CHANGE OF SERUM FREE RADICALS AND ANTIOXIDANT POTENTIAL IN REBREATHER DIVERS

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ABSTRACT

BACKGROUND: No study exists on the estimated risk from oxidative stress induced by rebreather diving activities, although some observations suggest that inhaled oxygen under hyperbaric treatment increase oxidative stress on living body. This study intended to evaluate oxidative stress and antioxidant potential of divers during rebreather diving activity, and to estimate the effect of reactive oxygen and antioxidant potential.
MATERIALS AND METHODS: The subjects were 10 healthy rebreather divers. Diving profile is depth of 30m for 30 min, total diving time was 60 minutes including 10 min of descending time and 20 min of decompression time. Subjects were equipped with their closed circuit apparatus keeping PO_2 as 1.3ATA (constant). Subjects were asked to swim about 1200m underwater for 60 min. Blood samples were collected from divers' forearm vein before and after diving. Immediately after having the samples, a portable free radical and antioxidant potential determination device called FRASR (Free Radical Analytical System) was used to measure the reactive oxygen metabolites (ROM) and the biological antioxidant potential (BAP).
RESULTS: ROM, which is the indexes of evaluating reactive oxygen

species, had no significant difference (before: 284.5 ± 49.9 CARR.U., after: 284.5 ± 51.2 CARR.U.) (ROM × Hct/43; before: 294.8 ± 48.4 CARR.U., after: 289.5 ± 50.6 CARR.U.). BAP, which indicate antioxidant potential, significantly increased 10.7% after diving (before: 2221.6 ± 466.5 μmol/l, after: 2458.4 ± 363.5 μmol/l) ($P<0.05$).
CONCLUSIONS: In this study, it was confirmed that there was a time frame during which serum BAP increased in rebreather diving, which suggests that antioxidant potential would be induced from some tissues.

INTRODUCTION

Introduced about 10 years ago, rebreather diving apparatus has acquired some popularity in Japanese expert diving community or diving enthusiasts. Rebreather diving is an activity conducted underwater with underwater rebreathing apparatus, removing CO_2 in the expiration and compensating the metabolised O_2. Breathing high pressure oxygen supply can bring higher risk of reactive oxygen damage.

It is well known that reactive oxygen is a cause of significant cell damage from the oxidation of membranes or by altering critical enzyme pathways and systems. Many studies point out that oxidative stress appears to be associated with increased production of reactive oxygen radicals that alter the natural antioxidant defense mechanisms present in most cells and tissues.

Some reports states observations that inhaling oxygen under hyperbaric treatment increased oxidative stress on living body. However, there are no study on estimating the risk of oxidative stress caused by high pressure oxygen mixed gas inhalation by healthy subjects during rebreather diving. For divers' health care, it is an important issue whether rebreather diving activity increases oxidative stress or not. The aim of this study is to evaluate oxidative stress and antioxidant potential of divers during rebreather diving activity, and to estimate the effect of reactive oxygen on rebreather diving .

MATERIALS AND METHODS

Subjects

The subjects were 10 healthy male rebreather divers (age range 45.9 ± 9.6 years-old, smokers is not included). All subjects were informed of the aim of this study, and informed consent was obtained from all subjects.

Fig.1～3 are full closed circuit rebreather (CCR).

1: O_2, 2: diluent gas
A: expiration,
B: CO_2 absorbent,
C: O_2 addition,
D: inhalation

Fig.1 The system of full closed circuit rebreather.

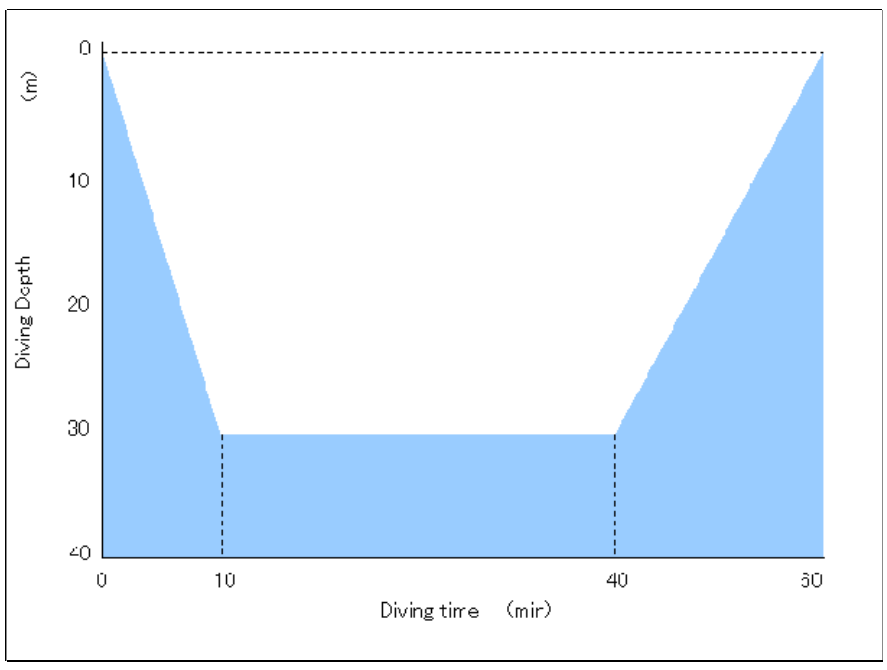

Fig.4 Depth and time in diving profile

Fig.3 Divers equipped With full closed circuit rebreater.

Fig.5 FRAS4

Diving profile

Diving profile was depth of 30m for 30 min, total diving time was 60 minutes including 10 min of descending time and 20 min of decompression time (Fig.4). Bottom temperature is 15℃. The diving profile is shown below.

Subjects were equipped with the full closed circuit apparatus keeping PO_2 as 1.3ATA (constant), and asked to swim across about 1200m underwater for 60 min.

Sample collection and measurements

Blood samples were collected from divers' forearm vein before and after diving. Immediately after gathering samples, a portable free radical and antioxidant potential determination device called free radical analytical system 4 (FRAS4®) (Diacron, srl., Italy) (Fig.5) was used to measure the reactive oxygen metabolites, ROM and the biological antioxidant potential, BAP, and blood chemistry examination; WBC, RBC, Hb, Hct, Plt, MCV, MCH, MCHC, thrombomodurin, and accelerated plethysmography (APG) (Heart rater SA-3000P®:) before and after diving.

Other examinations

During diving, each diver carried a pulse sensor (Seiko pulse graph® Seiko Watch Co., Japan) to confirm one's own heart rate to be below 110 bpm (Fig.6). They also carried diving computers (Citizen air diver®, Citizen Watch Co., Ltd.) to control the time and depth of diving (Fig.7).

Fig.7 Citizen air diver.

Fig.8 An example profile of rebreather divers.

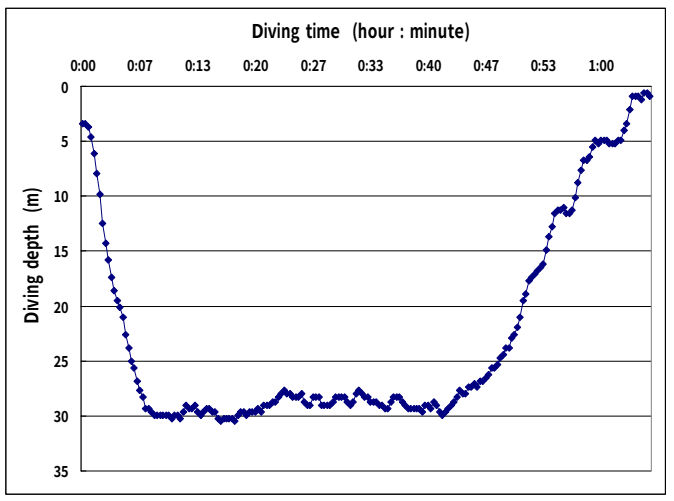

Fig.6 Seiko pulse graph.

Statistics

All data are expressed as mean ± SD. Statistical analysis was performed on a personal computer with the Microsoft Excel. Variables were analyzed by paired t-test to analyze change from baselines. *P* values of less than 0.05 were considered significant.

RESULTS

Fig.8 is an example of subjects in rebreather diving.

ROM, which is the indexes of evaluating reactive oxygen species, had no significant difference (before: 284.5 ± 49.9 CARR.U., after: 284.5 ± 51.2 CARR.U.) (ROM × Hct/43; before: 294.8 ± 48.4 CARR.U., after: 289.5 ± 50.6 CARR.U.) (Fig.9). BAP, which indicate antioxidant potential, significantly increased 10.7% after diving (before: 2221.6 ± 466.5 μmol/l, after: 2458.4 ± 363.5 μmol/l) ($P<0.05$) (Fig.10).

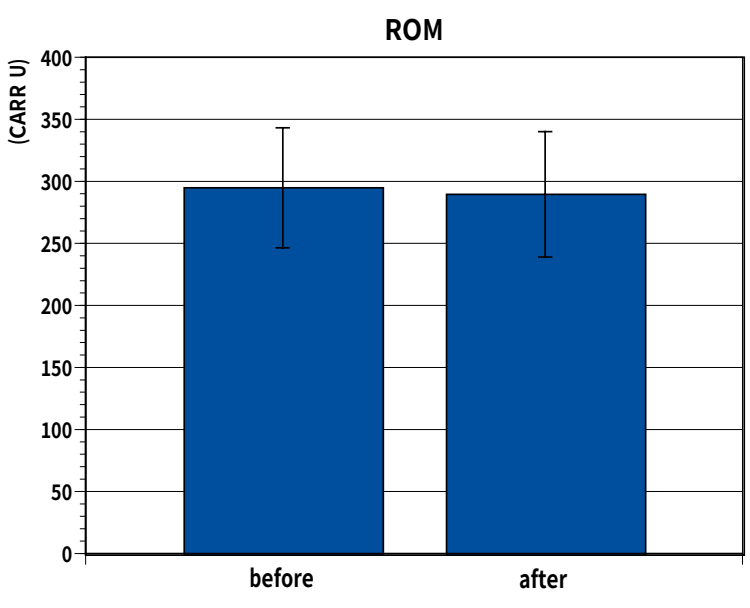

Fig.9 ROM

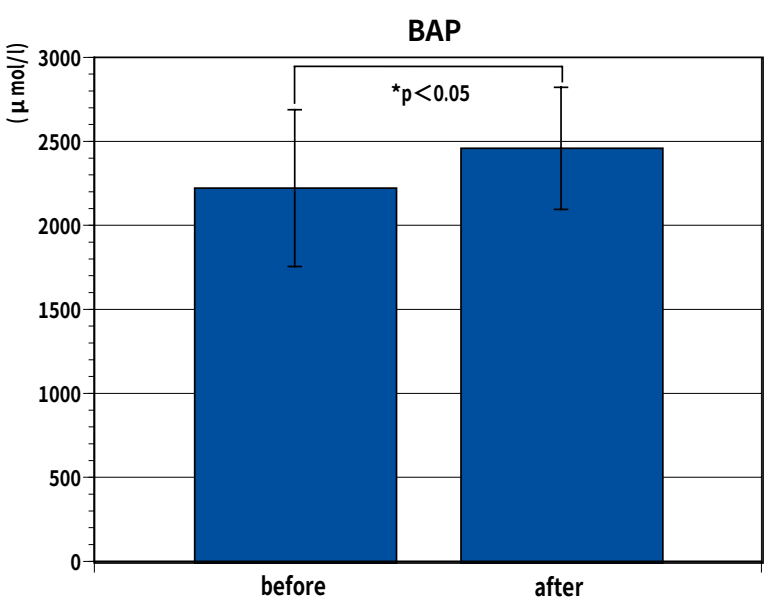

Fig.10 BAP

Table 1 Blood chemistry examination and APG before and after diving.

	before	after
WBC	5175.0±364.2	5437.5±1337.3
RBC	481.1±35.5	481.1±30.3
Hb	14.8±0.6	15.0±0.5
Ht	44.1±1.6	43.5±1.3
PLT	22.0±5.3	22.5±5.3
MCV	91.8±4.5	90.4±4.2
MCH	30.8±1.6	31.2±1.8
MCHC	33.6±0.6	34.5±0.8
TM	15.7±3.1	16.2±3.6
Acceleration plethysmogram	no significant	
Heart Rate	77.4±11.6	76.4±10.8
autonomic nervous system		
Time Domaim Analysis	no significant	
Frequency Domain Analysis	no significant	

DISCUSSION

In this rebreather divings, constant PO2 of 1.3ATA in the closed circuit apparatus was kept as respiration gas. So during the undersea activities, oxygen partial pressure of inhalation gas did not increase or decrease, although keeping higher compared to open circuit scuba. Differ from the open circuit scuba diving, where at the depth of 30 meters undersea, for example, the environmental pressure becomes 4 atmospheres, and the alveolar oxygen partial pressure theoretically becomes approximately 640 mmHg, which is almost 4 times of atmospheric pressure respiration, in this rebreather diving study, the alveolar oxygen partial pressure stayed approximately 1,000 mmHg, which is almost 6 times of atmospheric pressure respiration. Nevertheless, there have not been any studies or researches conducted in the topic of oxidative stress during rebreather diving.

Generally, it is said that the rise of inhalation oxygen partial pressure increases the generation of reactive oxygen by the effect of promoted oxygen metabolism in the body. When the generated reactive oxygen and free radicals are not appropriately eliminated, there should be a risk of oxidative damage in the body. Although it is said that physical exercise improves antioxidant potential, the change of antioxidant potential in rebreather diving activity wherein high pressure oxygen is inhaled, has not been clarified in relation to physical activities.

In this study, it was confirmed that there was a time frame when serum BAP increased in low stressed rebreather diving, which signifies that antioxidants were induced from some tissue to control oxygen stress, or that free radical damage may be reduced in rebreather diving activities under a certain conditions.

Usually, it is said that about 2% of oxygen consumed in respiration at rest becomes free radicals, but in rebreater diving wherein high partial pressure oxygen is inhaled, there are concerns that free radical generation may increase in accordance with the increased oxygen intake. However, as for physical exercise and lipid hyperoxidation, it is reported that exercise load up to 50% of maximum oxygen intake does not affect the lipid hyperoxidation.

Furthermore, as in the rebreather diving in this study, in diving activities wherein stress load was relatively low, radical generation might have not been so significant. Nonetheless, even taking this in consideration, many issues remain to be solved regarding the result that shows the no change of free radicals while ROM level was expected to rise during rebreather diving. One possibility is that although there may have been generation of free radicals during rebreather diving activities, the scavenger system in the divers' body was mobilized and that the BAP level was higher after diving. However, the reason why the scavenger system that overcomes radical generation was mobilized to higher the BAP generation cannot be explained from this study. There could be some specific features in rebreather diving that are different from physical exercise activities in atmospheric pressure environment, and it would be interesting to investigate if this is so. Also, the fact that samples who participated in this study were under a slight oxidative stress condition before diving, this could have complicated the interpretation of the result of this study, but it is thought that slight oxidative stress condition is due to the age factor of samples.

In this study, the fluctuating situation of the scavenger system during rebreather diving activities was not grasped, but it was confirmed that antioxidant potential show significant increase before and/or after a single rebreather diving activity. It is considered that

one of the reasons for that is that the samples of this study were all well experienced divers. According to studies related to antioxidant potential, people who exercise frequently have higher level of antioxidant enzyme and other antioxidant substances in tissue. The antioxidant potential that was measured in this study showed a high value before the experiment in many subjects. It is considered that this is because the samples had already acquired sufficient antioxidant potential in their daily exercise activities that includes diving.

In this study, ROM and BAP test was used to evaluate the oxidative stress or antioxidant potential of the samples. Conventionally, measurement of free radical was time consuming and also caused consumption of much expense, and the ROM test method itself was quite difficult, but the ROM and BAP test that was developed recently can measure free radicals or antioxidant potential in a very easy-to-use method, and the test can be done in a very short time with accurate evaluation of oxidative stress or antioxidant potential in human body. There are evidences that the results of d-ROMs test correlate with the results of ESR, and the ROM test is utilized in many studies as being a reliable testing method.

As a conclusion, in rebreather diving, there is a time frame when the serum BAP increase, and rise of oxidative stress due to high pressurized oxygen inhalation was not found. As one topic to follow, an investigation needs to be conducted on what influences the oxidative damage in cases when diving is conducted repeatedly, and how divers acquire the antioxidant features. Moreover, although evaluation of oxidative stress, inspiration oxygen pressure or exercise changes are difficult because of multiple factors that affect the relation among these parameters, it is thought that appropriate analysis is possible when accurate control is conducted.

ROM test: Test for oxidative stress level

Principle

The method is based upon metals transition capacity to catalize, once these metals are freed from their chelate protein transport forms and the deposit where they are normally found in plasma and cells, reactions from the formations of free radicals according to Fenton's reaction as in the following formula, or in radical propagation:

$H_2O_2 + Fe^{2+} + Fe^{3+} \rightarrow OH\bullet + OH^-$

The radicals which are produced, the quantity of which is directly proportional to the quantity of peroxide present in plasma, are chemically trapped by phenolic derivate molecules, and through the reaction, these peroxides are transformed into ions. The ion transformation colors the peroxides, and the color can be measured with photometers. Practically, a small amount of serum is diluted in an acid buffer solution (pH 4.8). The iron ions that were bonded to the serum proteins become available to catalyze in vitro the breakdown of blood hydroperoxides to alkoxyl and peroxyl radicals.

The reactions of ROMs test is as follows;

1A) $R\text{-}OOH + Fe^{2+} \rightarrow R\text{-}O^* + Fe^{3+} + OH^-$

1B) $R\text{-}O^* + A\text{-}NH_2 \rightarrow R\text{-}O^- + [A\text{-}NH_2{}^*]^+$

2A) $R\text{-}OOH + Fe^{3+} \rightarrow R\text{-}OO^* + Fe^{2+} + H^+$

2B) $R\text{-}OO^* + A\text{-}NH_2 \rightarrow R\text{-}OO^- + [A\text{-}NH_2{}^*]^+$

wherein;

R-OOH is a generic hydroperoxide

$R\text{-}O^*$ is the alkoxyl radical of a generic hydroperoxide

$R\text{-}OO^*$ s the hydroperoxyl radical of a generic hydroperoxide

$A\text{-}NH_2$ is N, N-diethyl-paraphenylendiamine, i. e. the chromogenic substrate of d-ROMs test

$[A\text{-}NH_2{}^*]+$ is the coloured radical cation of the chromogenic substrate

The chromogen (N,N,-diethylparaphenylen-diamine) is oxidized by hydroperoxyl and alkoxyl radicals, then change to colored cation detectable at 505 nm. The concentration of colored complex reflects (correspond, related) to the hydroperoxides levels of the tested biological sample. The results were expressed as CARR U., where 1 CARR U. corresponds to 0.08 mg/100 ml H_2O_2. The normal range has been determined as 250-300 CARR.U..

BAP test: Test for biological antioxidant potential

Principle

Based on the ability of a colored solution, containing a source of ferric (Fe^{3+}) ions bound to a chromogenic substrate (i.e. a thiocyanate derivative), to decolor when Fe^{3+} ions are reduced to ferrous ions (Fe^{2+}), as it occurs by adding a blood plasma sample. For the test, plasma sample already has been dissolved in a colored solution obtained by mixing a source of ferric ions (i.e. ferric chloride, $FeCl_3$).

Practically, a small amount of blood plasma (10µL) to be tested is dissolved in a colored solution, which has been previously obtained by mixing a source of ferric ions (i. e. ferric chloride, $FeCl_3$) with a special chromogenic substrate (i. e. a thiocyanate derivative)

After five minutes incubation at **37°C,** the solution will decolor, reflecting the ability of plasma to reduce ferric ions to ferrous ions, according to these reactions:

1. $FeCl_3$ + AT (uncolored) → $FeCl_3$ - AT (colored)
2. $FeCl_3$ - AT (colored) + BP(e-) → $FeCl_2$ + AT (uncolored) + BP

wherein:

$FeCl_3$ is ferric chloride

AT (uncolored) is a thiocyanate derivative (uncolored);

$FeCl_3$ - AT (colored) is the colored complex of ferric chloride with thethiocyanate derivative

BP (e-) is a molecule of blood plasma barrier with reducing/electrongiving/antioxidant activity against ferric ionsBP is the oxidized form of BP (e-)$FeCl_2$ is the ferrous chloride obtained by the reducing activity of BP (e-)

By photometrically assessing the intensity of decoloration, the amount of reduced ferric ions can be adequately evaluated, which shows antioxidant potential of tested sample.

The value more the 2,200 µmol/l, is estimated to physiological adequate.