

## Intermittent hypoxia hypobaric exposure minimized oxidative stress and antioxidants in brain cells of *Sprague Dawley* mice

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### Abstrak

**Latar belakang:** Hipoksia hypobaric meningkatkan produksi radikal bebas, terutama spesies oksigen reaktif (ROS). Peningkatan ROS akan menyebabkan stres oksidatif bila tidak disertai dengan peningkatan enzim antioksidan. Kondisi ini dapat dikurangi dengan hipoksia hipobarik intermiten (HHI). Tujuan penelitian ini mengidentifikasi frekuensi IHH yang dapat meminimalkan efek hipoksia hipobarik terhadap stres oksidatif dan aktivitas antioksidan spesifik pada tikus *Sprague Dawley*.

**Metode:** Penelitian eksperimental pada bulan Februari-April 2010, Subjek terdiri dari satu kelompok kontrol dan empat kelompok paparan pada mencit jantan *Sprague Dawley*. Setiap kelompok terdiri dari 5 tikus. Kelompok kontrol tidak terpapar IHH. Kelompok terpapar (dengan selang waktu satu minggu) terpapar sekali, dua kali, tiga kali, atau empat kali IHH. Semua kelompok paparan dipaparkan hipobarik setara dengan ketinggian: 35.000 ft (1 menit), 25.000 ft (5 menit), dan 18.000 ft (25 menit). Jaringan otak diperiksa untuk 8-OHdG dan SOD.

**Hasil:** Setelah tiga paparan IHH tingkat 8-OHdG sudah kembali ke nilai kontrol ( $P = 0,843$ ). Tingkat SOD meningkat secara progresif pada dua, tiga, dan empat kali paparan IHH. Bahkan setelah paparan kedua, tingkat SOD sudah sama dengan nilai kontrol,  $0,231 \pm 0,042$  ( $P = 0,191$ ).

**Kesimpulan:** Tiga kali IHH sudah dapat meminimalkan pengaruh hipoksia hipobarik terhadap stres oksidatif dan aktivitas spesifik antioksidan pada tikus *Sprague Dawley*.

**Kata kunci:** hipoksia hipobarik intermiten, stres oksidatif, antioksidan

### Abstract

**Background:** Hypoxia hypobaric increase the production of free radicals, especially reactive oxygen species (ROS). The increase in ROS would cause oxidative stress when not accompanied by an increase in antioxidant enzymes. This condition may minimize by intermittent hypobaric hypoxia (IHH). This study aimed to identify the number of IHH which may minimize the effect of hypoxia hypobaric on oxidative stress and the specific activity of antioxidants in *Sprague Dawley* male mice.

**Methods:** The experimental study was in February-April 2010 consisted of one control group and four exposed groups of male mice *Sprague Dawley*. Each groups consisted of 5 mice. The control group did not have IHH. The exposed groups (with an interval of one week) had once, twice, three, or four times IHH using a chamber flight. All exposed groups were treated hypobaric equivalent to: 35,000 ft altitude (1 minutes), 25,000 ft (5 minutes), and 18,000 ft (25 minutes). All of their brains had 8-OHdG and SOD measured.

**Results:** The 8-OHdG level among three time IHH exposures had already returned to the control value ( $P = 0.843$ ). The SOD level increased progressively among two, three, and four times IHH. However after the second exposure, it was found that the SOD level was similar to the control value,  $0.231 \pm 0.042$  ( $P = 0.191$ ).

**Conclusion:** In conclusion, three times of IHH may improve the effect of hypoxia hypobaric on oxidative stress and specific activity of antioxidants in *Sprague Dawley* male mice. The SOD level was increased at an earlier exposure, which was after one IHH exposure.

**Keywords:** intermittent hypoxia hypobaric, oxidative stress, antioxidants

Decrease of atmospheric pressure in altitude causes a decrease in the partial pressure of oxygen causing a reduction in the number of molecules of air that is inhaled. This will decrease blood hemoglobin saturation and result in low tissue oxygen levels, referred to as hypoxia.<sup>1,2</sup>

Hypoxic conditions at this altitude will affect the body's physiological metabolism and disrupt homeostasis. This disruption will affect function of the major organs in the body, like the brain.

In hypoxia there will be an increase the production of *reactive oxygen species* (ROS), such as superoxide ( $O_2^-$ ) and *hydrogen peroxide* ( $H_2O_2$ ).<sup>3,4</sup> Excessive levels of ROS can cause cell damage by oxidative stress. Because ROS are highly reactive in nature, it will react with macromolecules in the body such as proteins, lipids, and DNA. ROS will cause lipid peroxidation reaction, attack proteins and form a carbonyl as the end result, and destroy DNA which will form *8-hydroxydeoxyguanosine* (8-OHdG).<sup>5</sup>

Previous studies revealed that intermittent hypoxia hypobaric (IHH) may help to control the effects of hypoxia hypobaric, such as oxidative stress and the specific antioxidant activity in the mice heart.<sup>6</sup> However, the number of IHH needed which may minimized the effect of hypoxia hypobaric oxidative stress and the specific activity of antioxidants, in particular in brain is still unknown.

This study aimed to identify the number of IHH which minimize the effect of hypoxia hypobaric on oxidative stress 8-OHdG in brain tissue and superoxide dismutase (SOD) in Sprague Dawley male mice.

## METHODS

This was an in vivo experimental study on male, 2 month-old, Sprague Dawley mice, weighing between 150-200 grams. The study was held at the Institute of Aerospace Medicine Saryanto (Lakespra Saryanto) Jakarta.

The mice were divided into one control group and four exposure groups. Each group consisted of 5 mice. The control group was not exposed to IHH. The exposure groups were exposed to once, twice, three, or four times IHH (with an interval of one week) in a hypobaric chamber flight. Exposure was hypobaric hypoxia equivalent to 35,000 ft altitude (1 minutes), 25,000 ft (5 minutes), and 18,000 ft (25 minutes). 8-OHdG and MnSOD of the brains tissues were measured.

The examination for 8-OHdG in DNA oxidation used ELISA (8OHdG-EIA kit, Bioxytech<sup>®</sup>) with DNA isolation steps, reagents, and sample preparation kit according to instructions. ELISA results read at a wavelength of 450 nm.

For SOD enzyme measurement, brain tissue homogenates were examined for SOD activity using Ransod (Randox<sup>®</sup>). Then the absorption of light was read with a spectrophotometer at a wavelength of 505 nm.

Statistical analysis used t test with Stata software released 9. The study was approved by the Research Ethics Committee of the Medical Faculty of Medicine, Universitas Indonesia.

## RESULTS

In term of 8-OHdG level, Table 1 shows that compared with controls, subjects who had one and two times IHH exposures decreased their 8-OHdG level ( $P=0.107$  and  $P=0.126$  respectively). The 8-OHdG level among three time IHH exposures had already returned to the control value ( $P = 0.843$ ).

Furthermore, in term of SOD level, Table 1 shows that compared with controls, the SOD level decreased among the mice which had one time IHH only ( $P=0.138$ ). But the SOD level increased progressively among two, three, and four times IHH. However after the second exposure, it was found that the SOD level was similar to the control value,  $0.231 \pm 0.042$  ( $P = 0.191$ ).

Table 1. 8-OHdG and MnSOD levels control and treatment group in brain tissue of Sprague Dowley mice

Group	8-OHdG level		SOD level	
	Mean $\pm$ SD	P*	Mean $\pm$ SD	P*
Control Hypoxia hypobaric	470.37 $\pm$ 34.47	Reference	0.207 $\pm$ 0.013	Reference
One time	418.28 $\pm$ 23.74	0.107	0.186 $\pm$ 0.033	0.138
Two times	420.72 $\pm$ 24.56	0.126	0.231 $\pm$ 0.042	0.191
Three times	466.98 $\pm$ 20.23	0.843	0.233 $\pm$ 0.029	0.139
Four times	472.53 $\pm$ 5.78	0.891	0.310 $\pm$ 0.041	0.006

\* t test

## DISCUSSION

This analysis had several limitations, among others, the number of IHH exposures were limited to four times only. In addition, in assessing the damage due to oxidative stress only one compensator antioxidants.

It was found that the 8-OHdG increased and then decreased levels of 8-OHdG. However, the changes in the levels of 8-OHdG was statistically was not significantly. These results indicate that the levels of 8-OHdG in brain cells with IHH exposure was relatively stable and this activity was expected to reduce the risk of damage at the DNA level.

SOD levels in this study seemed to decrease with one exposure IHH compared with the control group. The levels of SOD further increased with exposure of IHH more than once. SOD levels in the IHH exposure significantly increased four exposure compared to the control group. The increased levels of SOD occurred was greater with increasing frequency of exposure of IHH. These results indicate that SOD activity increased with increased exposure IHH which may be specific for organ adaptation to hypoxic conditions, especially in the brain. The increased SOD was probably intended to neutralize the free radicals (ROS) formed by exposure to hypoxia hypobaric conditions.

Several biochemical reactions inside the cell, particularly in the mitochondria is the major source of ROS because of electron transport activity. A total of 0.2 to 2% of molecular oxygen is consumed during the process of mitochondrial respiration and converted into a major superoxide in the form of complex I and III.<sup>7,8</sup>

Increasing the concentration of oxygen in tissue reperfusion causes loss of the ability to reduce the cofactor in the respiratory chain and increase mitochondrial ROS formation. Furthermore, the decrease in complex I activity will show an increase in superoxide. The high density of mitochondria in cardiomyocytes and oxidative phosphorylation activity will increase the amount of superoxide. Under normal circumstances the superoxide will be detoxified by antioxidant enzyme such SOD, catalase, and glutathione peroxidase (GPX). Oxidative stress occurs when an increase in ROS was not anticipated by the antioxidant system, Smith and Murray concluded that SOD (superoxide dismutase) is very important in the regulation of superoxide as a result of oxidative phosphorylation.<sup>9</sup>

IHH results show the pattern of adaptation, in which reactive oxygen compounds carbonyl and MDA increase after one IHH one treatment. This suggested that the activity of lipid peroxides in the cell membrane rapidly increased and can damage cell membranes. But a slow decrease in damage is possible due to an increased antioxidant activity of catalase and SOD.

A previous study noted that serum levels of SOD in the hypoxic hypobaric continued to rise after the 5th day. While the 8-OHdG levels were likely to be stable in the brain cells exposed to three and four IHH.<sup>10</sup> Therefore, more the increase number of IHH exposure, will cause SOD to increased which was the body's effort to neutralize reactive oxygen indicated by 8 OHdG as a sign of oxidative damage.

In conclusion, three times of IHH may improved the effect of hypoxia hypobaric on oxidative stress and specific activity of antioxidants in Sprague Dawley male mice. The SOD level was increased at an earlier exposure, which was after one IHH exposure.

## Acknowledgments

The authors would like to thank Saryanto Institute for Aerospace Medicine (Lakespra Saryanto) and Department Biochemistry and Biomolecular Biology, Faculty of Medicine, Universitas Indonesia for allowing us to conduct the study. We appreciate Prof. Bastaman Basuki for technical assistant in data analysis and writing this article.

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