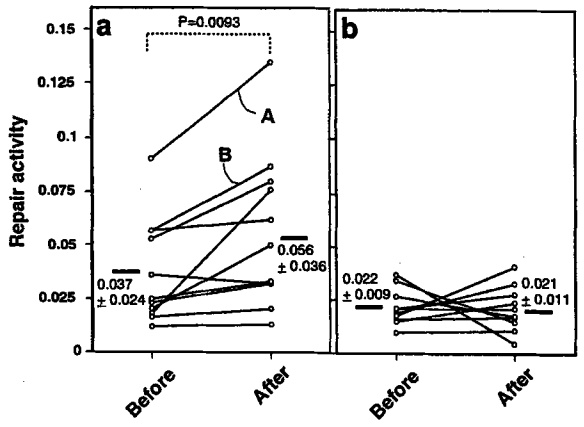

FIGURE 2 Effect of physical exercise on the 8-OH-Gua repair activity in human leukocytes. (a) untrained men; (b) trained athletes. Mean values ± SD are shown. The significance of the differences between the data of before and after the exercise was evaluated by a paired *t*-test and that between the data of untrained and trained men before the exercise was by an unpaired *t*-test.

Figure 2 shows the effect of the endurance exercise on the 8-OH-Gua repair activity in the human leukocytes. Before the exercise, the mean repair activity value of the untrained subjects was 0.037 ± 0.024 (mean ± SD), and 5 min after the exercise, it increased to 0.056 ± 0.036. The mean levels of repair activity significantly increased ($p = 0.0093$).

A 9.3-fold inter-individual difference was observed in the 8-OH-Gua repair activity, and a 7.8-fold inter-individual difference was observed in the 8-OH-Gua levels of the pre-exercise group. Particularly, higher levels of 8-OH-Gua in the pre-exercise group were found in untrained men (Figure 1). It is interesting to examine whether the increase in the repair activity correlates with the decrease in the 8-OH-Gua level in each individual. In particular, donor A showed the highest 8-OH-Gua repair activity before exercise, and its increase after exercise was also high (Figure 2(a)). The 8-OH-Gua level in the leukocyte DNA from donor A was also high before exercise (Figure 1(a)). A similar tendency was observed in donor B. On the other hand, both lower levels of 8-OH-Gua and its repair-activity were found in the moderately trained athletes before and after the exercise (Figures 1(b) and 2(b)). These results suggest that physical exercise enhance the cellular antioxidant defence enzyme system, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPx).

Living organisms are continuously exposed to reactive oxygen species, as a consequence of biochemical reactions and exposure to external factors, such as ionizing radiation and chemical carcinogens. 8-OH-Gua is one of the major forms of oxidative DNA damage,[8] and has been proposed as a key biomarker relevant to carcinogenesis.[9] Repair systems for 8-OH-Gua exist in *E. coli*[10] and mammalian cells[11,12] and are enhanced by oxidative stress.[13,14] In *E. coli*, the 8-OH-Gua repair enzyme is induced immediately 5 min after a shift from anaerobic to aerobic growth conditions.[14] The present study indicates that physical exercise causes a reduction of oxidative DNA damage in human leukocytes, by inducing its repair activity or by inducing antioxidant enzyme systems. It has been reported that 8-OH-Gua levels in human lymphocyte DNA are reduced after swimming.[15] The repair activity for oxidative DNA damage may increase not only in blood cells, but also in cells of various organs, because the oxygen concentration is known to increase in most human organs with physical exercise.[1,16] Regular physical exercise may, therefore, maintain a low level of oxidative DNA damage in various organs. It would be interesting to study the effects of various life styles on the 8-OH-Gua level in human leukocyte DNA as a new trial for the molecular epidemiology of cancer.

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References

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

[2] D. Kritchevsky (1990) Influence of caloric restriction and exercise on tumorigenesis in rats. *Proceedings of the Society of Experimental Biology and Medicine*, **93**, 35–38.

[3] R.J. Shephard (1990) Physical activity and cancer. *International Journal of Sports Medicine*, **11**, 413–420.

[4] I.M. Lee and R.S. Jr. Paffenbarger (1994) Physical activity and its relation to cancer risk: a prospective study of college alumni. *Medicine and Science in Sports and Exercise*, **26**, 831–837.

[5] E. Giovannucci, A. Ascherio, E.B. Rimm, G.A. Colditz, M.J. Stempfer and W.C. Willett (1995) Physical activity, obesity, and risk for colon cancer and adenoma in men. *Annals of Internal Medicine*, **122**, 327–334.

[6] S. Asami, T. Hirano, R. Yamaguchi, Y. Tsurudome, H. Itoh and H. Kasai (1998) Effects of forced and spontaneous exercise on 8-hydroxydeoxyguanosine levels in rat organs. *Biochemical and Biophysical Research Communications*, **243**, 678–682.

[7] D. Nakae, Y. Mizumoto, E. Kobayashi, O. Noguchi and Y. Konishi (1995) Improved genomic/nuclear DNA extraction for 8-hydroxydeoxyguanosine analysis of small amounts of rat liver tissue. *Cancer Letters*, **97**, 233–239.

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

[9] H. Kasai, S. Nishimura, Y. Kurokawa and Y. Hayashi (1987) Oral administration of the renal carcinogen, potassium bromate, specifically produces of 8-hydroxydeoxyguanine in rat target organ DNA. *Carcinogenesis*, **8**, 1959–1961.

[10] M.H. Chung, H. Kasai, D.S. Jones, H. Inoue, H. Ishikawa, E. Ohtsuka and S. Nishimura (1991) An endonuclease activity of *Escherichia coli* that specifically removes 8-hydroxyguanine residues from DNA. *Mutation Research*, **254**, 1–12.

[11] M.H. Chung, H.S. Kim, E. Ohtsuka, H. Kasai, F. Yamamoto and S. Nishimura (1991) An endonuclease activity in human polymorphonuclear neutrophils that removes 8-hydroxyguanine residues from DNA. *Biochemical and Biophysical Research Communications*, **178**, 1472–1478.

[12] F. Yamamoto, H. Kasai, T. Bessho, M.H. Chung, H. Inoue, E. Ohtsuka, T. Hori and S. Nishimura (1992) Ubiquitous presence in mammalian cells of enzymatic activity specifically cleaving 8-hydroxyguanine-containing DNA. *Japanese Journal of Cancer Research*, **83**, 351–357.

[13] R. Bases, W.A. Franklin, T. Moy and F. Mendenz (1992) Enhanced excision repair activity in mammalian cell after ionizing radiation. *International Journal of Radiation Biology*, **62**, 427–441.

[14] H.S. Kim, Y.W. Park, H. Kasai, S. Nishimura, C.H. Park, K.H. Choi and M.H. Chung (1996) Induction of *E. coli* oh^{8}Gua endonuclease by oxidative stress: its significance in aerobic life. *Mutation Research*, **363**, 115–124.

[15] T. Inoue, Z. Mu, K. Sumikawa, K. Adachi and T. Okochi (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.

[16] R.R. Jenkins (1988) Free radical chemistry, relationship to exercise. *Sports Medicine*, **5**, 156–170.