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HIGHLIGHTED TOPIC | *Free Radical Biology in Skeletal Muscle*

8-Oxoguanosine and uracil repair of nuclear and mitochondrial DNA in red and white skeletal muscle of exercise-trained old rats

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Radak Z, Kumagai S, Nakamoto H, Goto S. 8-Oxoguanosine and uracil repair of nuclear and mitochondrial DNA in red and white skeletal muscle of exercise-trained old rats. *J Appl Physiol* 102: 1696–1701, 2007. First published January 4, 2007; doi:10.1152/japplphysiol.01051.2006.—Oxoguanine DNA glycosylase (OGG1) and uracil DNA glycosylase (UDG) are two of the most important repair enzymes that are involved in the base excision repair processes to eliminate oxidative damage from mammalian DNA, which accumulates with aging. Red and white skeletal muscle fibers have very different antioxidant enzyme activities and resistance to oxidative stress. In this paper, we demonstrate that the activity of OGG1 is significantly higher in the red type of skeletal muscle compared with white fibers from old rats. Exercise training resulted in increased OGG1 activity in the nuclei of red fibers and decreased activity in nuclei of white fibers and in the mitochondria of both red and white fibers. The activities of UDG were similar in both red and white muscle fibers. Exercise training appears to increase the activity of UDG in the nuclei and mitochondria. However, exercise training affects the activity of OGG1 in nuclei and mitochondria differently, suggesting different regulation of the enzymes. In contrast, UDG showed similar activities in nuclei and mitochondrial extracts of exercise-trained animals. These data provide evidence for differential regulation of UDG and OGG1 in maintaining fidelity of DNA in oxidatively stressed cells.

oxoguanine DNA glycosylase; uracil DNA glycosylase; muscle fiber types; oxidative stress

CELLULAR DNA is continuously attacked by reactive oxygen species (ROS), leading to base and sugar modifications in the genome. If these damages are not repaired they may arrest transcription or lead to mutation and a variety of pathophysiological changes. 8-Hydroxy-2′-deoxyguanosine (8-OHdG) is one of the most abundant and well characterized DNA lesions. It has been estimated that about 180 guanines are oxidized to 8-OHdG by ROS on a daily basis in mammalian cells (17). 8-OHdG can pair with adenine leading to G:C to T:A transversion mutation (9). 8-OHdG accumulates in DNA with age, particularly in the mitochondrial genome, which can be more than ten times higher than in the nuclear DNA (29). 8-OHdG appears to contribute to the development of a wide range of pathological conditions including tumorigenesis, aging, and Alzheimer and Parkinson diseases (2). Therefore, to maintain genomic integrity it is of primary interest to eliminate this damaged DNA base. Oxidatively modified guanines and uracil are primarily repaired by the base excision repair (BER) pathway (4). BER consists of basic steps catalyzed by different enzymes. In mammals, oxidized guanines are primarily recognized and removed by oxoguanine DNA glycosylase (OGG1), when paired with cytosine. Similarly, the repair of uracil in DNA, at the beginning of the BER process, is primarily carried out by uracil DNA glycosylase (UDG). Uracil in DNA is produced from deamination of cytosine resulting in mutagenic U:G mispairs and misincorporation of dUMP, which results in a less mutagenic U:A pair. Both nuclei and mitochondria are equipped with OGG1 and UDG isoforms.

Physical exercise, above a certain intensity or duration, has been shown to increase the generation of ROS and the associated oxidative damage to DNA (1, 8, 24, 26). Moreover, it has also been demonstrated that exercise has the capability to increase the activity of OGG1, measured from crude cell extracts (28, 29, 32). Generally, it is believed that exercise induces ROS generation in the mitochondria and, consequently, oxidative damage to DNA in the skeletal muscle. To date, to our knowledge, there is no report on the oxidative damage to mitochondrial DNA in skeletal muscle, and this dearth of information, most probably, is due to the difficulties of finding valid artifact-free measurements. We recently found that, in the liver, mitochondrial 8-OHdG content decreased with exercise training and increased with aging (18). Although liver has been shown to be oxidatively stressed as a result of a single bout of exercise (5), skeletal muscle may respond differently to exercise-induced oxidative stress and adaptation. Oxygen flow and energy turnover rate in exercising skeletal muscle are the highest among all organs during intensive exercise. In addition, skeletal muscle consists of different fiber types, which have different threshold levels for activation, different levels of force output and resistance to fatigue, different capillarization, and different mitochondrial density, and therefore very different characteristics. Indeed, the antioxidant capacity of Type 1 fibers, red fibers, is significantly higher than that of white fibers (13, 25, 27). Moreover, the resistance of red and white fibers to exercise-induced oxidative damage varies significantly, and the assumption that the activity of damage repair enzymes is fiber dependent is particularly inviting.

Studies on the aging process have revealed that the activity of OGG1 changes in an organelle-dependent manner in liver (33), that is, increases in mitochondria and decreases in nuclei (33). The activity of UDG appears to decrease during aging, at

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least in liver (34). However, skeletal muscle is a poorly studied organ with respect to DNA repair.

When compared with liver or brain, skeletal muscle shows the opposite response to aging, in terms of the activity of antioxidant enzymes (13–15). The effects of aging on the activities of OGG1 and UDG in nuclear and mitochondrial fractions of skeletal muscle are generally unknown. Therefore, in the present investigation, we aimed to determine the activities of OGG1 and UDG from the nuclear and mitochondrial fractions of white and red portions of quadriceps femoris muscle of old exercised rats to clarify whether exercise training influences the activity of DNA repair and whether exercise training can protect against DNA damage through increased activities of DNA repair enzymes.

METHODS

Animals and Exercise Protocol

Male specific pathogen-free (SPF) F344/Du rats were purchased and transferred from the Tokyo Metropolitan Institute of Gerontology at age 21 mo and kept in a clean conventional animal room in the animal facility of the Faculty of Pharmaceutical Sciences at Toho University during the experimental period. Sixteen animals were familiarized to walk on a motor-driven treadmill for 5 days at 5–7 m/min, once a day for 10–20 min. After this acclimation period, eight animals were randomly assigned to either a sedentary control or an exercise training group. The training animals ran 30–40 min/day at 8–10 m/min on an 8% slope for the first 2 wk. Time and speed were gradually increased, up to 90 min/day at 14–15 m/min, for 8 wk. In the last 3 wk the slope was deceased by 5%, because the motivation to run appeared to be reduced in the animals. A soft brush, but no electric shock, was sometimes used manually to stimulate the animals to run. We showed previously (28) that this exercise protocol resulted in a 40% increase in maximal oxygen uptake for the same age group.

Body weight was measured once a week. The sedentary control animals walked for 5 min on the treadmill at the same frequency as the trained animals to minimize the possible effects of environmental factors, but this low level of physical activity did not result in exercise-induced adaptation. All animals were killed 48 h after the last training session.

Preparation of Nuclear and Mitochondrial Fractions

The quadriceps muscles were separated into red and white portions (23) and grouped as red control (RCT), red exercise (RE), white control (WCT), and white exercise (WE).

Portions were homogenized with buffer (HB) containing 20 mM of Tris (pH 8.0), 1 mM of EDTA, 1 mM of dithiothereitol, 0.5 mM of spermidine, 0.5 mM spermine, and 50% glycerol and protease inhibitors. The nuclear and mitochondrial fractions were separated by centrifugation. To prepare nuclear fractions, the homogenate was centrifuged at 1,000 *g* for 10 min at 4°C, and the pellet was suspended in HB and recentrifuged. Then, the pellet was resuspended in HB with 0.5% Nonidet P-40 and centrifuged. Next, the pellet was washed twice in HB. After centrifugation, the final nuclear pellet was rocked for 30 min after the addition of a 1/10 vol/vol of 2.5 M KCl and centrifuged at 14,000 rpm for 30 min. The supernatant was divided into aliquots and stored at −80°C. The protein levels were measured using the BCA method. For the isolation of mitochondria, the supernatant from the first centrifugation was centrifuged at 14,000 *g* for 30 min at 4°C. Then, the pellet was resuspended in HB and recentrifuged three times. The pellet was suspended in 0.5 ml HB. The final mitochondrial pellet was suspended in HB containing 0.5% Triton X-100 and was kept on ice for 20 min. The protein levels were measured using the BCA method.

Excision Assay

The assay was carried out according to the protocol described by Radak et al. (28). In brief, 20 pmol of synthetic substrate containing 8-OHdG or uracil (Trevigen, Gaithersburg, MD) was labeled with ^{32}P at the 5′ end using polynucleotide T4 kinase (Boeringer Mannheim). For the nicking reaction, protein extract (2 μg) was mixed with 20 μl of a reaction mixture containing 0.5 M of *N*-[2-hydroxyethel]piperazine-*N*′-[ethanesulfonic acid], 0.1 M EDTA, 5 mM of dithiolthreitol, 400 mM KCl, purified BSA, and labeled probe (~2,000 cpm). The reaction was carried out at 30°C for 15 min and stopped by placing the mixture in ice. Next, 30 μl chloroform was added, samples were centrifuged, and 15 μl of the aqueous layer was taken and mixed with loading buffer containing 90% formamide, 10 mM NaOH, and blue-orange dye. After 3 min heating at 95°C, samples were chilled and loaded into polyacrylamide gel (20%) with 7 M urea and 1× TBE and run at 400 mV for 2 h. Radioactive signals of the cleavage product of the labeled substrate were quantified using a STORM Bioimaging Analyzer (Molecular Dynamics). Radioactivity in the separated, cleaved product and intact oligo bands were quantified with a PhosphpoImager (Molecular Dynamics) loaded with Image Quant software. The activity to repair 8-OHdG was determined and expressed as a percentage of the substrate cleaved (28).

Statistical Analyses

Statistical significance was assessed by two-way ANOVA, followed by Tukey's post hoc test. The significance level was set at $P < 0.05$.

RESULTS

To assess the purity of the isolated nuclear and mitochondrial suspensions, the pooled samples from each group were subjected to Western blot analysis. Figure 1 shows no detectable cytochrome *c* in the nuclear extracts.

The data revealed that there is a difference in the activity of OGG1 between red and white skeletal muscle in the mitochondrial and nuclear extracts (Figs. 2 and 3). Red fibers have significantly higher activity of OGG1. The activity of OGG1, measured from nuclear extracts, was significantly higher in the red fibers of exercise-trained animals than controls (31% increase vs. control). On the other hand, the activity of enzyme decreased in the white fibers of trained rats (Fig. 2). Unexpectedly, exercise training resulted in nearly a 25% decrease in the activity of OGG1 in the mitochondrial extract of the red fibers (Fig. 3). The activities of UDG were not found to be significantly different in red and white fibers (Figs. 4 and 5). Exercise training resulted in a 43% increase in the activity of UDG in nuclei when compared with the values obtained from sedentary animals (Fig. 4). Similar to the nuclei, the UDG activity, measured from the mitochondrial fraction, was significantly higher in skeletal muscle of exercise-trained rats in both red and white fibers (Fig. 5).

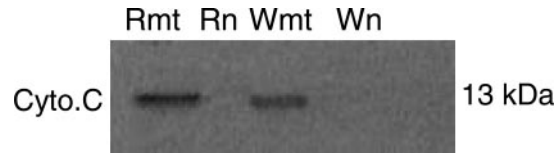

Fig. 1. Quality control of nuclear and mitochondrial preparations. Equal amounts (20 μg/lane) of lysates from red muscle mitochondria (Rmt), nuclei (Rn), white muscle mitochondria (Wmt), and nuclei (Wn) were subjected to Western blot analysis using cytochrome *c* antibody.

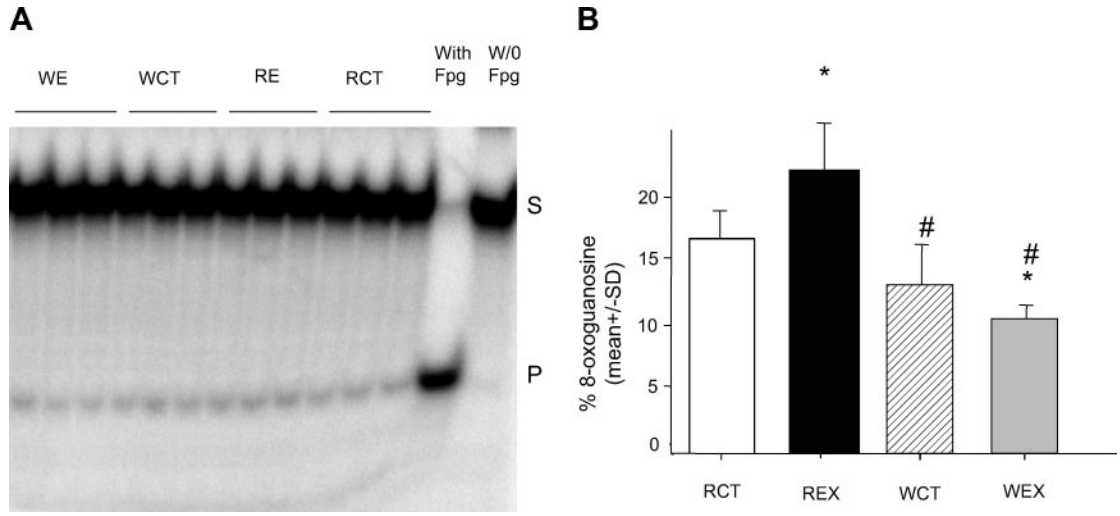

Fig. 2. Nuclear lysates of red fibers possess higher oxoguanine DNA glycosylase (OGG1) activity than white fibers. *A*: 2 μg of protein extract were mixed with labeled probe and the reaction was carried out at 30°C for 15 min. Chloroform-extracted samples loaded into 20% polyacrylamide gel containing 7 M urea, cleaved, and uncleaved probes were separated by electrophoresis. Radioactivity of cleaved product and intact probes were quantified with a PhosphpoImager. On the last 2 lanes, formamidopyrimidine-DNA glycosylase (Fpg) and probe were loaded. *B*: excision of 8-hydroxy-2′-deoxyguanosine (8-OHdG) was determined and expressed as a percentage of the substrate cleaved as in METHODS. Gels were run 3 times, and representative data of 3 animals from each group are shown in *A*. S, substrate; P, product. Sample groups: red control (RCT), red exercise (RE), white control (WCT), white exercise (WE). $*P < 0.05$ vs. control, $\#P < 0.05$ vs. red fibers.

DISCUSSION

Human cells generate $\sim 10^9$ ROS per cell on a daily basis and this results in $\sim 10^6$ oxidative DNA damage (3). A large part of this damage is prevented by enzymatic and nonenzymatic antioxidants. It is suggested that the extent of DNA damage is reduced to 10^2 alterations per cell per day by the efficient work of BER and other repair systems. The immune system, differentiation, apoptosis, and necrosis almost completely eliminate the damage so that an average of one to three mutations per cell per day remain and accumulate throughout life (21, 22).

Regular exercise has been shown to decrease the age-associated increase in the accumulation of 8-OHdG in the nucleus (28), and this could be due to the increased activity of OGG1. Indeed, in the present study, we found that OGG1 activity is significantly increased in nuclei of red fibers of skeletal muscle in exercise-trained rats compared with control animals. On the other hand, it appears that the activity of OGG1 decreased in nuclei of white fibers and in the mitochondrial fractions as a result of exercise training. It cannot be stated that red and white fibers were used to the same extent during the given exercise protocol. Therefore, the difference in the activity of OGG1 in the nuclei of red and white fibers could simply be related to the difference in metabolic rate. On the other hand, it cannot be excluded that endurance training resulted in a shift of Type IIx fibers to Type I red fibers, which could occur as a consequence of adaptation (21). We suggest that this could happen in the present study and, as a result, the

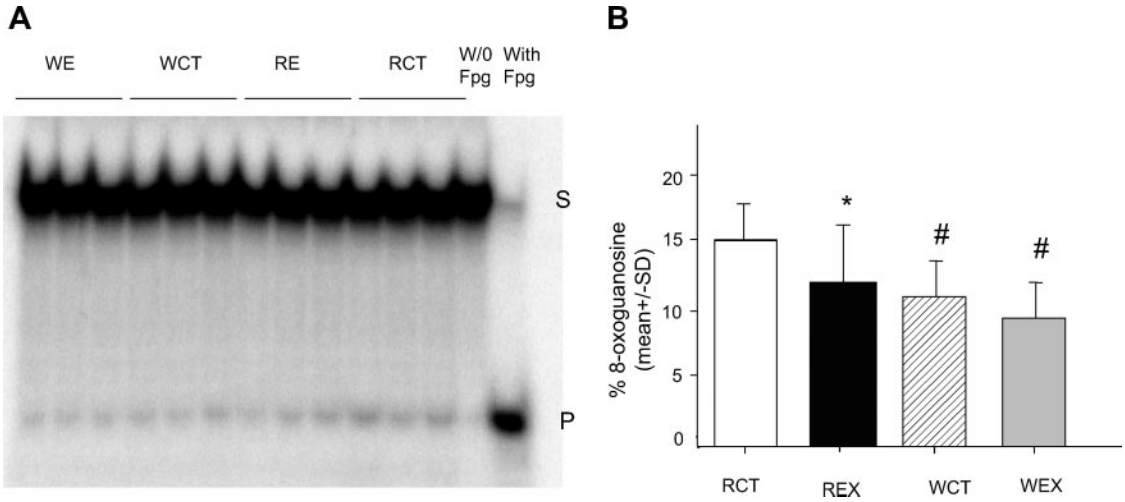

Fig. 3. The activity of OGG1 in the mitochondria decreased as a result of exercise training. OGG1 activities were determined as in legend to Fig. 2. *A*: representative data of 3 animals from each group are shown. On the last 2 lanes, Fpg and probes were loaded. *B*: densitometric data obtained as described in the METHODS. Gels were run 3 times, and representative data of 3 animals from each group are shown in *A*. $*P < 0.05$ vs. control, $\#P < 0.05$ vs. red fibers.

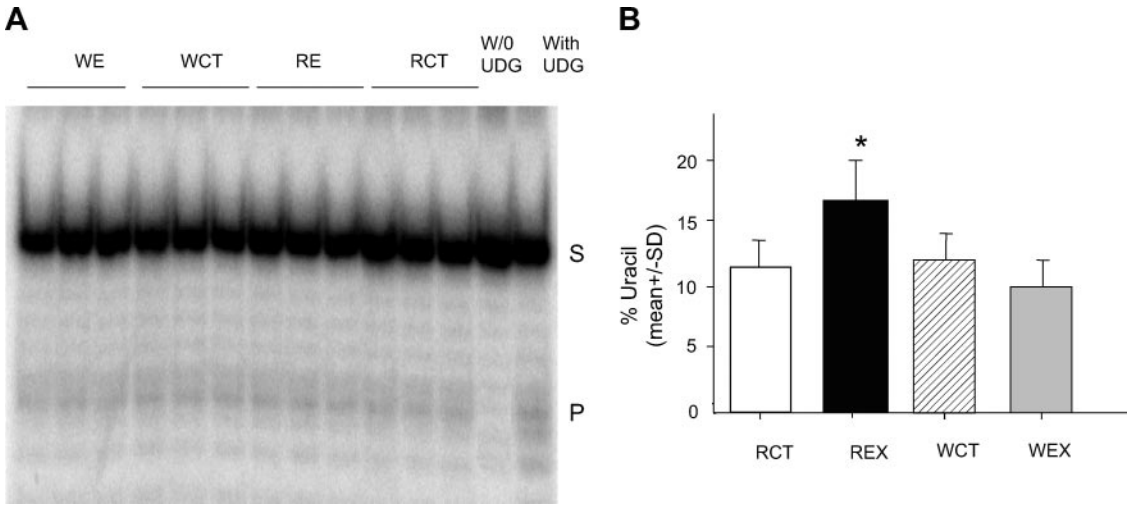

Fig. 4. Changes in uracil DNA glycosylase (UDG) activity in nuclear extracts after exercise training. *A*: activity of UDG from nuclear extract increased with exercise training. Excision activities of lysates for UDG were determined as in legend to Fig 2. *B*: data obtained by densitometric analysis of *A*. Gels were run 3 times and representative data of 3 animals from each group are shown in *A*. $*P < 0.05$ vs. control.

remaining white fibers would be more homogeneous and the shift of mixed fibers into red fibers would cause decreases in metabolic rate and activity of OGG1 in the nuclei. However, this hypothesis needs verification.

Our findings underscore the possibility of a fiber type-dependent or requirement-dependent adaptation process that occurs with exercise training, which includes the regulation of the mechanism of DNA repair. It thus could be suggested that aerobic training, which targets the more efficient involvement of slow-twitch fibers, increases the protection of nuclear DNA by enhancing the activity of OGG1. In addition, the age-associated shift to red fibers could be regarded as a protective mechanism by which the organism aims to decrease the vulnerability of DNA from oxidative damage.

The decreased activity of OGG1 in the mitochondrial extracts of exercised-trained animals is somewhat surprising, but is in accordance with our earlier observations on skeletal muscle (Radak Z, Nakamoto H, Goto S, unpublished observations) and liver (18). Recent studies have shown the decreased activity of OGG1 in aged tissues (6, 16, 33). Decreased OGG1 activity could be due to the impaired import processes of OGG1 from the ribosome to the mitochondrial matrix, which target complexes of mitochondrial outer and inner membranes (34, 35). It would be interesting to elucidate the mechanism by which mitochondrial OGG1 activity is decreased in exercising animals. It is known that the damage itself can initiate the repair process (7, 10, 11) and the only known exercise-related study, which measured 8-OHdG in mitochondrial DNA, found decreased levels of lesions in the liver (18). This finding indicates that decreased levels of OGG1 activity in the mitochondria do not ultimately mean higher concentration of 8-OHdG in exercise studies. However, we and others have shown that regular exercise decreases the 8-OHdG level in nuclear DNA, and we have repeatedly found increased OGG1

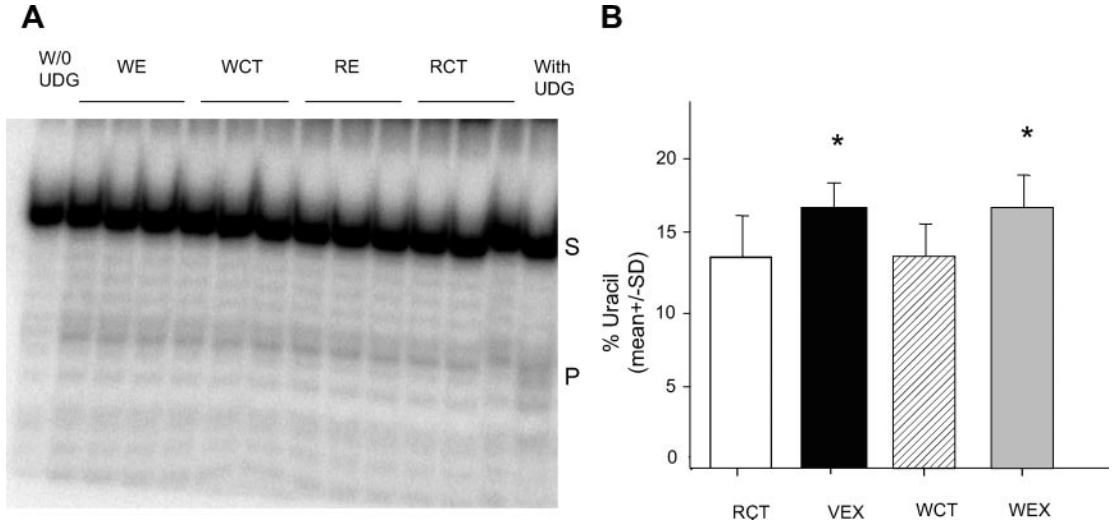

Fig. 5. Changes in UDG activity in mitochondrial extracts. *A*: UDG in the mitochondrial extracts show increased activity in exercise-trained animals in both red and white fibers. Activities were determined as in legend to Fig. 1*A*. *B*: data of the densitometric analysis of radioactive signals shown in *A*. Gels were run 3 times and representative data of 3 animals from each group are shown in the *A*. $*P < 0.05$ vs. control.

activity (28, 32). These findings strongly indicate that the regulation of nuclear and mitochondrial OGG1 could be different as a result of exercise training.

The findings of the present study have revealed, for the first time, that red fibers have greater OGG1 activity than that found in white fibers. It appears that white fibers are more sensitive to oxidative stress than red fibers because of the antioxidant enzymatic defense (19), and our data indicate that the difference in the activity of OGG1 shows a similar pattern. It is important to note that the antioxidant system, working in parallel with the repair system and exercise training, is a known inducer of antioxidant enzymes, and the fiber type-dependent response is also well documented (13, 15, 19).

The changes in activity of UDG appear to be very different than OGG1. There is no significant difference in the activities of UDG in red and white fibers. Generally, exercise training increases the activity of UDG in both organelles in fast and slow muscle fibers. The increased activity of UDG in the mitochondria as a result of exercise training suggests that the decreased OGG1 activity is not due to the impaired transfer processes of repair enzymes from the site of protein assembly to nuclei or mitochondria. This adaptive increase in UDG activity could result in more efficient repair of cellular DNA resulting in fewer mutagenic lesions and could contribute to the beneficial effects of exercise for the patient suffering from mitochondrial myopathy, mitochondrial DNA defects, and/or aging (12, 20, 36).

In summary, the findings of the present study make it apparent that the changes in activity of nuclear and mitochondrial OGG1 and UDG, as a result of exercise training, are different. Moreover, the exercise training-induced adaptation process on OGG1 activity is related to skeletal muscle fiber type, at least for nuclei. Exercise training enhances the activity of UDG in nuclei and mitochondria. This finding indicates that exercise increases repair of DNA damage and could contribute to the maintenance of the fidelity of cellular DNA.

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