INFLUENCE OF ENDURANCE RUNNING ON PLASMA 8-HYDROXY-DEOXYGUANOSINE LEVELS IN HUMANS

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Abstract

We have determined the plasma 8-hydroxy-deoxyguanosine (8-OHdG) levels during endurance exercise. Eight untrained male subjects completed a 10-km run. Plasma 8-OHdG, total coenzyme Q10 (CoQ10), ubiquinol, and thiobarbituric acid (TBA) concentrations were measured after exercise. Plasma 8-OHdG concentrations significantly decreased immediately after (0.21 ± 0.13 ng/ml, p < 0.01) and 1 hr after (0.23 ± 0.09 ng/ml, p < 0.05) the run compared to the resting values (0.36 ± 0.09 ng/ml). Both plasma CoQ10 and ubiquinol concentrations significantly increased (p < 0.05) immediately after the run compared to the resting values. On the other hand, plasma TBA concentrations did not change significantly at any point after the run. These results suggest that, during and after submaximal endurance exercise in this study, an augmented antioxidant defence system such as CoQ10 might thus play a role in the decrease of 8-OHdG in the plasma, and that exercise might stimulate the repair of oxidative damage to DNA.


Key word: 8-hydroxy-deoxyguanosine, coenzyme Q10, thiobarbituric acid, oxidative damage, endurance exercise

Introduction

It is well known that the energy demand during physical exercise markedly increases oxygen uptake and supply to active tissues, which may increase the rate of reactive oxygen species (ROS), such as superoxide, hydroxyl radical and singlet oxygen.14 The imbalance between the increased ROS generation and the scavenging capacity of the host is termed oxidative stress, which has been gathering attention as being associated with many pathological processes.15 Oxidative stress during physical exercise damages normal cell function, such as proteins, DNA, and membrane lipids.

8-hydroxy-deoxyguanosine (8-OHdG) is a product of oxidative DNA damage following specific enzymatic cleavage after 8-hydroxylation of the guanine base. Singlet oxygen, photodynamic action, or hydroxyl radicals are responsible for the formation of 8-OHdG. The oxidized DNA is continuously repaired and the excised deoxyribonucleosides are excreted in the blood and urine. At present, 8-OHdG is one of the most commonly used markers for evaluating oxidative damage in the whole body, because the excretion of 8-OHdG reflects the integrated rate of oxidative DNA damage and the repair of DNA. Increased 8-OHdG levels have been observed in human leukocytes after a half-marathon8 and in urinary concentrations during a four-day supramarathon period12. However, contradictory data was also reported by different authors. The urinary excretion of 8-OHdG did not change significantly after a single bout of maximal treadmill running and cycling exercise13. Viguie et al.15 observed no change in urinary 8-OHdG excretion for three days after 90 min of moderate exercise at 65% peak $O_2$ uptake. These discrepancies may partly arise from the method used to express 8-OHdG urinary excretions13. Because the collection of urine for prolonged periods may present some practical problems, spot

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urine samples have been sometimes collected for creatinine and a correction procedure using the amount of creatinine excreted has been used as a reference marker. Differences in exercise duration and intensity also seem to contribute to inconsistent results and training may influence susceptibility to oxidative injury by some components of the antioxidant system.

To our knowledge, no one has reported changes in the blood 8-OHdG concentrations after exercise. Information on oxidative DNA damage obtained from blood samples is very important, because it indicates the rate of oxidative damage and the repair of DNA much more precisely than urine samples. Therefore, the aim of the present study was to evaluate plasma 8-OHdG levels as a marker of oxidative damage to DNA during and after a single bout of endurance running. To elucidate the mechanism of the antioxidative capacity during endurance exercise, total coenzyme Q10 (CoQ10) and ubiquinol (reduced form of CoQ10) in the plasma were also determined.

Methods

Subjects

Eight non-smoking male students (19–24 years old) volunteered to participate in this study. The purpose and protocol of the study and possible risks were fully explained to the subjects before each one signed an informed consent agreement. Each subject met the following eligibility requirements: 1) did not currently smoke; 2) took no medications, vitamins or minerals; 3) did not participate in any specific exercise training for a year; 4) passed medical evaluations for the absence of cardiovascular, pulmonary, or metabolic diseases, lower back problems, or existent musculoskeletal conditions that might worsen with running; 4) had a negative maximal treadmill stress test for inducible myocardial ischemia or cardiac dysrhythmias. Physical characteristics and the mean running time of the subjects were shown in Table 1.

Experimental Design

The subjects performed a 10-km run on a road at 75% heart rate max (HRmax). Each subject monitored his own heart rates continuously using a heart monitor (S610i, Polar Electro, Finland) and thus kept his heart rates between 70 and 80% of HRmax during the 10-km running.

Blood sampling and analysis

Blood samples were collected from an antecubital vein using a 21-gauge needle at rest, immediately after, and 24 hrs after the 10-km run. The samples were centrifuged at 3000 rpm for 10 min, and plasma was divided into aliquots and stored at −80°C until analysis. Plasma 8-OHdG levels were determined with a competitive enzyme-linked immunosorbent assay kit (8-OHdG check, highly sensitive 8-OHdG check, Japan Institute for the Control of Aging, Shizuoka, Japan). Total CoQ10 and ubiquinol concentrations were measured by high-performance liquid chromatography (HPLC). The lipid peroxidation products in the plasma were evaluated by the thiobarbituric acid (TBA) reaction method.

Statistical analysis

A repeated measures analysis of variance (ANOVA) was performed using the Scheffe–post hoc comparison to test for significant differences in the means. The level of significance was set at p < 0.05. All data are represented as mean and standard deviation (SD).

| Table 1. Physical characteristics and running time of the subjects. |
|---|---|---|---|---|---|
| N | Age (years) | Height (cm) | Mass (kg) | %Fat (%) | 10km Running Time (min) |
| Mean | 8 | 21.8 | 172.4 | 67.7 | 20.9 | 65.0 |
| SD | 2.1 | 5.6 | 8.8 | 4.8 | 10.0 |
Results

Fig. 1 shows the changes of plasma 8-OHdG and TBA concentrations at rest and after exercise. Plasma 8-OHdG concentrations tended to decrease after exercise, and a significant difference was found immediately after (0.21 ± 0.13 ng/ml; mean ± SD, p < 0.01) and 1 hr after (0.23 ± 0.09 ng/ml, p < 0.05) the 10-km run compared to the resting values (0.36 ± 0.09 ng/ml). The 8-OHdG concentrations were still lower 24 hrs after the 10-km run than at the resting levels, but there was no significant difference between the resting values and the values after 24 hrs. On the other hand, plasma TBA concentrations did not change significantly at any point after the 10-km run.

Fig. 2. Plasma total CoQ10 (A) and ubiquinol (B) concentrations at rest, immediately after, 1 and 24 hrs after the 10-km run. Values are means ± SD. Significantly different from rest; *(p < 0.05)

Discussion

We examined the effects of the 10-km run on blood 8-OHdG levels and antioxidant status in humans. A previous study has demonstrated a close association between oxidative DNA damage as assessed by the urinary excretion of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) and oxygen consumption in healthy premenopausal women.
Therefore, we hypothesized that the 10-km submaximal running would induce sufficient oxidative stress to alter the profiles of the antioxidants and increase the indexes of oxidative DNA damage in the plasma. However, we found significantly lower 8-OHdG levels after the 10-km run compared to the resting values. Additionally, plasma lipid peroxide levels did not change significantly after the exercise. These results indicate that the oxidative stress incurred during and after the 10-km run was insufficient to result in increased oxidative damage to DNA. Our data partly correlated with Viguie et al.\textsuperscript{15} who observed a tendency of a decrease in 8-OHdG excretion with no significant change in human urine, a marker of oxidative RNA damage, for three days after 90 min of moderate exercise at 65% peak O\textsubscript{2} uptake. Sumida et al.\textsuperscript{13} also reported no significant changes in the urinary excretion of 8-OHdG after a single bout of maximal treadmill running and cycling exercise. A recent study has reported that the two DNA repair enzymes, hOGG1 and MTH1, were slightly up regulated upon marathon exercise, and concluded that the up-regulation of DNA repair enzymes might be an important part of the regular exercise induced adaptation process\textsuperscript{11}. Because the excretion of 8-OHdG reflects the integrated rate of oxidative DNA damage and the repair of DNA in the whole body, significantly lower 8-OHdG levels after the 10-km run in the present study can be interpreted to suggest that exercise might stimulate the repair of oxidative damage to DNA. In contrast to our results, increased 8-OHdG levels have been observed in human leukocytes after a half-marathon\textsuperscript{8} and in urinary concentrations during a four-day supra-marathon period\textsuperscript{12}. These discrepancies may partly depend on differences in exercise duration and intensity. Whether other inducible repair mechanisms exist for oxidative protein damage remains to be established, although increased proteasome activity after exercise has been reported\textsuperscript{9}. This may, however, depend on the type of exercise and time after exercise\textsuperscript{6,16}. Therefore, the duration and intensity of exercise performed in this study might be shorter and lower to increase oxidative damage to DNA. Furthermore, the differences in samples used and the method used to express 8-OHdG may be other reasons for these discrepancies, because the present study is the first study reporting changes in human blood 8-OHdG levels after submaximal endurance exercise. On the other hand, the site where 8-OHdG formation took place during and after the exercise could not be exactly identified from our data. Exercising skeletal muscles seems to be the most likely candidate for DNA damage, however, oxidative damage to other organs and exercise-induced inflammation during and after exercise cannot be excluded. Thus, plasma 8-OHdG concentrations in this study might represent a whole body response, albeit organ specific accumulation of DNA damage could occur\textsuperscript{12}.

In the present study, observed increases in plasma CoQ10 and ubiquinol concentrations indicate the presence of oxidative stress during the 10-km submaximal run. CoQ10 is a lipid-soluble compound comprised of a redox-active quinoid nucleus and a hydrophobic side chain containing a number of monounsaturated trans-isoprenoid units. It has been well known that CoQ10 acts as an electron carrier of the respiratory chain in mitochondria and prevents lipid peroxidation in biological membranes. Ubiquinol is the two-electron reduction product of CoQ10 and is about as effective in preventing peroxidative damage to lipids as \( \alpha \)-tocopherol, which is considered the best lipid-soluble antioxidant in humans\textsuperscript{3}. Faff et al.\textsuperscript{27} have demonstrated that CoQ10 supplementation in rats markedly suppresses exercise-induced lipid peroxidation in the liver, heart and gastrocnemius muscle. Futhermore, previous studies\textsuperscript{5,10} have shown that the level of plasma free radical scavenging properties such as tocopherol, ascorbate, CoQ10 and whole blood glutathione concentrations increased during intensive exercise, and that mobilization of those could help to prevent lipoperoxidation phenomena occurring in exercising skeletal muscle. Therefore, changes in plasma total CoQ10 and ubiquinol concentrations can be
interpreted to suggest that despite ROS formation during the 10-km run there exists sufficient antioxidant buffering capacity in the subjects to protect against ROS-induced damage in exercising skeletal muscle and other tissues.

Our results indicate that healthy untrained young men seem to be well protected from oxidative DNA damage during and after 10-km submaximal running. We conclude that, during and after submaximal endurance exercise in this study, an augmented antioxidant defense system such as CoQ10 might thus play a role in the decrease of 8-OHdG in the plasma, and that exercise might stimulate the repair of oxidative damage to DNA.

References


