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A study regarding alveolar-capillary gas exchanges in hyperbarism

Studiu privind schimburile gazoase alveolo-capilare în condiţii de hiperbarism

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Abstract

Professional divers are exposed to possible important negative effects of hyperbaric oxygen and this is the reason for research in specialized centers to find objective tests which can predict and prevent major hyperbaric effects.

The studies of oxygen diffusion in hyperbaric conditions and of the effects of different O_2 saturations on respiratory exchanges and clinical consequences were performed both under laboratory conditions (on white NMRI mice) and under real clinical conditions (on healthy experimented divers).

The authors propose a synthesis of the physiology and pathology of continuous or intermittent exposure to hyperbaric oxygen on the cardiorespiratory system. Based on laboratory and histological results, the conclusions of the study give a practical solution for testing hyperbaric hyperoxia tolerance in order to minimize and prevent diving accidents, being useful for all specialists involved in this field.

Key words: hyperbaric hyperoxia; oxygen tolerance test.

Rezumat

Scafandrii profesionişti sunt expuşi unor posibile efecte negative cu implicaţii majore asupra sănătăţii datorate oxigenului hiperbaric, motiv pentru care, de-a lungul anilor, s-au desfăşurat cercetări în centre specializate, pentru a găsi teste obiective care pot preveni accidentele hiperbare.

Studiile de difuziune ale oxigenului în condiţii de hiperbarism, a efectelor diferitelor saturaţii de oxigen asupra schimburilor respiratorii şi a consecinţelor clinice s-au desfăşurat atât în condiţii de laborator (pe şoareci albi rasa NMRI), cât şi în condiţii clinice (pe scafandrii sănătoşi pofesionişti).

Colectivul de autori propune o abordare sintetică a fiziologiei şi patologiei expunerii prelungite sau intermitente a sistemului cardio-respirator la acţiunea oxigenului hiperbar.

Bazate pe rezultate de laborator şi histologice, concluziile studiului reprezintă o soluţie practică de a testa toleranţa la hiperoxia hiperbară, astfel minimizând sau prevenind accidentele de scufundare şi putând fi de un real folos tuturor specialiştilor implicaţi în acest domeniu.

Cuvinte cheie: hiperoxia hiperbară, test de toleranţă la oxigen.

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Introduction

The entrance and activity in underwater conditions has always been a challenge, breathing and hyperbaric adaptation possibilities representing a major concern in a continuous evolution. The list of present current hyperbaric activities includes sport diving, commercial, military or industrial purposes, archeological and experimental diving and also, assistive and therapeutic hyperbaric medicine (Gill & Bell, 2004; Khandelwal & Kaide, 2008).

There are three major categories for underwater interventions (Bove & Davis, 2003):

a) Direct diving: the most used type of intervention, based on direct contact with water. Depending on the depth of diving, it can be classified into:

- Direct diving at small and medium depths – can be free diving in apnea (limited by the time of breath keeping and by the mechanical effect of lung distortions under pressure) or diving using special equipment to increase the underwater time (SCUBA=self contained underwater breathing apparatus – with an underwater autonomous breathing unit using air/oxygen, with a light or heavy diving suit) (Kayle, 2009).

- Direct diving at great depths – unit diving in a decompression chamber or saturation diving

b) Indirect diving: in rigid, sealed, pressure-resisting chambers, where the diver breathes in normal atmospheric pressure. Underwater devices with men on board can be non-autonomous (linked to surface by communication cables) or autonomous (with atmospheric regeneration and rescue possibilities, self-propulsion, navigation, communication).

Breathing is the fundamental problem on which life and work in hyperbaric conditions depend and is the reason for which the gas mixture breathed by the diver has a major importance (Tezlaff & Thorsen, 2005). The most used gases in hyperbaric conditions are: O_2, H_2, CO_2, CO, N_2, He, Ar, Ne, and the gas mixtures are double-gases (Nitrox – N_2/O_2, Heliox - He/O_2, H_2/O_2) or triple-gases ($N_2/He/O_2$, $Ne/He/O_2$). Particularities (Edmonds et al., 2005):

- *O_2* breathed at high pressure has a toxic effect on the body. The tolerance time depends on its partial pressure from inspired air. Because O_2 is not practically dissolving in the blood, it is not included in decompression tables.

- *CO_2* concentration over 5% in an inhaled gas mixture will determine hyperventilation, which will reach the maximum at the concentration of 9% CO_2. Because of its toxicity, CO_2 can be used in diving activities only under some limits of $ppCO_2$, between 6 mbar (optimum percentage) and 70 mbar (maximum percentage) in a chamber.

- *CO* has a 210-300 times greater affinity for hemoglobin than O_2, forming with it carboxyhemoglobin (HBCO), which at 5-10% concentration in peripheric blood determines clinical manifestations of CO poisoning, and at 40% HbCO concentration, loss of consciounsness and death will occur. CO toxicity is based on an extremely slow speed for HbCO dissociation, the half-time is 4 hours in normal ventilation persons and 1 hour in pure O_2 atmospheric breathing. In saturation chambers, pCO must be under 30 µbar.

- *N_2* has a relatively slow diffusion and a slower pulmonary release. The density of gas N_2 increases significantly with depth, hindering the ventilation mechanical work. The increase of pN_2 during breathing at 5-6 ATA will have a negative effect known as nitrogen narcosis. It is demonstrated that the first performance decrease appears at pN_2 = 3.2 ATA, corresponding to 30 m depth.

- *Helium* is the most used inert gas for gas mixtures in diving at great depth, because of some advantages: it is the lightest gas after H_2 and has a low density compared to N_2, these properties ensuring a better comfort at higher pressures, when lower densities of inhaled gases decrease the nitrogen effect by using it as an O_2 diluent in experiments and saturation operations.

- *H_2* is the lightest gas, cheap and easy to get. Its low density recommends it for usage at medium and great depths instead of helium. It has low narcotic properties – only a quarter of the CNS depression capacity of N_2.

- *Ar, Xe, Ne* have been experimented for being used as oxygen-diluents in gas mixtures for deep immersions. Some of them have serious disavantages – argon is narcotic even at 1 ATA.

Pulmonary ventilation under hyperbaric conditions

During hyperbarism, there are many particularities of ventilation physiology (Carmichael, 2008):

- *Respiratory type*: In upside-down diving, abdominal breathing is accentuated and the inferior pulmonary lobes are more ventilated, compared to the relative decrease of the pulmonary apex, due to intense diaphragm contraction. At the comeback ascent, with head up, the base of the thorax is constrained, superior rib breathing appears, with a higher thoracic antero-posterior diameter, diaphragm ascension and a relative hyperventilation of the lung apex.

- *Inhalation*: During immersion, the inhalation phase has a bigger amplitude, and the increase of the air/gas mixture density will put a higher strain on inhalation at greater depths. Forced inhalation can appear in divers as a result of effort dyspnea or CO_2 intoxication dyspnea.

- *Exhalation*: Its amplitude is increased proportionally to respiratory gas density and it can be noticed at small depths (nitrox or air diving) or bigger depths (heliox diving). Active exhalation can prevent overpressure during diving.

- *Respiratory frequency*: In hyperbarism using air, the respiratory frequency decreases during rest, posing the problem of adaptation of ventilation through exercise for professional divers. The reduction of breathing frequency at 3-5 ATA with air compared to 1 ATA is obvious during rest and also, during maximal effort. The increase of respiratory movement amplitude and the decrease of ventilation frequency during air inhalation in hyperbarism appear as a spontaneous adaptation of the diver's body to mechanical work. The increase of ventilation frequency during diving may appear during effort or an accidental increase of CO_2.

- *Changes in pulmonary volumes and capacities*: The decrease of vital capacity (VC) by 8-9% and reduced changes of residual volume (RV) due to the respiratory factor (hydrostatic pressure exerted on the thorax and

abdomen determines diaphragm ascension) and to the blood factor (redistribution of blood volume due to hydrostatic pressure, with thoracic blood volume and decrease of pulmonary blood volume). In 1973, Dahlback and Lundgren (Wattel, 2006) demonstrated that diving up to shoulder level can produce the ”air trapping” phenomenon (like in emphysematous people) because of the reduction of transmural forces in the airways when the torax is compressed by the surrounding water and the reduction of pulmonary volume. For preventing ”air trapping” signs, the pulmonary volume must be raised at 40% of vital capacity, and breathing must have a higher frequency for opposing partial airflow obstruction.

- *Respiratory flow*: minimum changes occur during rest in hyperbarism, variations being noticed after effort or heliox exposure. Decreases in the respiratory flow may appear at a higher gas density and hydrostatic pressure; increases in the respiratory flow may appear due to heat loss through convection. Maximum expiratory volume per second decreases in air hyperbarism even up to 70% of the inferior normal limit, representing a decrease of the bronchial permeability index. Maximum ventilation decreases too, in hyperbarism, because of the decrease of the current volume and of the respiratory rate.

Effects of hyperbaric oxygen exposure and reactive oxygen species

Using hyperbaric oxygen at high pressures and concentrations over long diving periods is limited and requires prudence due to its toxicity (Christiani, 2011). Partial oxygen pressure (ppO_2) in air increases with depth, so at 10 ATA corresponding to 90 m, ppO_2 will be 2.1 ATA O_2. Using O_2 at a pressure higher than 0.4 ATA can produce acute or chronic hyperoxia, depending on the time of exposure.

Acute hyperoxia: known as the Paul Bert effect or hyperoxic seizures, first described in 1873 as a form of CNS hyperbaric oxygen toxicity at 1.7-2 ATA. Based on observations on humans and mice (Tache et al., 1994), there are three stages of symptoms:

a) The stage of metabolic cellular changes, when metabolic disruptions are initiated in most of the organ’s essential parts implicated in energy production, essential for a good cellular functionality.

b) The prodromal stage with the first clinical signs of oxygen toxicity (nausea, trembling, dizziness, retrosternal pain, breathing acceleration, tachycardia, cramps in facial and limb muscles, discomfort, cold sweating, problems of concentration and work, mydriasis, tinnitus, visual field narrowing with tunnel effect, anxiety, confusion, fatigue, euphoria or depression, amnesia). Physical effort during underwater work increases the susceptibility to oxygen toxicity, and the increase of oxygen consumption by trembling can double toxicity.

c) The clinical stage consists of a tonic period followed by a clonic period and loss of consciousness (Dean et al., 2003). Continuing inhalation will decrease the intervals between seizures, until death, because of tissue hypoxia due to hyperbaric hyperoxia. Stopping inhalation and reducing pO_2 may stop the seizures without consequences. Short exposures to an increased pO_2 atmosphere are not harmful, but useful by increasing tissue oxygenation due to the increase of arterial pO_2 – the result of the increase of plasma dissolved O_2, the good effect used in hyperbaric oxygen therapy.

Chronic hyperoxia, known as the Lorrain Smith effect, first described in 1897, appears after multiple and long exposures (hours) to pO_2 at 0.4-1.7 ATA, especially after saturation diving, in hyperbaric chambers with hyperoxic atmosphere for the persons inside (Christiani, 2011). Clinical signs are called ”oxygen pneumonia”, with specific pulmonary lesions - congestion, bronchoalveolar edema, epithelial degeneration and scaling, fibrin deposits on the airways and pneumonia. The small bronchi obstruction, accentuated by bronchospasm, is followed by atelectasis due to gas resorption in non-ventilated areas. The first pathological changes are those of the pulmonary surfactant, followed by capillary endothelium disruption and invasion of blood elements in the alveolae and pulmonary interstitium.

Hyperbaric oxygen has a bi-stressing effect, meaning it has a double toxicity for the human body due to the hyperbaric factor and due to the hyperoxic factor (Mathieu et al., 2006).

The hyperbaric factor is directly proportional to depth, with every 10 meters underwater the pressure increases by 1 ATA. The air from the chamber or in the breathing mask for divers must be at the same pressure as that of the water around the divers, to prevent thorax flatting. This means that for every 10 meters underwater, pO_2 will increase by 1 ATA (ATA = absolute atmosphere, a term used for working divers). At sea level, the pressure is 1 ATA, and at 10 meters depth the pressure reaches 2 ATA. Working divers, who breathe pure oxygen from closed devices, have a limitation at 7 meters depth for a safety immersion. (Mitchell & Bennett, 2008).

The hyperoxic factor – the experiments that tried to dissociate hyperoxia from hyperbarism, showed that the major disturbances were due to hyperoxia. The term ”oxygen intoxication” or ”oxygen toxicity” and its effects must be well understood. Different intoxication levels are tolerated by different tissues when hyperoxia is used in therapy or usual diving operations. Oxygen toxicity studies on pulmonary tissue have described three stages (Clark & Whelan, 2004):

- Initial disturbances consist of histological changes that occur 40-60 hours post-exposure to 1 ATA O_2 100%: endothelial cell tumefaction, neutrophil aggregation, cytoplasmic vacuolization and the first hyperoxic pulmonary lesions.

- The transitional stage consists of the increase in the number of cells in the alveolar interstitium due to inflammatory cells accumulated in the capillaries, endothelial cell swelling and major disturbances of liquid permeability.

- The late stage is characterized by a destructive endothelial process expansion to type 1 and 2 pneumocells and basal membrane exposure, with the increase of cellular infiltrate and interstitial matrix volume.

Clark and Lambertsen demonstrated in 1971 that breathing pure oxygen at 1 ATA for longer periods of time will determine most frequently death through alveolar

flooding (Gocan et al.,1993). In mice, the exposure is deadly through pulmonary edema in 50% of animals after 66-72 hours. The lung is the only organ in direct contact with the atmosphere and is the most exposed to hyperoxic attack. Under hyperoxia conditions, the O_2 amount that reaches other organs is due to the high plasma dissolved form of the gas. Beyond 2.5 ATA O_2, oxygen toxicity can cause death through CNS effects - seizures and coma (Newton, 2001).

Hyperbaric oxygen is a form of oxidative stress, an extensively studied phenomenon in the last 20 years for its biochemical and pathophysiological aspects. The weapons of hyperbaric hyperoxia at cell level are the so-called "reactive oxygen species" – singlet oxygen, superoxide anion, oxygenated water and hydroxyl radical (Clark & Whelan, 2004). The reactive O_2 species have a common characteristic – the presence of an uneven electron called "bachelor electron" on the last orbital, whose presence is noted by a point in the right upper part of the substance's chemical symbol. These active oxygen derivates are formed by different processes (Courtiere, 2006):

a) Activation of atmospheric oxygen, which cannot be used in this form by the cells and needs to be activated through a reduction process, consisting of electron attachment:

- One electron attached – superoxide anion (O_2^*)
- Two electrons attached – form peroxide, which in conjunction with water forms oxygenated water (H_2O_2)
- Three electrons attached form hydroxyl radical (OH^*)
- Four electrons attached – form water (H_2O)

b) Under the action of ultraviolet rays or hypochloride anion, it will form singlet oxygen or molecular activated oxygen ($^1O_2$)

c) Nitric oxide (NO) is considered another reactive oxygen species, with an uneven electron on the last orbital.

Reactive oxygen species or active oxygen derivates last only for a few seconds, but are extremely toxic by attacking different structural parts of the cell, with a major impact on cell functionality (Clark & Whelan, 2004; Courtiere, 2006):

- On DNA: oxidative lesions on nucleic acids are cumulative and are implicated in cancer genesis and aging.
- The reaction of active oxygen derivates on proteins will form carbonylated proteins, which partially lose the functional properties of the initial proteins (contractility, electric charge, ion channel function, receptor function).
- Glycoproteins are also a target for reactive oxygen species, with functional disturbances for cells.
- The effects of active oxygen derivates on lipids are extremely toxic, because lipids are important components in the structure of all cell membranes and in the myelin sheath of nervous fibers. The results are lipoperoxides, very harmful for cell membrane function. A stable derivative of the lipo-oxygenation process is malondialdehyde, which persists for a long time in the serum and which can be measured as an indirect sign of the oxidative attack intensity.

Endogenous and exogenous aggression of the reactive oxygen species on metabolic active cells has different oxidative stress forms and determines the activation of all antioxidant defense mechanisms in order to prevent or limit oxidative lesions (Tache et al., 1994). These antioxidative mechanisms prevent the formation of free radicals, transform the oxidants into less toxic species and repair molecular lesions induced by free radicals. Preventing excessive free radical formation is the first major step for living cells, because potential toxic O_2 metabolites are continuously forming in the breathing process during normal oxidative phosphorylation. Mitochondrial cytochrome oxidase is using 90% of O_2 in pulmonary cells and for the metabolization of excess free radicals. The partitioning of free radical formation through metal ions such as iron and copper may have an important role in oxidant-induced tissue lesions through lipid peroxidation stimulation, which generates cytotoxic aldehydes. The antioxidants are called "free radical scavangers" and represent the most significant defense mechanisms for oxidative stress. For lungs, the major antioxidants are:

- Enzymatic systems: catalase, superoxide dismutase, glutathione redox cycle GSH-GSH peroxidase, G-6-PDH
- Liposoluble antioxidants: vitamin E, beta-carotene, bilirubin
- Hydrosoluble antioxidants: vitamin C, uric acid, glucose, glutathione, cysteine and cysteamine, taurine
- High-molecular weight antioxidants: albumin, tracheobronchial mucus.

The selection of divers must be based on individual antioxidant capacity, especially for those who perform physical effort underwater (Tache & Manea, 1996).

Oxygen tolerance test

Oxygen tolerance may be formulated in terms of biochemical and microbiological, mathematical and graphical aspects, as a dose-effect relationship, meaning administered dose (pO_2) and pharmachological/toxic response/effect. Like in every dose-effect relationship, in hyperbaric hyperoxia the effects of small doses tend to be unmeasurable, even if they exist, and these very small initial O_2 effects are the most important for trying to stop the toxic effect and to extend oxygen tolerance (Piantadosi, 2008).

Regarding oxygen, the toxic dose capacity is related to pO_2 (Ratzenhofer-Komenda et al., 2006). In continuous exposures, the effect is extended to the characteristics of the dose-effect relationship, meaning that for the initial phase, it is also influenced by pressure and the time of exposure. These three-dimensional circumstances of initial oxygen toxicity have been since 1962 the base for Dickens' concept regarding the hyperbolic rectangular relationship between the time of pO_2 exposure and death. Based on this concept, Lambertsen and Clark designed in 1967 the pulmonary O_2 tolerance test for the human body during rest, with a 5% reduction of vital capacity observed in the groups after exposure to pO_2 less than 1 ATA. The data indicated the absence of objective changes after exposures to less than 0.6 ATA (Tache & Manea, 1996). These oxygen tolerance tests are widely used in diving, spatial medicine and clinical hyperbaric medicine, where exposure to more than 1 ATA is needed for work or therapy. The tolerance curves during continuous exposure to hyperoxia are statistical regression lines. For measuring the toxicity of oxygen at different pressures and during different periods of time,

the unit pulmonary toxic dose (UPTD) and the cumulative pulmonary toxic dose (CPTD) (Piantadosi, 2008) are defined.

Different oxygen intoxication levels are tolerated in tissues when hyperoxia is used in usual diving or in therapy (Hardy, 2008). In order to compare the response of different tissues and organs to the hyperoxic attack, real sensitivity must be differentiated from the oxygen exposure dose.

The objective toxicity changes observed by researchers are: decreased vital capacity, breathing frequency perturbation, increased airway resistance, decrease of maximum expiratory volume per second, increase of residual expiratory volume and decrease of CO_2 diffusion capacity. These disturbances appear 4-6 hours after exposure to 2 ATA O_2, or after saturation exposures of 45 ATA for 11 days, or 48 hours after finishing a 12-day exposure to 45 ATA (Levett & Miller, 2008). If we accept that the decrease of vital capacity is more than just fatigue, atelectasis and direct pulmonary lesions can be incriminated in these changes. The attack oxygen dose is not a standard value for an organ in case of exposure to a constant medium or to a constant pO_2 over lungs. The morphological and functional particularities of the response are the result of exposure to hyperbaric hyperoxia. The maximum hyperbaric O_2 dose acts on the cells exposed to equal doses of inhaled pO_2 at arterial and alveolar level: tracheobronchial epithelium, alveolar-capillary membrane, venous pulmonary endothelium, endocardium, arterial endothelium, renal glomerular capillaries, carotid corpuscle, microcirculation (Jain, 2009).

Correlating theory with practice, we can express oxygen tolerance during a prolonged exposure in quantitative terms such as the decreasing speed of a mathematical function. The tolerance of tissues and cells varies quantitatively and qualitatively for different pO_2 - the reason for expressing oxygen tolerance depending on its pressure.

Individual correlations regarding hyperbaric oxygen tolerance based on latency time for intoxication signs imposed testing divers for oxygen tolerance as a selection probe. The oxygen tolerance test, first used by the American Navy, is used nowadays in all control and selection centers for professional divers and as a mandatory test for those who stop diving for more than a year (Kayle, 2009).

The test is performed in a dry hyperbaric chamber, where the subjects are exposed to conditions that simulate diving at 18 meters underwater (2.8 ATA) and they breathe pure oxygen through a tight facial mask for 30 minutes. Cardiac frequency is also monitored in order to prevent a hyperoxia crisis and to stop hyperbaric O_2 administration in time. Seizures are a major sign of toxicity and also, the presence of muscular cramps, which are considered signs for stopping the test. Data from Butler and Knafelc (1986) show that the reproducibility of oxygen sensitivity in the examined American marine divers is not identical to that from the initial oxygen tolerance test. Other conclusions (Tache & Manea, 1996):

- Tolerance varies due to individual variations.
- The test does not identify all oxygen-susceptible subjects.
- Subjects who successfully passed the tolerance test and who use pure oxygen have an acceptable oxygen security level.
- CNS toxicity signs are limiting factors during diving operations.

Control tests of diver's training

The diversity of human diving activities at different depths underwater imposes severe rules for somatic and physical health safety, which should be periodically tested. Mandatory periodic control of divers includes (Tache & Manea, 1996; Edmonds et al., 2005): history of diving activities, medical diving-related problems, personal and family medical problems and a complete clinical examination regarding:

- General examination – anthropometry, somato-sensory integrity (ophthalmology, ENT, cardiovascular, respiratory, renal, gastrointestinal, dermatological, endocrine, locomotor, neurological control) and mental integrity (psychological and psychiatric control).
- Non-specific tests: swimming test, effort tests (ASTRAND and MARGARIA), underwater activity tests and EEG changes induced by hyperpnea.
- Specific tests: oxygen tolerance test, narcosis test, CO_2 tolerance test, heliox tolerance test, apnea test.

Personal studies. Under real diving conditions, when using hyperbaric O_2, besides the depth of the water and O_2 pressure, there are also some contributing factors to acute intoxication risk, such as water contact, decrease of blood pH, increase of pCO_2, the diver's physical activity.

The authors aimed to evaluate the training level of divers based on complex additional tests, which can be used to predict hyperbaric O_2 toxicity signs and to prevent possible accidents. This was the reason for a study carried out by the researchers on three groups of professional divers, in the Naval Medicine Center in Constanta (Tache & Manea, 1996). Each group consisted of 20 healthy male subjects, with a mean age of 28 years and 7-8 years of experience in diving.

Training and physical status control was performed by exposure to hyperbaric conditions, preceded and followed by the ASTRAND test for submaximal physical effort. Exposure lasted 30 minutes, in a dry hyperbaric chamber RDO 1500 Ulis Comex, at the Hyperbaric Center. The subjects were medically assisted and underwent hyperbaric exposure in groups of two, as follows:

Group A – continuous exposure to 5.2 ATA Nitrox

Group B – continuous exposure to 2.8 ATA O_2 – oxygen test

Group C – intermittent exposure to 2.8 ATA O_2 with the program on/off for 10 minutes at 2.8 ATA O_2 / 5 minutes at 1 ATA air, total period of exposure to 2.8 ATA O_2 lasting 30 minutes.

Breathing pure oxygen during the test was possible by using a facial tight mask, during rest, in recumbent position, with heart rate monitoring at 5 minutes (radial allure).

Before and after exposure to hyperbarism, the subjects were tested for submaximal effort during cycling on an ergobike for 5 minutes at 1 ATA air, keeping constant the effort intensity at 150 watts and speed at 60/minute (Astrand test). Before and after the effort test, cardiac frequency and brachial arterial pressure at rest were measured, within 15 seconds post-effort and 5 minutes post-effort,

and urine samples during rest and one hour post-effort were also analyzed. Based on anthropometry and heart rate, using Astrand tables and nomograms, the following were determined: maximal effort oxygen consumption – VO_2max, expressed in ml/kgc, also used for determination of ideal vital capacity values, expiratory maximal volume and expiratory maximal volume per second.

After finishing the effort test, the spirometric values of pulmonary volumes - current volume (CV), vital capacity (VC), inhaling and exhaling reserve volumes (IRV and ERV) and also, the respiratory capacities – maximum expiratory volume per second (MEVS) and maximum ventilation per minute (MVM) were determined. Real values of vital capacity, maximum expiratory volume per second and maximum ventilation per minute were referred to ideal values for each person and expressed in percentage. Real values (ATSP-Ambient Temperature and Saturated Pressure) of vital capacity, maximum expiratory volume per second and maximum ventilation per minute were corrected for BTPS (Body Temperature and Saturated Pressure). Based on the recorded indices, other important values were measured:

- Maximal oxygen consumption in effort (ml/kg and ml/min)
- Difference between maximum and minimum arterial pressure values (mmHg)
- Tiffneau-Pinelli index (in percentage) after the formula MEVS x 100/VC
- Effort respiratory economy, in absolute values, using the formula of Anthony ventilation equivalent = MEV (l) / VO_2max (l)

The urine dosages of urinary amines – adrenaline, noradrenaline, their basic and acid catabolists, histamine, serotonin, thiamine were analyzed. The dosing used the Kakimoto & Armstrong method, with values expressed in microg/min. The data of the study were statistically processed using the Wilcoxon test and the results were presented as tables, graphics and probability curves.

Continuous exposure to hyperbarism, for 30 minutes, at 5.2 ATA Nitrox and 2.8 ATA O_2, and also, intermittent exposure to hyperbarism at 2.8 ATA O_2 determine significant decreases of radial pressure compared with pre-exposure after 5-10 minutes of hyperbaric exposure, with the restoration of rest values at 10 minutes post-exposure. The normal bradycardic effect of hyperbarism, more intense in trained subjects, is not a permanent effect; it was first described by Hardenbergh in 1973, Pastuch in 1974 and Schipke & Pelzer in scuba-divers in 20001 (Schipke & Pelzer, 2001). The restoration of cardiac frequency 5 minutes after the cessation of effort, pre- and post-exposure, is seen in 80% of the subjects, meaning a good cardiovascular adaptation of the tested divers.

Maximal oxygen consumption will decrease after exposure to 5.2 ATA Nitrox and will rise after exposure to 2.8 ATA - significant changes versus pre- and post-exposure values. Maximal oxygen consumption as a parameter for maximal aerobic power indicates disturbances in normal effort capacity after hyperbaric exposure. Intermittent exposures do not show significant changes compared to continuous exposures, even if intermittent exposure programs (studied especially in animals) are recommended for increasing hyperbaric hyperoxia tolerance (Harabi, 1988; Bove, 2003). A group of researchers conducted by Mahon (Mahon et al., 2009) demonstrated that short oxygen prebreathing periods reduce or prevent severe decompression sickness in a 70-kg swine saturation model.

Urine amine elimination increases significantly after effort in pre-exposure, after hyperbarism exposure and after the post-exposure effort test. Physical effort determines in well-trained hyperbaric divers, the activation of the sympathoadrenal system, with amine urine elimination. Moderate hyperbarism exposure to 5.2 ATA Nitrox or 2.8 ATA O_2 is considered an additional effort for the body, with elevated values of amines before and after exposure and with decreasing values one hour post-exposure. The data regarding increased amine elimination after effort and after hyperbaric exposure are in accordance with the literature observations regarding urinary catecholamine, vanyl-mandelic acid or serotonin excretion (Davis 1977; Therminaris 1979). A group of Scandinavian researchers demonstrated that hyperbaric oxygen increases parasympathetic activity in professional divers (Lund et al., 2000).

Ventilated air volumes and vital capacity increase after submaximal effort, after hyperbaric hyperoxia exposure; continuous exposure to 2.8 ATA O_2 determines an increase of maximum volume exhaled per second, maximum ventilation per minute and Tiffneau index, but intermittent exposure to the same conditions does not. The respiratory response of the human body, studied after submaximal effort on an ergobike at 1 ATA air, is very little influenced by pre-exposure to hyperbaric continuous or intermittent oxygen. Intermittent exposure to 2.8 ATA O_2 in on/off system does not influence the oxygen tolerance test.

Pulmonary histological changes in laboratory animals after exposure to hyperbaric oxygen

Pulmonary changes after hyperbaric hyperoxia are the result of the toxicity of free O_2 radicals, because they are produced in excess and exceed the antioxidant capacity of the body. The authors continued the previous researches of the members of the Physiology Unit of the Cluj Medicine University and observed the pulmonary histological disturbances correlated with biochemical and clinical signs of toxicity in mice. They also studied the dose-effect relationship between the hyperbaric oxygen-toxic effect (convulsions, coma, death) in mice exposed to pressures over 3 ATA O_2 and the advantages of intermittent exposures with an on/off ratio smaller than 2/1 for pressures over 3 ATA O_2 (Gocan et al.,1993; Tache et al.,1994).

The research was carried out in young white male NMRI mice, with a weight of 20-30 grams, which were normally fed. The experiments progressed on 180 mice grouped in 11 cohorts, exposed to hyperoxic hyperbarism in an Engelke Konrad chamber, in clusters of 5 animals and a control group, permanently observed through the window in order to detect the beginning of seizures, coma progression and death. During the experiment, the O_2 concentration was constantly between 97-99%. In continuous exposures (groups 1-6), the animals were observed until the death of most of the mice, and in intermittent exposures (groups

7-11), until the death of 50% of the mice, value marked as lethal dose 50 (LD 50). Lung fragments were analyzed using the Scherle method; results (Gocan et al.,1993; Tache et al.,1994):

- Beyond 3 ATA O_2, massive pulmonary histological changes will occur such as intra-alveolar hemorrhage and toxic acute pulmonary edema, responsible for the death of the animals exposed to continuous and intermittent pressure. The animal that survived to intermittent exposures had moderate lesions, such as interstitial capillary stasis and thickening of the interalveolar septum.
- Pulmonary histological signs that caused death were similar and did not depend on the lethal moment or on the pressure value 3 ATA O_2.
- The value of 3 ATA O_2 can be considered as a level-limit beyond which pulmonary structural and functional irreversible disturbances will develop.

Studies on animals regarding the improvement of hyperbaric hyperoxia resistance – peer review

Increasing tolerance to hyperbaric oxygen has been a goal aimed by many researches during the course of time. In 1980, Lambertsen succeeded in raising animal tolerance to hyperbaric oxygen from 4 hours to 14 hours, by repetitive exposures to hyperbarism, followed in 1989 by White & co, who found similar results after the pre-exposure of mice to normobaric hyperoxia 85% O_2, or an air-ozone mixture or after injection with endotoxins. In 1986, Jamieson tried injections with superoxide-dismutase, catalase and glutathione-peroxidase, but did not succeed in increasing hyperbaric resistance because the injected enzymes are not able to penetrate the cell membrane. In 1989, White successfully increased hyperbaric oxygen tolerance by the pre-exposure of the animals to hypoxia 10-12% O_2 for 4-7 days (Wattel, 2006).

The results of the studies performed by the members of the Physiology Unit of "Iuliu Haţieganu" University of Medicine in Cluj (Tache & Manea, 1996) demonstrated a significant improvement of effort capacity in mice after previous exposure to hypobaric hypoxia corresponding to an altitude of 2000 meters, for 48 hours. Even such moderate hypobaric hypoxia is capable to modify the blood concentration of liver and muscle (lactic dehydrogenase, succinate dehydrogenase, glutathione dehydrogenase, cytochrome oxidase, ATP-ase). Based on the fact that the increase of hyperbaric oxygen tolerance needs changes in specific intracellular enzymes, the authors studied the effect of normobaric hypoxia exposure on mice.

The study was carried out on 30 healthy male Wistar Bratislava rats, divided into three groups of 10 animals each. The first group was the control group, exposed to hyperbaric oxygen 95-97% O_2 for 59-75 minutes. The second group was exposed for 48 hours to normobaric hypoxia 10% O_2 corresponding to 5500 m altitude. The third group was exposed for 48 hours to normobaric moderate hypoxia 16.5% O_2 corresponding to 2000 m altitude. Normobaric hypoxia conditions were possible by using an Engelke Konrad chamber in the Naval Medical Center in Constanta, then the animals were maintained in normobaric conditions for 48 hours and after that, returned to the chamber and exposed to hyperbaric hyperoxia at 4.5 ATA, similar to a depth of 35 m underwater. Before exposing the animals to hyperbaric hyperoxia and at the time of restoration of normobarism, blood samples from the retro-orbital sinus were analyzed for dosing glucose, lipids, proteins, ceruloplasmin. The rats were permanently observed through a window and at the first signs of oxygen toxicity (convulsions noticed at least in one animal from a group of five), hyperoxia was stopped. A number of animals died when returning to normobaric conditions, because of seizures followed by coma or because of acute pulmonary edema. Results discussion:

Confirmation of Torbati's theory since 1987 that oxygen intoxication induces hyperglycemia before convulsions - clinically and on EEG (Newton, 2001). Both in the control group and in animals pre-exposed to hypoxia, a direct relationship between reduced oxygen tolerance (clinical signs) and hyperglycemia ($p<0.02$) was observed, so blood glucose analysis during the oxygen tolerance test is a convenient and cheap indicator for oxygen sensitivity.

Rat exposure to moderate normobaric hypoxia (O_2 16.5%) will increase resistance to hyperbaric oxygen toxicity, and this protective effect of hypoxia lasts for 48 hours after returning to normobarism.

The significant decrease in blood lipids can be explained by lipid peroxidation and accelerated metabolization. Ceruloplasmin, one of the important plasma antioxidants, decreased in the control group and in the moderate hypoxia exposure group, meaning that it was consumed during reactions against free radicals. The phenomenon by which hypoxia increases oxygen tolerance can be a change in the concentration of some intracellular enzymes, used in the fight against reactive oxygen species.

Hyperoxia determines disturbances of alveolar cells, disruptions of pulmonary endothelial cells and alters the capacity of serotonin capture and angiotensin I hydrolization by the pulmonary endothelium. Pre-exposure to hypoxia at 10-12% O_2 has no direct effect on the concentration of the conversion enzyme, but partially prevents the decrease of the enzyme through consumption due to hyperoxia. A previous exposure of animals to hypoxia 10% O_2 was associated with a significant increase of pulmonary glutathione-peroxidase, G-6-DG, superoxide dismutase and catalase, suggesting that increased hyperoxia tolerance may be partially based on the glutathione redox system.

Conclusions

1. The changes of oxygen-diffusion capacity at pulmonary level represent a more sensitive toxicity index compared to vital capacity, considered as an essential index for oxygen tolerance.
2. The determination of submaximal effort capacity on an ergobike at 1 ATA is only in a small percentage influenced by the oxygen tolerance test at 2.8 ATA, for 30 minutes, continuous or intermittent exposure.
3. Studies on mice demonstrated that the oxygen tolerance test can be extended from 2.8 ATA to 3 ATA without major consequences.
4. For preventing the harmful effects of hyperbaric hyperoxia, the pre-exposure of animals to moderate hypoxia of 16.5% O_2, corresponding to 2000 m altitude, can be used, and this protective effect lasts for 48 hours.
5. Blood glucose analysis in dynamics during the

oxygen tolerance test can be a good predictor for oxygen sensitivity.

6. Pulmonary histological investigations in mice exposed to pressures over 3 ATA O_2 indicate inflammatory lesions.

7. The pressure of 3 ATA O_2 can be considered as a level-value beyond which irreversible pulmonary lesions will appear.

Conflicts of interests

Nothing to declare.

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