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Research Article

CHANGES IN THE OXIDATIVE STRESS OF DIVERS FOLLOWING A DIET WITHOUT FRUITS AND VEGETABLES

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ABSTRACT

The increased oxidative stress is the basis of the pathogenesis of many socially significant diseases such as atherosclerosis, carcinogenesis, cardiovascular diseases, metabolic disorders, etc. Also, the effects of scuba diving on health are reflected through the oxidative stress, which may be caused by demanding physical activity, hyperoxia and exposure to cold temperatures. Fruits and vegetables are rich in vitamins, which are natural antioxidants. The aim of this study was to establish whether a disturbance in oxidative stress and antioxidant defense is observed in divers subjected to a diet without vegetables and fruits and how this affects the decompression risk. The study involved 20 divers. The indicators of oxidative stress (dROMs) and biological antioxidant capacity (antiROMs) were measured before and after conducting a strict diet without vegetables and fruits in a period of 5 days. The results show a statistically significant increase in oxidative stress after diet, but the reduction in antioxidant barrier has not shown a significant reduction, due to the short diet period, the available stocks and non-depletion of important antioxidant compounds in the body.

INTRODUCTION

Modern man is constantly exposed to the influence of environmental factors leading to the production of a large amount of free radicals. They are extremely reactive, damaging the cell membranes and the DNA molecule. Oxidative stress is a term to denote the imbalance between the concentration of reactive oxygen and nitrogen radicals and body antioxidant defense mechanisms. The increased oxidative stress is the basis of the pathogenesis of many socially significant diseases such as atherosclerosis, carcinogenesis, cardiovascular diseases, metabolic disorders, etc. The presence of unpaired electron in reactive oxygen species (ROS) makes them highly unstable and reactive molecules, initiating a series of chain reactions leading to significant damage to macromolecules and tissues [12]. ROS include the superoxide anion (O_2^-), the hydroxyl anion (OH^-), hydrogen peroxide (H_2O_2), nitrogen oxide (NO), hypochlorus acid (HOCL), ozone (O_3), singlet oxygen ($^1O_2$) and other. Most ROS react as strong oxidants and their potent reactivity allows reactions with lipids, sugars, proteins and nucleic acids. Physiologically free radicals are formed in the normal metabolic processes in the cell. They play a role in regulating essential functions and act as signal molecules in transduction cascades or pathways for various growth factors, cytokines and hormones. Oxidative "blast" is absolutely necessary for the start of immunological reactions, activation of neutrophil cells and phagocytosis as the primary immune response of the organism against foreign antigens. Reactive species can generate either "positive" or "negative" effects depending on their concentration and intracellular localization. Loss of balance between ROS production and antioxidant level results in "oxidative stress", which lies at the root of many local and systemic diseases.

Antioxidant is any substance or compound that has the ability to bind free oxygen species or inhibit the oxidation process in the cell. The level of antioxidant compounds present in cells and extracellular should be sufficient to counteract the toxic effects of ROS and maintain normal physiology. This balance can be lost due to the overproduction of free radicals or insufficient intake of nutrients containing antioxidant molecules.

Antioxidants can be divided into two groups - antioxidants in plasma and intracellular antioxidants. The primary role of plasma antioxidant protection is to connect ions to transition metals, such as iron and copper, thus reducing their plasma concentration and reducing the free radical generation reactions. This prevents the formation of hydroxyl radicals that are more reactive and lead to the peroxidation of lipids and metabolites. Plasma antioxidants are transferrin, lactoferrin, ceruloplasmin, albumin, uric acid, haptoglobins and hemopexin. Ascorbic acid (Vitamin C), alpha-tocopherol

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(vitamin E), β-carotene and vitamin A are also plasma antioxidants that can clear water-soluble peroxyl radicals and other ROSs and prevent the chain reaction of lipid peroxidation.

The major intracellular antioxidant enzymes are superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase. Human plasma has very little catalase but contains low activities of SOD and GSH-Px. SOD is localized both in the cytosol (CuZn-SOD) and mitochondria and acts as a dismutase for superoxides to hydrogen peroxide and molecular oxygen. SOD is considered a major intracellular enzyme because it is capable of reducing the richest free radical, the superoxide anion ($\bullet O_2^-$).

Vegetables and fruits are rich in vitamins (Vitamin C, Vitamin A, flavonoids, and carotenes), which are natural antioxidants. Enriched with fruit and vegetable diet has a protective role. Oxidative stress affects you all the time. It can cause the following problems, tiredness, obesity, sick, and old, and the ache in your knees after hard training, may affect the physical condition of athletes, etc. [13, 18].

High oxygen concentrations, such as in hyperbaric conditions, act pro-oxidant and increase the production of reactive oxygen species. Currently, the data on oxidative status under these conditions and the health of people under high pressure are quite controversial. Some studies describe positive effects and other undesirable effects [20]. Along with the increase in oxidative stress, antioxidant protection (catalase, GPx, SOD), which has the role of reducing ROS levels, is affected.

Purpose

The aim of this study was to establish whether a disturbance in oxidative stress and antioxidant barrier is observed in divers subjected to a diet without vegetables and fruits and how this affects the decompression risk.

MATERIAL AND METHODS

In the survey participate 20 military divers, all men, conducting scuba diving at small and medium depths, and the breathing gas used is a compressed air. Initially, oxidative stress (dROMs) and antioxidant capacity (antiROMs), and plasma protection were studied. By observing a strict diet without vegetables and fruits within 5 days, the oxidative stress and antioxidant barrier are redefined. At this time, individuals are routinely performing their professional activities.

For the characterization of the group the mean meanings of the signs (means) and the standard deviation are used (M±SD) - in case of parametric distribution, and medium and interquartile range (Me (LQ – UQ)) - for nonparametric data. The total length of service of the divers is (Me (LQ – UQ)): 10 (5 – 16) years, underwater experience: 2000 (500 – 3500) hours, average depth of underwater dives: 13 (12 – 16) meters, maximum depth of dives 40 (30 – 60) meters. The physiological characteristics of the group are as follows:

- Age, years: 35 ± 10
- Height, cm: 179 ± 7
- Weight, kg: 84 ± 11
- BMI, kg/m^2: 25.8 ± 4.6
- Number of smokers: 5 (25%)

For our study the procedure had been modified by using an Olympus multiplex analyzer. dROMs (Diacron International Italy) is a photometric test for the overall assessment of the oxidative stress oxidation component [5]. Determines the concentration of reactive oxygen metabolites and, in particular, the hydroperoxides (ROOH) generated in the cell by the oxidative action of ROS on various biochemical substrates (lipids, amino acids, peptides, proteins, nucleic acids). Available hydroperoxides in the sample are placed under conditions provided by the Fenton reaction, where the iron released from the plasma proteins by acidic buffer is capable of generating in vitro hydroperoxyl and alloxy radicals [7, 9]. These radicals are capable of oxidizing an alkyl-substituted aromatic amine (N, N- diethyl-para-phenylendiamine) to give a pink colored derivative photometrating at 505 nm [1].

The intensity of the stain is proportional to the concentration of the dROM and the results are expressed in arbitrary Carratelli units (CARR U; derived from the name of the chemist who invented the test - 1 CARR U = 0.08 mg of hydrogen peroxide/dL) [2, 3, 4]. Values above 300 Unit CARR are an indicator of oxidative stress.

AntiROMs (Diacron International Italy) is a photometric test to determine the total antioxidant capacity of plasma [5]. The test is based on the ability of antioxidants to reduce iron by converting it from Fe^{3+} to Fe^{2+}. Reaction with the aa′-dipyridyl compound results in a red-purple coloration which can be quantified photometrically at a wavelength of 505 nm at 37°C. The intensity of the color increases proportionally depending on the amount of iron reduced by the antioxidants present in the sample [10]. The results are expressed in micromoles of reduced iron/liter or microequivalent/liter using ascorbic acid as a standard. The result is expressed in two different values. Determined the amount of "fast" (most often these are exogenously imported compounds - Vitamin C, Vitamin A, tocopherol, flavonoids, etc.) and "slow" plasma antioxidants - uric acid, thiols, cysteine etc. Fast antioxidants immediately intervene to remove the ROS formed while the slow ones are involved at a later stage of antioxidant protection. Value below 200 μEq/l for the first, and below 1000 μEq/l for the second is an indicator of oxidative stress state. When we have a reduction in fast antioxidant protective mechanisms, it is most often associated with reduced bioavailability of vitamin C, tocopherols or polyphenols, looking for dietary disorders or food intake in the gastrointestinal tract. Low levels of slow antioxidants are most often due to a decrease in thiols and uric acid and reflect disturbances in the endogenous metabolism and motor activity of the body. The tests were made in serum. The blood taken is centrifuged and the serum freezes at -20°C. Samples were made in 2 steps. The periodic quality control (the intra-assay variation) of two control standardized samples with different concentration ranges showed a variation coefficient (VK) of 3.2% and 4.6% and d% 3.4%

Data analysis was performed using a statistical program IBM SPSS Statistics Version 25. The study was conducted in accordance with the Helsinki Declaration.

RESULTS

Tables 1 and 2 give the reference values of oxidative stress and the limits of oxidative protection.

Table 1 Reference values of oxidative stress

dROMs in CARR U	Oxidative stress
250-300	Normal range
301-320	Border values
321-340	Low level
341-400	Moderate level
401-500	High level
above 500	Very high level

Table 2 Impairment degree of plasma antioxidant barrier

AntiROMs "Fast" in µEq/l	AntiROMs "Slow" in µEq/l	Antioxidant barrier
>200	>1000	Optimal value
	900-1000	Border line condition
150-200	700-900	Slight reduction
100-150	500-700	Moderate reduction
<100	<500	Strong reduction

Our results of oxidative stress and antioxidant protection obtained before the diet (1) and after the diet (2) are shown in Table 3.

Table 3 Results of oxidative stress and antioxidant barrier

	dROMs CARR U		antiROMs "Fast" µEq/l		antiROMs "Slow" µEq/l	
before 1 after 2	1	2	1	2	1	2
Mean	384	414	205	179	1039	1020
Std. Dev.	±56.6	±46.6	±56.3	±46.7	±199.2	±144.7
p	0.0076		0.83		4.8	

DISCUSSION

The results show a statistically significant increase in oxidative stress after diet, but the reduction in antioxidant barrier does not suffer a significant reduction due to the short course of the diet, stored stocks and non-depletion of important antioxidant compounds in the body. More perceivable is the change in so-called fast antioxidants, where the vitamins taken with food account for a large share of protective antioxidant compounds.

Oxidative stress testing is of fundamental importance for preventive medicine and health care, as well as a preventive method for assessing the decompression risk of divers. Also, the effects of scuba diving on health are reflected through the oxidative stress, which may be caused by demanding physical activity, hyperoxia and exposure to cold temperatures [17].

In the study group, a moderate level of oxidative stress was initially (348, SD ± 56.6 CARR U). This may be due to various reasons, most often smoking, alcohol use, overweight, etc. A number of studies have shown the impact of scuba diving on endothelial function [14, 16], lymphocyte antioxidant system [8], and various other effects of increased ambient pressure, which may also cause higher levels of oxidative stress in professional divers. Our previous study showed a decrease in nitrogen oxide levels after diving [19]. Repeat study marks an increase in the range of high-level oxidative stress (414, SD ± 46.6 CARR U), which is statistically significant from the first study.

Initially, the antioxidant barrier values show optimal protection ranges for "fast" (205, SD ± 56.3) and 1039, SD ± 199.2 µEq/l for "slow" antioxidants. After the 5-day non-vegetable and fruit diet, the "fast" antioxidant values change more sensitively, to a range of slight deficiency (179, SD ± 46.7 µEq/l). For endogenous antioxidants, significant change is not observed and values remain within the optimal range of antioxidant protection (1020, SD ± 144.7 µEq/l).

CONCLUSION

Despite the proven, significantly higher levels of free oxygen radicals and post-dietary lipid peroxidation, the markers of antioxidant protection did not change significantly. The topic of antioxidants is particularly relevant nowadays due to their protective effect and prevention of cellular damage by the effects of free radicals. This is all the more true for people who are subjected to heavy physical efforts such as professional divers. Fruits and vegetables contain numerous antioxidants such as vitamins C, E, beta-carotene, etc., which are absolutely necessary in a nutritional and balanced dietary regimen in people working under hyperbaric conditions, especially when available and harmful habits.

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