**Exercise and oxidative stress in hypoxia**

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Received: August 31, 2013 / Accepted: September 9, 2013

**Abstract** There is considerable indirect proof that a hypobaric-hypoxic environment increases oxidative stress, which is usually reflected by an increase in hepatic TBARS levels and a decrease in Mn-SOD levels. In a hypobaric chamber experiment designed to simulate the summit of Mt. Fuji, we detected an increase in hydroperoxide, an oxidative stress marker, although the percentage increase was lower than that observed at Mt. Fuji. This highlights the compounding effects of environmental factors (ultraviolet rays, temperature differences, etc.) and indicates the importance of conducting measurements in the field. Although the production of oxygen radicals increases with accelerated aerobic metabolism, it has been reported that oxidative stress increases even in hypoxic environments. Activation of xanthine oxidase (XO), that accompanies ischemia-reperfusion (I/R) or an increase in white blood cells, etc. are considered as potential mechanisms by which oxidative stress increases in hypoxic environments. However, these mechanisms have not been fully clarified.

**Keywords**: oxidative stress, hypoxia, mountain climbing, d-ROMs

**Introduction**

In recent times, there has been a boom in mountain climbing. The 2012 statistics indicated that a record high of approximately 321,000 people climbed Mt. Fuji during the summer. In 2013, the number of climbers is expected to exceed 400,000 as Mt. Fuji has been registered as a World Heritage site, and the number of climbers is increasing dramatically.

Oxidative stress is known to increase when descending from high altitudes. Although there has been an expansion in the market for “high altitude tours” as a type of healthy sport, considerable research needs to be done on altitude adaptation and the physiology of mountain climbing. Physical exercise creates a hypoxic environment in the body with an increased demand for oxygen. The hypoxia caused by mountain climbing may potentially have an additive and synergistic effect on elevating oxidative stress. This review evaluates the effects of reactive oxygen on the body in a hypoxic environment by evaluating the levels of HIF-1 mRNA expression. It also discusses how hypoxic stress leads to oxidative stress and whether or not oxidative stress from mountain climbing affects health.

**Radical generation in organisms**

The adverse effects of oxygen-induced damage to biological tissue have long been known, as evidenced by the markedly reduced lifespan of small animals raised in environments with high oxygen concentrations. Oxidative stress-induced damage is thought to be related to a majority of diseases such as cancer, cerebral or myocardial infarction, ulcers, hypertension, atopic dermatitis, diabetes, dementia, and similar lifestyle-related diseases. Hence, defending against oxidative stress is important for the maintenance of a healthy lifespan.

Oxidative stress refers to a state where an imbalance occurs between the generation of reactive oxygen species (ROS) and antioxidant capabilities. ROS includes superoxide radicals, hydroxyl radicals, non-radical hydrogen peroxide, and singlet oxygen. These ROS have a high reactivity as they are more activated than ordinary oxygen. ROS generation and antioxidant capabilities are usually balanced. However, exercise and stress, which may lead to excessive ROS generation or reduced antioxidant capabilities, may disrupt the balance between ROS generation and defense, thus leading to diseases including cancer, and affecting the aging of tissues. In recent years, ROS has drawn significant attention owing to its functional role in muscle contraction and signal transduction. It is evident that excessive ROS generation accompanying physical exercise has the potential to cause extensive damage to biological tissues and cells. However, the associated confounding factors need to be investigated in terms of their influence on the oxidative stress induced by exercise.

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Generation of reactive oxygen from physical exercise

Since 1980, research has focused on the generation of oxidative stress after physical exercise\(^1\). It has been noted that in addition to the acceleration of aerobic metabolism, the generation of radicals is increased through an increase in foreign matter due to increased respiratory volume and through muscle inflammation. In general, it is estimated that approximately 2-5% of the oxygen used in the mitochondria in aerobic metabolism is transformed into superoxide anion radicals (\(O_2^-\)). In recent years, there have been reports that the generation of superoxide in the mitochondria is much lower than or at most 0.15% of the oxygen volume\(^2\).

As the intensity of physical activity increases with aerobic exercise, the volume of oxygen consumed increases by 10-20 times. This generates excessive oxygen radicals that cannot be scavenged during metabolism, and it has been suggested that these radicals cause cell damage. For example, it has been demonstrated that after exercising to the point of exhaustion, the volume of radicals in the muscles and the liver increases by 2-3 times, damaging the sarcoplasmic reticulum\(^3\). It is difficult to directly measure ROS generation during physical exercise. As reported by Sen et al.\(^4\), however, there has been indirect proof based on the anaerobic threshold that oxidative stress increases with an increase in the intensity of physical activity, as evidenced by the increase in the ratio of oxidized glutathione/total glutathione depending on the intensity of physical exercise. Within organisms, radicals are constantly generated through the autoxidation of reducing substances. Moreover, the accelerated metabolism due to exercise leads to an increase in autoxidation of catecholamines and hemoglobin; and the activation of NADPH oxidase facilitated by inflammation (\(\text{NADPH} + 2\text{O}_2 \leftrightarrow \text{NADP}^+ + 2\text{O}_2^- + \text{H}^+\)), uneven distribution of blood flow, and ischemia-reperfusion (xanthine oxidase activation; Fig. 1) lead to ROS generation. Dekkers et al.\(^5\) have reported an increase in ESR signals in the muscle tissue homogenate immediately after maximum exercise. Furthermore, many studies have shown an increase in thiobarbituric acid reactive substances (TBARS), which are an indicator of lipid peroxidation, in the blood and skeletal muscles\(^6\). Thus, it is thought that the volume of ROS generation generally increases depending on the intensity of exercise\(^7\).

Oxidative stress occurs as a result of the imbalance between various antioxidant substances and stressors. Therefore, if antioxidant capabilities exceed the damaging potential of the stressors, it is thought that oxidative stress damage will not occur. D-ROMs is employed to assess the sum total of peroxides in the blood (mainly hydroperoxide concentration: LOOH) (Fig. 2). Relative to the intensity of the exercise, the concentration of d-ROMs begins to rise from approximately 100 watts on an average. Fig. 3 indicates the concentration of hexanonyl lysine (HEL), which is a marker of oxidative stress in the urine, and d-ROMs in the blood. While both d-ROMs and HEL increase after pedaling a bicycle at 150 W for 30 minutes (intermittent line), both tend to decrease below resting levels when exercising at 50 W for 30 minutes (continuous line) (there was a significant decrease in HEL).

Exercise and oxidative stress at high altitude in a hypoxic environment

At sea level, the atmospheric pressure is 1013 hPa, while at high altitudes of 1,000 m, it is 899 hPa; at 2,000 m, it is 795 hPa; and at 3,000 m, it is 701 hPa, indicating that the atmospheric pressure decreases gradually by about 100 hPa with every 1,000 m. At the summit of Mt. Fuji, the highest peak in Japan, the average atmospheric pressure is approximately between 626 and 648 hPa, according to the data recorded by the Japan Meteorological Agency. The corresponding oxygen concentration is 13-13.5% compared to the 20.9% detected on the ground, or roughly two-thirds of that at ground level. Atmospheric pressure at 5,500 m usually drops to half of what is observed at ground level. In measurements taken from 2 alpinists in a hypobaric chamber, simulating the summit of Mt. Everest (337 hPa), the resting levels of PaO\(_2\) were 30.3 ± 2.1 mmHg and 30.6 ± 1.4 mmHg\(^8\).

Wozniak et al.\(^9\) suggested that oxidative stress is accentuated at high altitudes. In their research on kayakers during high altitude training, they observed an increase in serum TBARS levels and a significant increase in SOD.
and catalase activation. During mountain climbing, the potential for radical generation increases further with the additional environmental factors of UV exposure and changes in temperature. In addition, atmospheric oxygen concentration likely changes with the ascent, time spent at the summit, and descent that affects the response to hypoxic stress.

Although the contribution of high altitude has not been fully proven, as shown in Fig. 1, it has been suggested that the generation of \( \cdot \text{O}_2 \) radicals derived from xanthine oxidase (XO) may increase \(^{10,11}\). Therefore, when oxygen is inhaled during physical discomfort at high altitudes, it results in a rapid influx of oxygen to hypoxic tissues and potential exacerbation of oxidative stress by the excessive generation of \( \cdot \text{O}_2 \) radicals through the same mechanism as in ischemia-reperfusion.

Joanny et al. \(^{12}\) conducted an experiment in which 8 subjects were exposed to hypobaric and hypoxic conditions for 31 days in a reduced pressure chamber. The concentration of fatty acid peroxides in the blood increased depending on the extent of the hypoxic environment, with an average increase of 23\% (\( P < 0.05 \)) at 6,000 m, 79\% (\( P < 0.01 \)) at 8,000 m, and 94\% (\( P < 0.01 \)) at 8,848 m. Moreover, observations, based on the redox-sensitive probes that have been developed in recent years, show an actual increase in ROS in the mitochondria in hypoxic environments \(^{13}\).

Research conducted by the present authors evaluated the changes over time in urinary levels of 8-OHdG, an oxidative stress marker of DNA, in elderly subjects (average age 63 years; \( n = 4 \)) while climbing Mt. Fuji (unpublished: Fig. 4). Although this high altitude exercise of just one ascent of Mt. Fuji did not result in oxidative stress that would risk cellular damage, the continuous consumption
of small amounts of an antioxidant (Oligonol from Amino Up Chemical Co., Ltd.) significantly reduced 8-OHdG levels. This finding suggests that the consumption of antioxidants should be considered from the perspective of protecting the body from the effects of age, adverse physical conditions, and other environmental factors.

In recent years, the major source of radical generation in a hypoxic environment was identified as electron leaks from the electron transport chain in the mitochondria. The major site was thought to be complex III (Fig. 5) in the mitochondrial electron transport chain. Although it is not known how a hypoxic condition increases the emission of ROS from complex III, during the last stage of the electron transport chain, there are fewer oxygen molecules that serve as electron acceptors, and as a result, electrons accumulate excessively in the mitochondria. These free electrons are more prone to being reduced to superoxides.

**Oxidative stress and transcription factors**

At the summit of Mt. Fuji, the oxygen concentration is approximately 13%, and physical exercise (mountain climbing) gives rise to a more hypoxic environment for the tissues. In an environment where the oxygen supply to tissues is reduced, erythropoietin (EPO), hypoxia-inducible factor (HIF-1), and HIF-1 mRNA are induced as hypoxic sensors. HIF-1 is a heterodimer protein composed of an α- and β-subunit. In an ordinary oxygenated environment, hydroxyl radicals from hydrogen peroxide and prolyl hydroxylase transfer a hydroxyl group to HIF-1α proteins, and after being ubiquitinated, they are broken on the summit.

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**Fig. 4** Concentrations of the urinary 8-OHdG during the mountain climbing. *p<0.05

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**Fig. 5** Complex I and II generate superoxide within the mitochondrial matrix. Complex III can generate superoxide in both the intermembrane space and the matrix. Hypoxia elicits the release of superoxide from complex III into the cytosol, where it is converted to H$_2$O$_2$ to activate oxidant-dependent signaling pathways resulting in the activation of HIF.
down by proteasomes. The half-life of HIF-1α in an ordinary oxygenated environment is approximately 5 minutes\(^{16}\). In a hypoxic environment, as hydrogen peroxide and prolyl hydroxylase are oxidized through mitochondrial activity, a hydroxyl group is not always transferred to HIF-1α proteins. As a result, HIF-1α proteins are not broken down, and form a dimer with HIF-1β, which acts on the DNA as a transcription factor to promote erythropoietin generation\(^{17}\). Similarly, kelch-like ECH-associated protein 1 (Keap1) senses oxidative stress and stops the fusion/breakdown of transcription factors (also called nuclear factor [erythroid-derived 2]-like 2, Nrf2), thereby promoting the shift to the NFE2L2 nucleus and inducing enzymes that detoxify radicals. Such changes in the transcription factors become the starting point for a variety of metabolic reactions that follow, and in addition to their role as the metabolic products resulting from oxidative stress (malondialdehyde, d-ROMs, etc.), they are recognized as hypoxic and oxidative stress sensors.

Jusman et al.\(^{18}\) kept rats in an environment of 10% normobaric hypoxia for 1-14 days and measured the antioxidant enzymes and metabolic products in the liver. A significant (P < 0.05) increase in MDA levels in liver extracts was detected from day 1 of hypoxic exposure, and these levels continued to increase until day 14. Simultaneously, there was a gradual decrease in GSH (P < 0.05), indicating that a hypoxic environment induces oxidative stress. At the same time, in the initial stages of hypoxic exposure, an increase in HIF1-α mRNA levels, ROS generation, and GSH concentration in the liver were observed, indicating a correlation between HIF1-α and oxidative stress. Furthermore, Pialoux et al.\(^{19}\) examined the relationship between ROS and HIF-1α gene expression and that between the HIF-1α target gene EPO and vascular endothelial growth factor. These studies indicated the possibility that ROS is involved in HIF-1α transcription (Fig. 6) and that a hypoxic environment causes excessive ROS generation and induces oxidative stress.

Our studies investigated whether the production of erythropoietin induced by HIF-1α mRNA and HIF-1 as a response to hypoxic stress was elevated while climbing Mt. Fuji. The findings of this study suggest that there was no major change in HIF-1α mRNA expression (real-time RT-PCR). However, the serum erythropoietin concentration (RIA) was significantly higher (P < 0.05) on the morning after arrival at the summit (86.8 ± 9.6 mIU/ml) and after descent (82.0 ± 23) than that before the ascent (16.1 ± 0.4). It has been previously shown that HIF-1 deactivates the transcription activating factors in erythropoietin genes, and it is thought that erythropoietin transcription is influenced more through the reduction in hydrogen peroxide production due to hypoxia than through changes in HIF-1 itself (Fig. 7).

At high altitudes, erythropoietin production from HIF-1 is caused by suppression of reactive oxygen generation in the kidneys. Therefore, oxidative stress is thought to be related to adaptation to high altitudes.

**Fig. 6** Relationship between HIF-1α mRNA in leukocytes and 8-hydroxy-2′-deoxyguanosine (8-OHdG) concentration in plasma during the acute increase phase of HIF-1α\(^{16}\).

**Fig. 7** Concentrations of the erythropoietin during the mountain climbing, a vs. before climbing b vs. just after climbing.
References