Effects of Hypoxia and Hypoxic Training on 8-Hydroxydeoxyguanosine and Glutathione Levels in the Liver

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The effects of hypoxia and hypoxic training on 8-hydroxydeoxyguanosine (8-OHdG), reduced glutathione (GSH), and oxidized glutathione (GSSG) levels and on glutathione reductase (GR) activity in the liver of rats were evaluated. Rats were divided into 3 groups: a hypoxia and exercise (HE) group, a hypoxia and sedentary (HS) group, and a normoxia and sedentary (NS) group. The liver 8-OHdG levels were lower in the HE and HS groups compared with the NS group ($P < .05$). No significant difference between in the liver 8-OHdG levels in the HE and HS groups were found. However, the liver GSH level in the HS group was lower than that in the NS group ($P < .05$), and the HE group had significantly higher levels of liver GSH than the HS group ($P < .01$). The activity of liver GR in the HS group was lower than that of the NS group ($P < .05$). Moreover, the liver GR activity of the HE group was significantly higher than that of the HS group ($P < .01$). No significant difference in liver GR activity between the HE and NS groups was noted. In conclusion, the present study confirmed that moderate hypoxia and hypoxic training attenuated liver DNA damage and decreased liver GSH levels and GR activity. These results indicate that moderate hypoxia and hypoxic training result in decreased oxidative stress.

NUMEROUS STUDIES have demonstrated that antioxidant capacity changes as a result of such factors as hypoxia and hypoxic training.[1-5] High-altitude hypoxia increases the concentration of reduced glutathione (GSH) in the plasma of rabbits,[6] but decreases it in blood.[7] Singh et al[8] observed a significant decrease in GSH and an increase in oxidized glutathione (GSSG) in the muscles and blood of hypoxia-exposed rats in comparison with unexposed rats. Glutathione reductase (GR) and glutathione S-transferase (GST) activities decreased in the liver and erythrocytes of hypoxia-exposed rats, but increased significantly in muscle.[8] Exercise training has been reported to enhance the basal GSH level in rats.[9,10] Nakanishi et al[11] observed a significant decrease in glutathione peroxidase (GSH-Px) activity in the liver during hypoxia.

8-hydroxydeoxyguanosine (8-OHdG) has been recognized as a marker of oxidative DNA damage caused by oxygen radicals.[12,13] It has also been demonstrated that 8-OHdG levels decrease in human lymphocytes after intermittent swimming[14] and remain unchanged in skeletal muscle of adult rats after exercise training,[15] but decrease in old rats.[15] In a study on the effect of hypoxia on 8-OHdG levels, Schmidt et al[16] showed that urine 8-OHdG increases after training in a cold environment at a moderate altitude.

The liver is more vulnerable than other organs to oxidative stress under hypoxia.[11] It is also an important site for GSH synthesis; approximately 90% of circulating GSH is synthesized in the liver and dumped into plasma and other tissues requiring GSH.[17] GSH is the most prevalent intracellular thiol and is well known to be one of the most important nonenzymatic antioxidants. It also plays a critical role in protecting cells from oxidative stress. Liver GSH levels increase after exercise training.[9,10] Endurance exercise may contribute to a 2-fold to 3-fold increase in free radical concentrations in the liver.[18] However, little is known about the effects of hypoxia and hypoxic training on 8-OHdG and GSH levels, and little to nothing is known about GR activity in the liver.

The present study was undertaken to investigate the effect of hypoxia on 8-OHdG, GSH, and GSSG levels and on GR activity in the liver of rats.

MATERIALS AND METHODS

Chemicals

GSH, GSSG, and β-nicotinamide adenine dinucleotide phosphate (NADPH) were purchased from Sigma (St Louis, MO). Methanol for high-performance liquid chromatography (HPLC) and sodium 1-octanesulfonate (SOS), perchloric acid (PCA), sodium metabisulfite, monochloroacetic acid, and ethylenediaminetetraacetic acid (EDTA) were purchased from Katayama (Osaka, Japan).

Animal Care

All animals were housed and bred in similar conditions at Nihon Clea (Fuji-gun, Japan). The animal room was climatized at 24°C with a 12:12 hour light-dark cycle. The animals were allowed free access to standard food and water. All experiments were performed according to the ethical principles laid down by Nihon Clea for the care of animals. In addition, Nihon Clea's Ethical Committee approved the protocols for the animal experiments. Wistar rats were provided by Nihon Clea. Male Wistar rats at 4 weeks of age were randomly assigned to one of the following: the hypoxia and exercise (HE) group, the hypoxia and sedentary (HS) group, or the normoxia and sedentary (NS) group. The HE group was allowed 8 weeks of unlimited access to a running wheel from the age of 4 weeks. The number of revolutions of the wheel (Natsume, Tokyo, Japan), which was 1.16 m in circumference, was recorded daily using a mechanical counter. The hypoxia groups (HE and HS) underwent experiments under hypoxia (16.0% O_2) using a normobaric hypoxic chamber. The hypoxic control system consisted of an oxygen control unit (YHS-CO5 B; YKS, Nara, Japan) and air compressor (SLP-22 CO; YKS, Nara, Japan).

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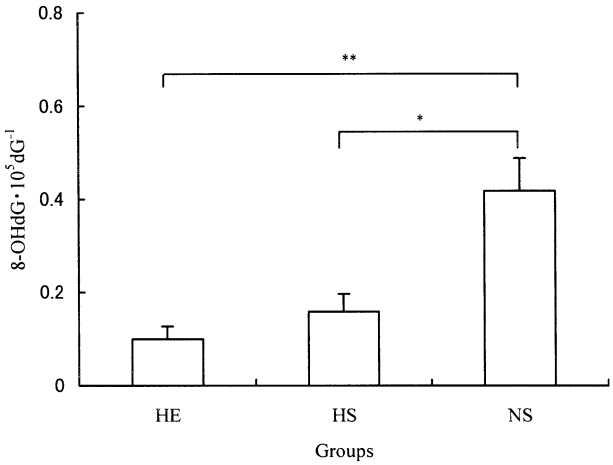

Fig 1. The effect of hypoxia and hypoxic training on the liver 8-OHdG levels. The values are mean ± SEM. *$P < .05$ and **$P < .01$: significant difference between both groups. HE, hypoxia and exercise group; HS, hypoxia and sedentary group; and NS, normoxia and sedentary group.

Tissue Sampling

All rats were euthanized with ether, and the liver was then rapidly dissected. The samples obtained were immediately washed with chilled phosphate-buffered saline and then frozen in liquid nitrogen until analysis. These samples were weighed and homogenized with 10 vol distilled water per wet weight of tissue. For quantitative analyses of GSH, GSSG, and GR activity, the homogenate (450 μL) was mixed with PCA solution (50 μL) and centrifuged for 10 minutes at 22,000 × *g*. The supernatant was filtered though a Millipore filter (pore size: 0.45 μm; Millipore Corp, Bedford, MA).

Measurement of 8-OHdG in DNA Prepared From Liver

The DNA of the liver was extracted using a commercial kit (DNA extractor WB; Wako Pure Chemical, Osaka, Japan). Liver 8-OHdG was measured using an enzyme-linked immunosorbent assay according to the kit instructions (Wako Pure Chemical Industries). The absorbance was read at 450 nm using a microplate reader. All assays were performed in triplicate.

Determination of GSH and GSSG by HPLC-Electrochemical Detection

GSH and GSSG levels were determined with an LC-4C amperometric detector (Bioanalytical System, West Lafayette, IN). The mobile phase consisted of 100 μmol/L monochloroacetic acid buffer (pH 3.0) bubbled with nitrogen gas. A biophase ODS-4 analytical column (5 μm by 4 mm by 110 mm; Bioanalytical Systems) was used. The flow rate was 0.7 mL · min^{-1}, and the injection volume was 20 μL. To detect of GSH and GSSG, 2 mercury-gold electrodes were set in a series. After elution from the column, GSSG was reduced to GSH at the upstream electrode at −1.3 V, and GSH was detected at the downstream electrode at an oxidation voltage of +75 mV. GSH and GSSG were quantified by comparing each peak area with their corresponding standard. All assays were performed in duplicate.

Determination of GR Activity by HPLC-Electrochemical Detection

GR activity was measured by HPLC-electrochemical detection (ECD).[19] An enzyme sample of about 1 to 5 μg of protein was incubated in a reaction mixture (total volume of 100 μL) with 100 mmol/L sodium phosphate buffer (pH 7.0) containing 1 mmol/L GSSG and 100 μmol/L β-NADPH. Incubation was performed at 37°C with gentle shaking for 20 minutes. The reaction was terminated by adding 100 μL 100-mmol/L PCA containing 100 μmol/L EDTA and 100 μmol/L sodium metabisulfite. After mixing, the solution was allowed to stand for 10 minutes in an ice bath, then centrifuged at 22,000 × *g* for 10 minutes at 4°C. The supernatant was filtered through a Millipore filter. GSH was quantitatively assayed by HPLC-ECD, as described above. All assays were performed in duplicate.

Statistical Analysis

Statistical analysis was performed using a 1-way analysis of variance followed by a Scheffe post hoc test. A difference of $P < .05$ was considered to be statistically significant. All values were represented as the mean and standard errors of the mean (M ± SEM).

RESULTS

Rat body weights were 390 ± 13 g for HE, 401 ± 15 g for HS, and 415 ± 13 g for NS. There were no significant differences between the 3 groups. After 8 weeks, the average distance of the HE group was 2276.9 ± 423.0 m · d^{-1}.

In the HE and HS groups, the levels of liver 8-OHdG were lower than the level for the NS group ($P < .05$), as shown in Fig 1. Although there was no significant difference ($P < .07$), the liver 8-OHdG levels in the HE group decreased compared with the HS group.

The levels of liver GSH in the HS group were lower than those of the control group ($P < .05$), as shown in Fig 2. In addition, the HE group had significantly higher levels of liver GSH than the HS group ($P < .01$). There was no significant difference in the liver GSH level between the HE and the control group. No significant differences were found in liver GSSG levels between the 3 groups.

The activity of liver GR was lower in the HS group than in the NS group ($P < .05$), as shown in Fig 3. In addition, the liver

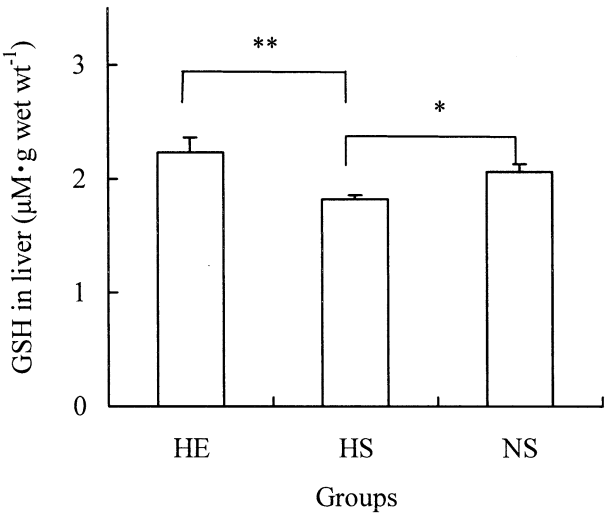

Fig 2. The effect of hypoxia and exercise on liver GSH levels. The values are mean ± SEM. *$P < .05$, **$P < .01$: significant difference between both groups. HE, hypoxia and exercise group; HS, hypoxia and sedentary group; and NS, normoxia and sedentary group.

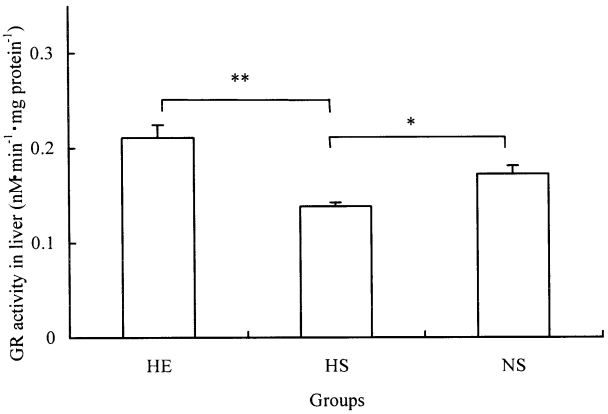

Fig 3. The effect of hypoxia and exercise on liver GR activity. The values are mean ± SEM. *$P < .05$, **$P < .01$: significant difference between both groups. HE, hypoxia and exercise group; HS, hypoxia and sedentary group; and NS, normoxia and sedentary group.

GR activity in the HE group was significantly higher than in the HS group ($P < .01$). However, there was no significant difference in liver GR activity between the HE and NS groups.

DISCUSSION

One of the major findings in the present study was that hypoxia and hypoxic training decreased the level of liver 8-OHdG compared with that of the control group. This result indicated that the hypoxia and hypoxic training attenuated DNA damage in the liver. It has been demonstrated that oxygen consumption during exercise is lower at high altitudes than at sea level[20,21] and that the formation of reactive oxygen species (ROS) decreases as oxygen partial pressure decreases.[22,23] De Groot et al[2] also noted that hypoxia usually decreases the formation of ROS as the partial oxygen pressure in biological systems decreases. From these previous reports, we can suggest that the decrease in the liver 8-OHdG levels in the present study might be due to a decrease in the generation of ROS as a result of the moderate hypoxic condition. It is conceivable that the decrease in liver 8-OHdG results in increased oxidative DNA damage and/or enhanced DNA repair.[14] Radàk et al[15] reported that exercise training increased DNA repair in old rats. Asami et al[24] also described that the repair activity of the cells of various organs (heart, lung, and liver) against oxidative DNA damage may be increased by regular voluntary exercise. We can infer from these investigations that decreased liver 8-OHdG levels in HE and HS rats might be caused by reduced ROS generation as oxygen partial pressure decreased[22,23] and/or DNA repair was enhanced. In this study, there was no significant difference in the liver 8-OHdG levels of hypoxic exercised rats compared with hypoxic sedentary rats. The effect of hypoxia may counteract that of exercise on 8-OHdG levels. However, further investigations of these factors are necessary.

In the present study, the levels of GSH in the liver decreased due to hypoxia. The data was in agreement with the findings of Shan et al.[25,26] They observed that liver cells isolated from rats exposed to hypoxia had lower synthetic rates than cells from normoxic sedentary rats. SaiRam et al[7] showed that hypoxia decreased the GSH level in blood. The hypoxia-induced decrease in the GSH levels in the liver seems to be due to low oxygen partial pressure, which decreases the generation of free radical species.[2] The decrease in the liver GSH level after hypoxia is also due to a decrease in the GR activity, which is caused by glutathione recycling enzymes (Fig 3). However, Sridharan et al[6] reported that an increase in the GSH level in plasma was observed in hypoxia-exposed group when compared with an unexposed group. Singh et al[8] showed that hypoxic conditions did not change the liver GSH level compared with that for unexposed rats. These conflicting results may be due to a difference in the severity of hypoxia. In the experiments by Singh et al[8] and Sridharan et al,[6] rats were exposed to simulated altitudes at 7,620 m and 7,000 m, respectively. In contrast, the oxygen level in our study was 16.0%. Therefore, severe hypoxia may cause a greater production of ROS than moderate hypoxia. The data in the present study was in agreement with that of Nakanishi et al,[11] who observed that the activities of antioxidant enzymes (Mn-SOD and GSH-Px) in liver were decreased after 21 days at 5,500 m. It is possible to assume that the altitude at 5,500 m depressed the activities of antioxidant enzymes in liver. However, as Nakanishi et al[11] suggest, the response to oxidative stress under hypoxia might also be affected by the length of exposure to hypoxia, measured antioxidants, antioxidant enzymes, and various organs. It is well known that the liver is more vulnerable than other organs to oxidative stress under hypoxia.[11] Moreover, results of this study might be caused by the long-term exposure to hypoxia. Previously, the effects of hypoxia on antioxidants and antioxidant enzymes have been studies for up to 4 weeks.[6,7,8,11,26,27] In our study, rats were exposed to hypoxia over 8 weeks.

To our knowledge there have been no studies on the effects of hypoxic training on the GSH levels in liver. The present study confirmed that the HE group had enhanced liver GSH levels compared with that of the HS group. We previously demonstrated that exercise training in normoxia induced an increase in the liver GSH concentration at the basal level.[9,10] The increase in the GSH concentration in the HE group compared with that of the HS group results from GR activity in the liver (Fig 3). We recently found that the levels of liver GR activity were significantly higher in voluntary exercised rats than in sedentary rats.[10]

Radàk et al[27] showed that the activity of antioxidant enzymes such as Mn-SOD, in both white and red types of skeletal muscle increased after exercise training at a high altitude. This increase in activity may be an adaptive response to exercise-induced oxidative stress.

In conclusion, this study confirmed that moderate hypoxia and hypoxic training attenuated the liver DNA damage and decreased the liver GSH levels and GR activity. These results indicate that moderate hypoxia and hypoxic training result in decreased oxidative stress.

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