



Marathon running alters the DNA base excision repair in human skeletal muscle

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Abstract

Reactive oxygen and nitrogen species generated either as products of aerobic metabolism or as a consequence of environmental mutagens, oxidatively modify DNA. Formamidopyrimidine-DNA glycosylase (Fpg) and endonuclease III (endo III) or their functional mammalian homologues repair 7,8-dihydro-8-oxoguanine (8-oxoG) and damaged pyrimidines, respectively, to curb the deleterious effects of oxidative DNA alterations. A single bout of physical exercise can induce oxidative DNA damage. However, its effect on the activity of repair enzymes is not known. Here we report that the activity of a functional homolog of Fpg, human 8-oxoG DNA glycosylase (hOGG1), is increased significantly, as measured by the excision of ³²P labeled damaged oligonucleotide, in human skeletal muscle after a marathon race. The AP site repair enzyme did not change significantly. Despite the large individual differences among the six subjects measured, data suggest that a single-bout of aerobic exercise increases the activity of hOGG1 which is responsible for the excision of 8-oxoG. The up-regulation of DNA repair enzymes might be an important part of the regular exercise induced adaptation process.

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Introduction

Mammalian DNA is exposed to continuous attack by reactive oxygen species (ROS), resulting in oxidative damage if the production of ROS exceeds the body's ability to repair and protect [1]. In addition, endogenous and exogenous agents such as radiation, genotoxic chemicals and smoking are known to be linked to increased oxidative DNA damage [1]. Hence protective systems have been developed to maintain the vital integrity of DNA. Among the protective systems, base excision repair (BER) is specialized to repair the damaged bases. The DNA *N*-glycosylases involved in BER after the recognition of modified bases, flip them and cleave their *N*-glycosylic bond, which results in formation of an apurinic/apyrimidinic (AP) site [2]. BER is initiated with excision of damaged bases by numerous DNA glycosylase/AP lyases, which cleave a wide range of substrates. However, enzymes have preferred substrates. The 8-oxoguanine (8-oxoG) is repaired primarily by 8-oxoG-DNA glycosylase (OGG1) in mammalian cells and by formamidopyrimidine-DNA glycosylase (Fpg) in bacteria [3]. Formamidopyrimidines, derived from purines by ROS, are also mutagenic like 8-oxoG and in bacteria are repaired by endonuclease III (endo III). The mammalian homolog of the Endo III is hNTH1, which catalyses the excision of DNA damage including *Cis-trans*-thymine glycol, 5,6-dihydrothymine, 5,6-dihydrouracil, 5-hydroxyuracil, and AP sites [2,4]. The proper function of OGG1 and hNTH1 is important to prevent the mutagenic effects of DNA damage [1].

It is known that a single bout of physical exercise increases the production of ROS, which could lead to oxidative damage to DNA [5–7]. On the other hand, regular exercise causes adaptations, which decrease the incidence of a number of oxidative stress related diseases [8]. Therefore, it appears unlikely that regular physical exercise induced oxidative DNA modification results in an increased base-level of oxidative DNA damage.

Exhaustive running has been shown to increase oxidative DNA damage, as measured by 8-oxodG content from leukocytes or urine samples of runners [9,10]. One of the suggested sources of the oxidative damage is skeletal muscle, since oxygen flow increases many fold during intensive exercise. Therefore, the present study was driven by the hypothesis that exhaustive running, such as the marathon, alters the activity of DNA glycosylases, including hOGG1 and endo III in skeletal muscle.

Methods

Six trained male physical education students from Semmelweis University, Budapest (age 22–24) 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 the guidelines of The Declaration of Helsinki for Research on Human Subjects. The runners participated in the Budapest marathon race. Needle biopsy samples from the quadriceps femoris muscles were taken using the method described earlier [11]. The sampling was done four days before the race, after a day of rest, to obtain data for the control level and 16–18 h after the marathon race. Sampling immediately after the marathon race was not possible for technical reasons. It has been shown, using human lung alveolar cells, that the 8-oxoG repair enzyme activity peaks 18 h after exposure to a carcinogen [12].

Excision assays

The obtained biopsy samples were homogenized with buffer containing 20 mM of Tris (pH 8.0), 1 mM of EDTA, 1 mM of dithiothreitol, 0.5 mM of spermidine, 0.5 mM spermine, 50% glycerol and protease inhibitors. Homogenates were rocked for 30 min after the addition of a 1/10 vol/vol 2.5 M of KCl and centrifuged at 14,000 rpm for 30 min. The supernatant was aliquoted and stored at -80°C . The protein levels were measured by the BCA method. The assay was carried out according to the protocol described by Cardozo-Peaez et al. [13]. In brief, twenty picomoles of synthetic probe containing either 8-oxoG or AP site (Trevigen, Gaithersburg, MD, USA) were labeled with P^{32} at the 5' end using polynucleotide T4 kinase (Boehringer Mannheim, Germany). For the nicking reaction, protein extract (2–4 μg) was mixed with 20 μg of a reaction mixture containing 0.5 M of *N*-[2-hydroxyethyl]piperazine-*N'*-[ethanesulfonic acid], 0.1 M EDTA, 5 mM of dithiothreitol, 400 mM KCl, purified BSA and labeled probe (approximately 2000 cpm). The reaction was carried out at 30°C for 5–15 min and stopped by placing the solution in ice. Then 30 μl chloroform were added and samples were centrifuged and 15 μl taken and mixed with loading buffer containing 90% formamide, 10 mM NaOH, and blue-orange dye. After three min heating at 95°C , samples were chilled and loaded into polyacrylamide gel (20%) with 7 M urea and $1 \times$ TBE and run at 400 mV for 2 h. Gels were quantified using a BAS 2000 Bioimaging Analyzer (Fuji Film Co., Japan). Radioactive signal densities were determined using the software designed for this system.

Parametric paired Student' test and the non-parametric Mann-Whitney test were used to compare the pre and post marathon data. Significance was set at $P < 0.05$.

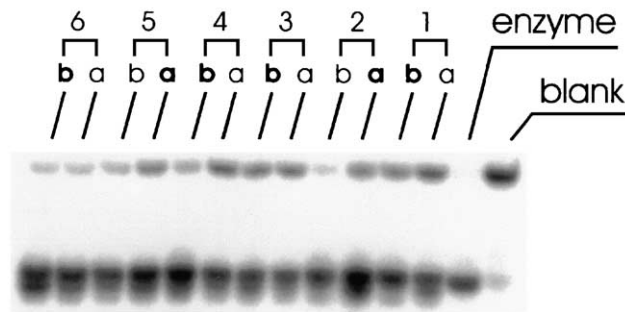
Results

All runners completed the marathon within 4:30 h (3:35–4:28 h). The activity of human endo III increased in the skeletal muscle of four runners after the race. However no significant alteration was detected in the activity of this AP site repair enzyme (Fig. 1A). On the other hand, the activity of hOOG1 increased in skeletal muscle of runners after the race suggesting an adaptive response to exercise-induced oxidative DNA damage. Five runners of the six had increased hOOG1 activity in quadriceps femoris muscle after the marathon race (Fig. 1B). The semi-quantitative densitometric data, evaluated by parametric and non-parametric statistical methods, provided significant ($P < 0.05$) differences between pre- and post-marathon running in the activity of hOOG1. Large individual differences, which were observed in the activity of DNA repair enzymes, might be due to a variety of factors including training status, stress tolerance, genetical differences etc.

Discussion

We are reporting here for the first time, that severe aerobic exercise, in this case marathon, running alters the activity of DNA repair enzymes in skeletal muscle. To our knowledge, the BER enzymes has not been measured in studies involving physical exercise. The repair enzymes can be increased by DNA damage or by damage causing agents [14]. The induction of the enzyme responsible for the removal of 8-oxoG in mammalian tissue cloned, as 8-oxoguanine DNA glycosylase [15], suggests an

A AP site repair



B 8-oxodG repair

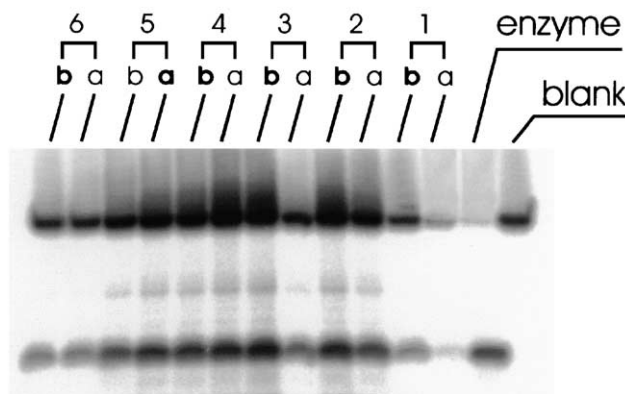


Fig. 1. For the AP site damage repair oligonucleotide with the following sequence $\text{CCTGCCCTG}_{\text{AP}}\text{GCAGCTGTGGG}$ and for 8-oxoG repair $5' \text{GA}(\text{ACTAGTGOATCCCCGGGCTGC}) 3'$ were labeled with ^{32}P . The TBE gels from the right were loaded with the following order, blank, enzymes, endo III for panel A, and Fpg for panel B and then six subject pre (a) and post (b) marathon running samples. Bold letters indicate increased density between pre and post marathon samples in each individual. Four runners have increased endo III activity after the race compared to pre-race value. The activity of 8-oxoG repair enzyme increased in the quadriceps femoris of five runner after the marathon race compared to resting level.

adaptive response to the increased formation of 8-oxoG as a result of marathon running. The up-regulation of 8-oxoG repair is an important process because of the mutagenic potential of 8-oxoG [16] which could lead to G:C transversion to T:A [17]. Acute physical exercise has been shown to increase the content of 8-oxoG in leukocytes and skeletal muscle of human subjects [7,10]. A reduction of 8-oxoG levels was reported in leukocytes of human subjects and in skeletal muscle of rats due to regular exercise training [11,18]. The finding of the present study suggests that this could be accounted for by the exercise-induced activity of the 8-oxoG repair enzyme, OGG1. The observed increase was not large. However, in physiology even a small change in the long term could exert a very significant effect and could play a vital role in some physiological function. The hypothesis, that the exercise mediated increase of the DNA repair system is one of the mechanisms by which exercise reduces the incidence of cancer, needs to be investigated in future studies.

The steady-state level of AP site damage, which is suggested to be 50,000–200,000 lesions per mammalian cell under normal physiological condition [19], reflects the balance between the formation

and repair of AP sites. The repair of AP sites involves the orchestrated work of two–three pathways and it has been shown that the following order likely occur for the BER enzymatic activities: AP endonuclease, DNA synthesis, deoxyribonucleotide phosphate lysase activity followed by ligation of DNA [20].

In the present study the activity of human endo III, was also altered. It increased in some subjects and decreased in others as a response to the marathon race. Therefore, the findings of the present study are not sufficient to draw a significant conclusion on the activity of human endo III after a marathon race. The current investigation is a human study, which limits the number of subjects, and individual differences are also apparent. Theoretically, increased activity of *N*-glycosylase would lead to increased formation of AP sites [20–22]. The removal of AP sites is important because they can be mutagenic and cause cell death [23–25]. The independent changes in the activity of 8-oxoG and AP repair might result in accumulation of AP sites [2]. Therefore, it cannot be excluded that marathon running disturbs the harmony between the DNA damage repair enzymes. On the other hand, a recent study suggest that human endo III, hNTH1, is involved in the removal of 8-oxoG from 8-oxoguanine/guanine mispairs in DNA [26]. Therefore, the difference in the activity of hOOG1 and human endo III might be due to the competition for substrate. The findings of this study do not allow us to make a statement on the exact mechanism behind the discrepancy in the activities of hOOG1 and human endo III. However, this remains to be elucidated.

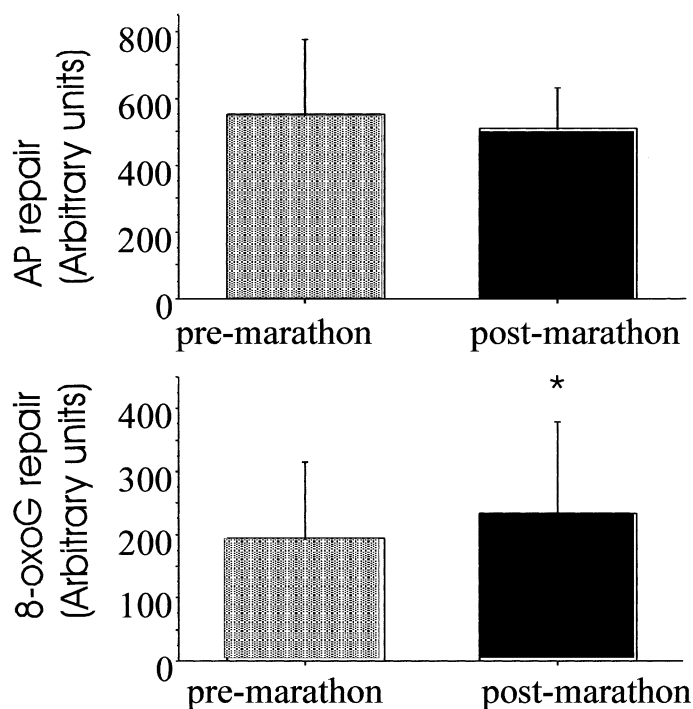


Fig. 2. The graph shows the densitometric data obtained by a BAS 2000 system. Pre and post marathon samples are compared. The statistical evaluation revealed that the activity of hOOG1 significantly increased after the marathon race. Values reported are the mean \pm standard deviation ($n = 6$). * $P < 0.05$.

In summary, the data of the present study reveal, for the first time, that a single-bout of physical exercise increases the activity of 8-oxoG repair enzyme in human skeletal muscle. Therefore, it cannot be excluded that regular exercise could result in an up-regulation of this enzyme leading to a decreased base level of 8-oxoG, as observed in other studies [11,18]. The changes in the activity of the AP site repair enzyme are not fully characterized but suggest some disruption in the coordinative activity of repair enzymes. (Fig. 2)

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