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CHANGES IN URINE 8-HYDROXYDEOXYGUANOSINE LEVELS OF SUPER-MARATHON RUNNERS DURING A FOUR-DAY RACE PERIOD

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Summary

We have determined the urinary 8-hydroxydeoxyguanosine (8-OHdG) levels of five well trained supra-marathon runners during a four-day race. The daily running distances of the four-day race were the following; 93 km, 120 km, 56 km and 59 km, respectively. Pre-race and post-race urine samples were collected on each day and analyzed by a monoclonal antibody technique. The urinary 8-OHdG content increased significantly on the first day and tended to decrease from the third day. By the fourth day 8-OHdG content was significantly less than measured on the first three days. The serum creatine kinase activity changed in a similar fashion, showing a large increase ($P<0.001$) up to the third day when it decreased significantly from the peak value ($P<0.05$). We conclude that extreme physical exercise causes oxidative DNA damage to well trained athletes. However, repeated extreme exercise-induced oxidative stress does not propagate on increase of urinary 8-OHdG, but rather causes an adaptation leading to normalization of oxidative DNA damage.

Key Words: prolonged exercise, DNA damage, oxidative stress, free radicals, adaptation

Regular physical exercise is known to be beneficial to health, by reducing risk of a number of pathological disorders and extending the life-span of humans and laboratory animals (1, 2). However, it has been claimed that some types of prolonged physical exertion are detrimental to health because reactive oxygen species (ROS) are generated excessively by enhanced oxygen consumption (3) or by activation of the xanthine dehydrogenase/oxidase system (4). Although, ROS are important contributors and regulators of normal physiological processes, such as redox signaling and muscle contraction (5), significant increases in ROS concentration seriously could jeopardize normal cell function. Indeed, the cytotoxic effects of ROS include the oxidative damage of cellular DNA. 8-hydroxydeoxyguanosine (8-OHdG) is one of the DNA damaged products formed by the interaction of cellular genetic material and ROS (6). 8-OHdG is a very dangerous DNA adduct because its presence in the DNA template causes alpha-polymerase to miscode incorporation of nucleotides in the replicated strands (7).

A recent study of Asami et al. (8) suggests that enforced exercise causes increases in tissue 8-OHdG content in rats, and it has also been reported that muscle soreness results in increased 8-OHdG concentration in human skeletal muscle (9). On the other hand, the muscle 8-OHdG content of trained animals decreases (10) and the urinary concentration of 8-OHdG is not elevated in trained humans, after a single bout of exercise (11, 12), which may indicate an adaptive response. The increased resistance to oxidative challenge and the improved repair system following oxidative damage is an important part of the exercise-induced adaptation process (9). However it cannot be excluded that even very well trained athletes suffer from oxidative damage after strenuous exercise. Therefore, the aim of the present study was to assess the oxidative DNA damage, measured by 8-OHdG, in urine samples of very well trained supra-marathon runners during a four-day, 328 km race.

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Methods

Five male supra-marathon runners (age: 26-45) volunteered for the study after being informed of the aim and methods. The protocol of the study was reviewed and approved by the ethics committee and was done according to guidelines of The Declaration of Helsinki for Research on Human Subjects. The runners participated in the 7th Vienna-Budapest supra-marathon race. The daily running distance of the four-day race were the following; 93 km, 120 km, 56 km and 59 km, respectively. The athletes followed their own nutritional protocol to achieve their best results during the competition.

Sampling and assays. Venous blood was collected at rest and 1 h after finishing each daily running distance. After centrifugation, serum was immediately stored at -40 °C. The urine samples were collected in the last 12 h before the start of the competition and between each running (the time period ranged between 12-18 h). The collection of 24-h urine collection was impossible due to the schedule of the competition. Urine samples were centrifuged at 2000g for 10 min and stored at -40 °C. The urine content of the competitors have changed during the four-day race possible due to the difference in recovery period and dehydration, however athletes did their best to avoid massive fluid loss. By consistantly drinking water at selected sites supplied by the race organizers. The 8-OHdG level from the urine samples were analyzed by enzyme linked immuno assay (9, 13) as described by the supplier (Genox Corp. Baltimore, USA). In brief, 50 μl of reconstituted primary antibody was added to each well. After 1 h incubation at 37 °C the wells were washed three times in washing solution. Then the secondary antibody was added and incubated again for 1 h at 37 °C. After washing by washing solution chromatic enzyme substrate solution was added and incubated for 15 min and then the reaction was terminated. The absorbance was read at 450 nm using a microplate reader. Three wells were used for each samples and the mean value was used for statistical purposes. The results expressed as 8-OhdG ng/ml due to the applied technique and this might be a downside of this method. The exact determination of modified DNA bases are very difficult and we made everything to minimize of artifacts.

Serum creatine kinase (CK), a marker of muscle damage, was measured with a radioimmunoassay.

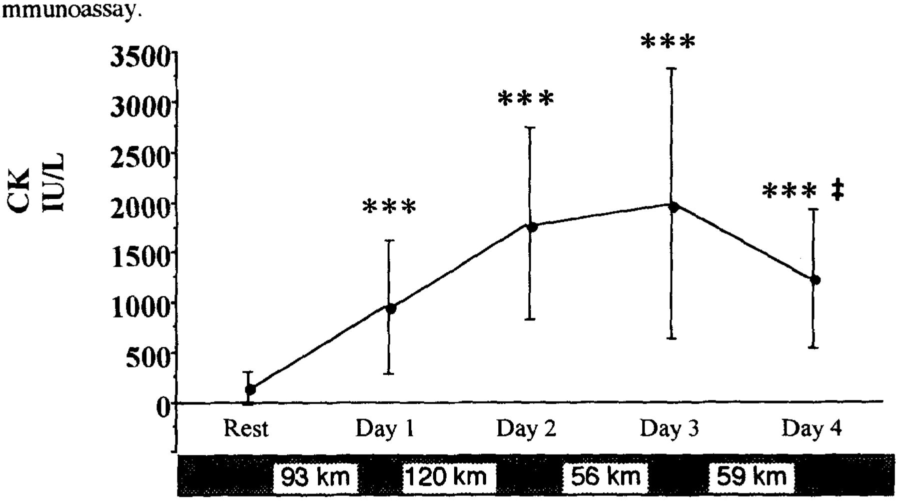

Fig. 1.

Blood samples were taken before the race (rest) and 1 h after the race in each day of the competition. Values are means ±SD of five runners. ***P < 0.001 vs. rest, ‡P < 0.05 vs. third day.

Statistical significance of the data was assessed by Anova, followed by Scheffe's post-hoc test. The significance was set at $P < 0.05$.

Results

The CK concentration increased significantly after the first, second, and third day ($P < .0001$) and decreased after the fourth bout (Fig 1). 8-OHdG concentration was elevated on the first day after running (Fig 2) and declined after the third day and was significantly less than that measured after running on the previous three days.

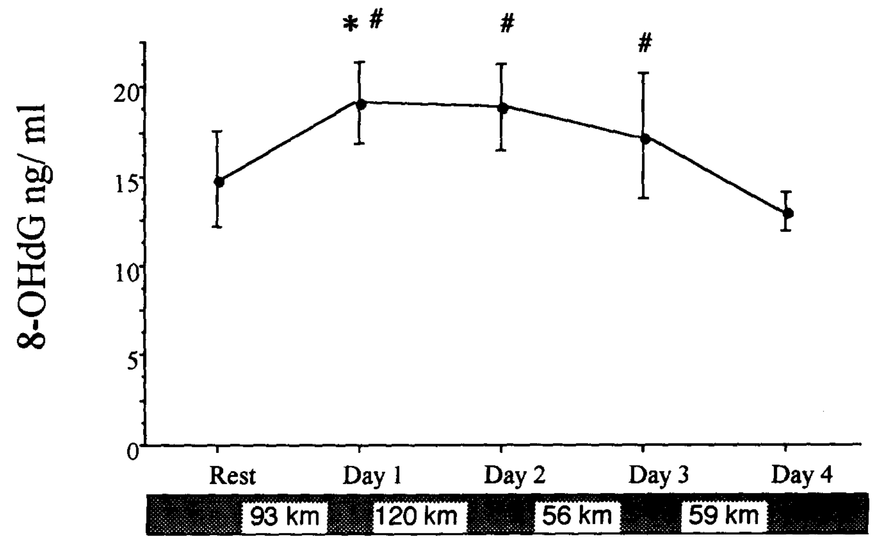

Fig. 2.

Urine samples were collected in the last 12 h before the start of the race (rest) and tin he period between each run. Values are means ±SD of five runners. $*P < 0.05$ vs. rest, $\#P < 0.05$ vs. day four

Discussion

The present study shows that after running a distance of 328 km during a four-day competition there is an increase in oxidative DNA damage, as measured by 8-OHdG, in urine samples of the runners. On the other hand, the pattern of the changes in urinary 8-OHdG levels and the activity of serum CK suggest the involvement of an adaptive process even during the race. The significant increase in 8-OHdG content and muscle damage marker, CK, after 93 km of running might be due to the unaccustomed size of the exercise stress. On the other hand, on the second day no further increase was observed, even though the running distance was increased to 120 km (almost three times longer as compare to a marathon!). Interestingly, a decrease was observed in 8-OHdG content and CK activity on days three or four following running distances of 56 and 59 km. The changes in the pattern of these two damage markers allow us to suggest that some adaptation process occurred. It is known that exercise increases the production of ROS and this could lead to the upregulation of the antioxidant system and the resistance to oxidative stress (14) and/or a reduction in the concentration of oxidative damage markers in the urine (11, 12, 15). This adaptive process might be very rapid and might be stimulated by damage and by-products of damage (3, 11, 16). It is believed that 8-OHdG is excreted very efficiently because of its toxic, mutagenic potential

(13). It is suggested that with the present experimental conditions the urinary levels of 8-OHdG represent a general oxidative damage marker for the whole body. The site of damage cannot be exactly identified from the data of this study. Obviously skeletal muscle seems to be the most likely candidate for DNA damage (the CK data also indicate muscle damage). However, oxidative damage to other organs like liver and kidney cannot be ruled out. It has been shown that liver and kidney suffer lipid peroxidation in untrained rats following exhaustive exercise (17). In addition, cytokine-derived ROS-generated oxidative damage on DNA can not be excluded, since it is known that strenuous exercise might cause inflammation (18). Although, there are number of ROS generating systems during strenuous exercise the urinary 8-OHdG concentration might represent a whole body response, albeit organ specific accumulation of DNA damage could occur.

All the subjects were well trained athletes and it seems that even their antioxidant/repair systems were not able to cope with the extreme challenge of the first day. On the second day we did not observe any alteration compared with the first day which suggests an active induction of the antioxidant/repair system. After the third and fourth days decreases of 8-OHdG content might indicate that the antioxidant and repair systems were able to efficiently prevent and eliminate the increases and increased levels of 8-OHdG.

Taken together these observations suggest that repeated extreme exercise-induced oxidative stress does not propagate an increase of urinary 8-OHdG, but rather causes an adaptation that leads to normalization of oxidative DNA damage.

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