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ORIGINAL ARTICLE

Is high-intensity resistance exercise-induced oxidative DNA damage related to OGG1 Ser326Cys polymorphism in athletes?

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Abstract The 8-oxoguanine DNA glycosylase-1 (OGG1) is one of the first lines of defense against 8-oxodg mutagenicity. The mutant hOGG1-Cys326 might be susceptible to a decrease in the repair function for oxidative DNA damage, which leads to higher oxidative DNA damage accumulation with excess oxidative stress. The purpose of this study was to investigate the effects of hOGG1 Ser326Cys polymorphisms on oxidative DNA damage following a single bout of resistance exercise (RE) protocol (7 sets of 4 exercises using 60–90 1 repetition maximum) in the flat pyramid loading pattern. Twenty-eight young resistance-trained men were allocated to two groups according to the hOGG1 gene polymorphism including the wild-type hOGG1-Ser326 genotype ($n = 12$) and the mutant hOGG1-Cys326 genotype ($n = 16$). Subjects performed a RE protocol (7 sets of 4 exercises using 60–90 1 repetition maximum) in the flat pyramid loading pattern. The hOGG1 genotypes were determined with PCR-RFLP methods at baseline blood samples of each subject. Urine samples taken before, immediately, and 24-h postexercise were analyzed for oxidative DNA damage as measured by urinary 8-hydroxy-2-deoxyguanosine (8-OHdG) excretion. The 8-OH-G levels in the mutant hOGG1-Cys326 genotype were significantly higher than those in the wild-type hOGG1-Ser326 genotype at pre, post and 24 h post high-intensity RE ($p = 0.001$, 0.03 and 0.01). Also, significant increase was observed in 8-OHdG level at 24 h post-RE compared with resting values ($p = 0.025$). Overall, the present study suggests that DNA sequence variations in the hOGG1 gene are associated with the magnitude effects of exercise on oxidative DNA damage in athletes. Genetic variation in the hOGG1 gene plays a role in oxidative stress responses to exercise in athletes. In conclusion, the Ser326Cys genotype in hOGG1 gene was found to be associated with higher oxidative DNA damage following high-intensity RE in athletes. Based on this information, athletes carrying the mutant hOGG1-Cys326 genotype are more susceptible to the induction of oxidative DNA damage induced by strenuous exercise and consequently, might benefit more from protection by dietary antioxidants.

Keywords 8-OHdG · hOGG1 gene polymorphism · Resistance exercise

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Introduction

Endogenous and exogenous sources of free radicals can cause oxidative DNA damage, if their production is beyond the body's antioxidant defense and repair [1]. 8-Hydroxy-2-deoxyguanosine (8-OHdG or 8-oxodg) is one of the DNA base modified products of cellular DNA and is a mutagenic agent causing GC-to-TA transversions [2] that is involved in the pathology of many diseases such as cancer, atherosclerosis and diabetics [3]. The harmful consequences of oxidative DNA damage can be prevented by efficient repair mechanisms. Oxidatively damaged bases are preferentially repaired by base excision enzymes, including 8-oxoguanine DNA glycosylase-1 (OGG1), NEIL1 (nei-like 1) and MUTYH (mutY homologue) relevant for 8-oxodg [4]. The OGG1 is one of the first lines of defense against 8-oxodg mutagenicity [5].

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OGG1 encoded by hOGG1 gene that is located on chromosome 3p25. The DNA glycosylase OGG1 protein recognize and catalyzes the removal of 8-OHdG from double-strand DNA, the major form of oxidative DNA damage induced by reactive oxygen species (ROS) [5]. Among several polymorphisms of the OGG1 gene, the Ser326Cys polymorphism is one of the most important, and it plays a major role in various types of cancers. C/G polymorphism at position 1245 in exon 7 of the OGG1 gene results in an amino acid substitution of serine with cysteine in codon 326 [6].

Some evidence exists suggesting that the wild-type hOGG1-Ser326 protein was more functional than the mutant hOGG1-Cys326 protein [7]. The hOGG1 Cys/Cys genotype has been associated with a 2.3-fold increased risk of squamous cell carcinoma of the lung [8]. Furthermore, lung cancer risk was found to be increased twofold in individuals with the homozygous hOGG1 Cys/Cys variant genotype [9]. Previous studies also demonstrated that 8-OHdG levels were higher in hOGG1 mutant carriers (genotypes of Ser/Cys and Cys/Cys) than wild-type carriers [10, 11].

Collectively, this data indicates that the mutant hOGG1-Cys326 might be susceptible to a decrease in the repair function for oxidative DNA damage, which leads to higher oxidative DNA damage accumulation with excess oxidative stress. Furthermore, a growing body of evidence suggests a bout of strenuous exercise increased oxidative DNA damage in trained and untrained individuals [12–14]. However, no studies, to date, have examined the association between genetic polymorphisms in the hOGG1 gene with exercise-induced oxidative DNA damage in athletes. The emphasis of the current study was not to evaluate oxidative DNA damage to resistance exercise (RE), but rather to evaluate the effects of genetic polymorphism in DNA repair gene (hOGG1) on oxidative DNA damage as measured by urinary 8-OHdG following high-intensity RE in athletes, of which there is no data in the literature. Thus, the purpose of this study was to investigate the effects of hOGG1 Ser326Cys polymorphisms on oxidative DNA damage following a single bout of high-intensity RE in athletes.

Methods

Subjects

Twenty-eight young resistance-trained men had given their written informed consent to participate in this study, which was approved by the research ethics committee of the department of exercise physiology. The subjects were regularly performing RE, 3 days/week for 2 years. The experimental procedure was explained in detail to all the subjects. Subjects were allocated to two groups according to the hOGG1 gene polymorphism including the wild-type hOGG1-Ser326 genotype ($n = 12$) and the mutant hOGG1-Cys326 genotype ($n = 16$) (Table 1). Exclusion criteria were consuming any supplementation, alcohol and tobacco products.

Procedures

One familiarization session was used to determine the maximal strength test (1RM) 1 week before the study. Participants then reported to the human performance laboratory on 3 separate weeks. During the first week, the participants performed the 1RM with the bench press, leg press, lat pull down, and seated rows [13]. Before RE protocols, all subjects performed a warm-up, which consisted of 3-min running, 5–10 repetitions at 50 % of perceived maximum and stretching period. Subjects in both groups performed same RE protocol as depicted in Fig. 1. The RE protocol performed in a flat pyramid loading pattern, which consisted of 7 sets of 3–6 repetitions of bench press, leg press, lat pull down, and seated rows with 80–90 % of 1RM [13] (Fig. 1). Body composition including weight, soft lean mass, fat mass were determined by body composition analyzer (InBody version 3.0; Biospace, Seoul, Korea).

Urine samples were collected before (pre), after (post), and 24 h after (24 h post) RE. After collection, urine samples were stored at −20 °C until analysis. Oxidative

Table 1 Physical characteristics of the subjects in heterozygote S/C ($n = 16$) and homozygote S/S ($n = 12$) groups

Variables	Genotype	Mean ± SD
Age (year)	Heterozygote S/C	20.00 ± 2.86
	Homozygote S/S	18.44 ± 2.12
Height (cm)	Heterozygote S/C	170.55 ± 6.42
	Homozygote S/S	174.78 ± 4.91
Weight (kg)	Heterozygote S/C	74.59 ± 15.19
	Homozygote S/S	76.31 ± 10.41
Soft lean mass (kg)	Heterozygote S/C	59.25 ± 8.71
	Homozygote S/S	63.57 ± 6.17
Fat mass (kg)	Heterozygote S/C	12.05 ± 6.72
	Homozygote S/S	9.23 ± 4.43
% Fat mass	Heterozygote S/C	15.31 ± 5.22
	Homozygote S/S	11.66 ± 4.21
BMI (kg/m^2)	Heterozygote S/C	25.85 ± 4.27
	Homozygote S/S	24.90 ± 2.53

There were no significant differences in physical characteristics of the subjects in two groups ($p > 0.05$)

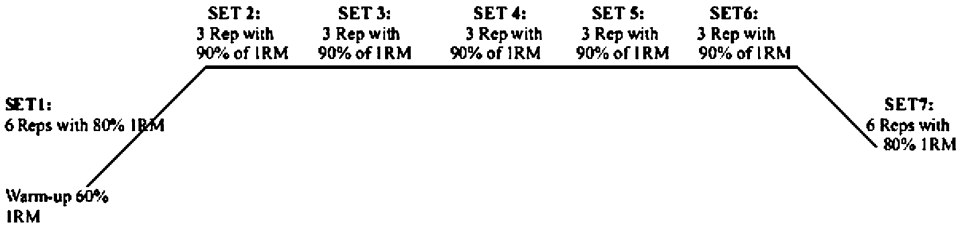

Fig. 1 Resistance exercise protocol with flat pyramid loading pattern

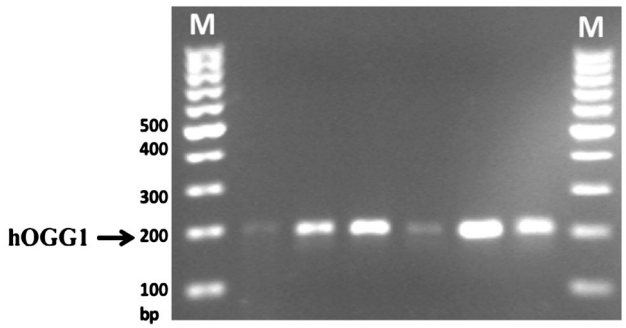

Fig. 2 Agarose gel electrophoresis of the hOGG1 gene PCR amplification products. Fragments of 200 bp indicate the hOGG1 gene

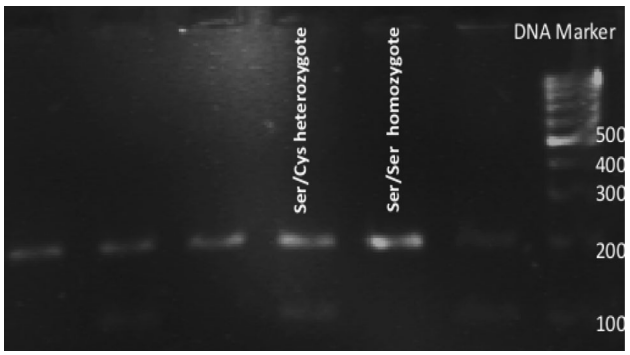

Fig. 3 Agarose gel electrophoresis of the hOGG1 gene RFLP-PCR amplification products. Lane DNA marker: 100 bp DNA lader; Lane Ser/Cys heterozygote includes two fragments of 100 and 200 bp. A fragment of 200 bp indicates the Ser/Ser homozygote

DNA damage was measured by urinary 8-OHdG level using the 8-OHdG EIA kit (Cayman Chemical, Catalog No. 589320, USA) based on the kit instructions. Blood samples were collected from each participant in EDTA-containing tubes. Genomic DNA was isolated from leukocyte using standard phenol–chloroform extraction by GPP^{TM} Solution kit (Gen Pajoohan Pouya CO.).

The hOGG1 Ser326Cys polymorphism in exon 7 described by Sugimura et al. [8] was assessed by PCR-RFLP using primers HOGG1F: 5-GGAAGGTGCTTGGG GAAT-3 and HOGG1R: 5-ACTGTCACTAGTCTCACC AG-3. Amplification consisted of a 5-min denaturation at 95 °C followed by 30 cycles of 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 1 min. An incubation step at 72 °C was added at the end of the reaction for 7 min. Instead of the single-strand conformational polymorphism method used by Sugimura et al. [8], we used a simple RFLP method to identify the Ser326Cys variant, because the C-to-G transversion creates a new Fnu4HI restriction site. The PCR product is 200 bp in length and is digested by the Fnu4HI restriction enzyme to two 100-bp fragments for the 326Cys allele and is undigested for the 326Ser allele. Fragments were separated on a 2 % NeuSieve GTG agarose gel (FMC Bioproducts, Rockland, ME) and compared with genotype standards (confirmed by direct sequencing). A representative gel is shown in Figs. 2 and 3.

Statistical analyses

Oxidative DNA damage related to different genotypes was analyzed by one-way analysis of variance with repeated measure (genotype × time). A p value <0.05 was considered statistically significant.

Results

The genetic polymorphism of hOGG1 in subjects was analyzed by PCR-RFLP and the results showed that the frequency of the heterozygous hOGG1 Ser/Cys genotypes and homozygotes hOGG1 Ser/Ser genotypes were 58 and 42 %, respectively. At baseline, subjects with the mutant hOGG1 Ser326Cys genotype had significantly higher oxidative DNA damage, measured by urinary 8-OHdG levels, at pre-exercise compared to the wild type of hOGG1 Ser326Ser genotype ($p = 0.025$) (Fig. 4). The 8-OH-G levels in the mutant hOGG1 Ser326Cys genotype were significantly higher than those in the wild-type hOGG1 Ser326Ser genotype at post and 24 h post high-intensity RE ($p = 0.03$ and 0.01) (Fig. 4). Also, the 8-OH-G levels in the mutant hOGG1 Ser326Cys genotype were significantly higher at 24 h post compared with pre-RE ($p = 0.025$). However, subjects with the wild type of hOGG1 Ser326Ser genotype had the only significant increase in urinary 8-OHdG levels in 24 h post-RE compared with pre-exercise ($p = 0.045$).

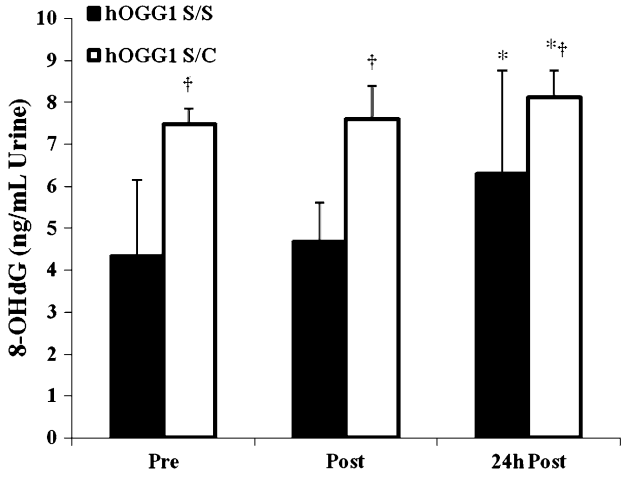

Fig. 4 The change in urinary 8-OHdG concentrations in subjects with the mutant hOGG1-Cys326 genotype and the wild-type hOGG1-Ser326 genotype at before (Pre), after (Post) and 24 h (24 h Post) after high-intensity RE. Values are mean ± SD. *Significant difference with pre-exercise at $p < 0.05$. †Significant difference between mutant hOGG1 Ser326Cys and wild-type hOGG1-Ser326 genotypes at $p < 0.05$

Discussion

Acute physical exercise has been shown to increase 8-OHdG that is one of the major oxidative products formed by radical-induced damage to DNA structure [13, 15]. The increased formation of 8-OHdG as a result of strenuous exercise is harmful to cells because of the mutagenic potential of 8-OHdG [2]. Previous studies have shown that oxidative DNA damages are repaired by base excision enzymes [4]. One of the first lines of defense against 8-OHdG mutagenicity involves the OGG1-initiated base excision repair (BER) pathway [5]. Several studies have shown that genetic polymorphisms in OGG1 may increase the susceptibility to oxidative DNA damage and carcinogenesis [4, 10, 11, 16]. To our knowledge, the genetic polymorphism BER enzymes have not been measured in studies involving exercise-induced oxidative DNA damage. We are reporting for the first time, that hOGG1 gene polymorphism alters the exercise-induced damage to DNA in athletes. The athletes with heterozygous hOGG1 Ser/Cys genotypes exhibited higher levels of oxidative DNA damage as measured by 8-OHdG compared with the Ser/Ser genotypes (wild type) before exercise (at rest). The urinary excretions of 8-OHdG and the base (8-oxoguanine), which is believed to arise from OGG1-mediated cleavage of DNA, are regarded as biomarkers of the steady-state level of oxidative DNA damage and repair in the whole body [11]. Several studies reported a relationship between high-excretion rates of 8-OHdG and risk of cancers [4, 11, 17]. Higher baseline levels of urinary 8-OHdG in athletes with the OGG1Cys326/Ser326 genotype are corroborated by Chen et al. [10] who found significant increase in 8-OHdG levels in individuals with heterozygotes and homozygotes genotypes of hOGG1 gene. Also, Hatt et al. [11] reported that subjects with the OGG1 Cys326/Cys326 genotype had the highest expression level of OGG1 mRNA and there was a positive association between the expression level and urinary 8-OHdG levels. As mentioned earlier, hOGG1 gene play a key role in the repair of 8-OHdG to prevent consequential mutations [16]. Based on these findings it could be speculated that inefficient DNA repair would lead to high levels of 8-OHdG and the development of diseases such as cancer, atherosclerosis and diabetics [3]. Furthermore, immediately post and 24 h post high-intensity RE, our findings showed that athletes with the OGG1 Cys326/Ser326 genotype had higher urinary 8-OHdG levels compared with athletes with the OGG1 Ser/Ser genotypes (wild type). This may have occurred owing to the lower repair capacity of Cys326-hOGG1 protein toward oxidized DNA lesions [18]. Our study is the first study on 8-OHdG repair-related gene in relation to physical exercise.

Despite the importance of oxidative stress following strenuous exercise, only two studies evaluated the influence of antioxidant enzyme gene polymorphisms on exercise-induced oxidative stress [19, 20]. Their results are also equivocal, with one study showing athletes with null genotype of Glutathione-S-transferase M 1 (GSTM1) gene have higher oxidative DNA damage and lipid peroxidation following cycling to exhaustion [20], and the other reporting GSTM1 polymorphisms did not affect oxidative stress immediately after the race in runners [19].

However, a number of clinical studies, in agreement with our results indicate that homozygous and heterozygous carriers of the OGG1 Ser326Cys polymorphism have higher oxidative DNA damage and lower repair capacity of 8-OHdG [21, 22] which is associated with risk of lung cancer [9, 11], breast cancer [23], nasopharyngeal carcinoma [24] Barrett's esophagus [25] and type 2 diabetes [10]. Although the mechanism of the reduced repair capacity of Cys326/Ser326-hOGG1 genotype remains to be elucidated, previous study assume that this may result from either loss of a putative regulatory serine phosphorylation site or the introduction of a redox-sensitive cysteine amino acid at position 326 [18, 26].

At pre-exercise (baseline), immediately post and 24 h post high-intensity RE athletes with the OGG1 Cys326/Ser326 genotype experienced more oxidative DNA damage as compared with athletes with wild-type genotype in OGG1 gene. These results imply that athletes with the OGG1 Cys326/Ser326 genotype are more prone to oxidative DNA damage during high-intensity RE. Therefore, we hypothesized that athletes carrying the unfavorable polymorphisms in OGG1 gene are more susceptible to the induction of oxidative DNA damage induced by strenuous

exercise, and consequently, will benefit more from protection by dietary antioxidants. In order to fully understand the role of DNA repair gene polymorphisms in oxidative DNA damage induced by high-intensity RE, further studies should aim at investigating DNA repair capacity, DNA repair genes mRNA, and DNA damage detection in large sample size.

Conclusions

In conclusion, increased oxidative damage to DNA in athletes carrying the OGG1 Cys326/Ser326 genotype following high-intensity RE indicates diminished ability to resist against oxidative DNA damage and repair. Taken together, these observations suggest that the OGG1 Cys326/Ser326 genotype may contribute to the exercise-induced damage to DNA in athletes. This may be due to the diminished repair capacity of OGG1 enzyme in athletes carrying mutant OGG1 genotype. Based on our findings, it could be speculated that hOGG1 genotypes may be a new genetic factor that influence sport performance.

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Conflict of interest The authors declare no conflict of interest.

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