LONG TERM PHYSIOPATHOLOGICAL CHANGES IN PROFESSIONAL DIVERS – A LONGITUDINAL STUDY

CEZARINA DINCU, A. DEFTU**, MARIA-LUISA FLONTA***

*Naval Medicine Center, 19, 1 Mai Blvd, Constanța, Romania
**Department of Physiology and Biophysics, University of Bucharest, 91–95, Splaiul Independenței, Bucharest, Romania

Abstract. In this study we have performed a retrospective survey of the records of blood biochemistry for a group of 16 professional divers in the "Divers Centrum of Constanța", over a 10 years period (2001–2010). In saturation diving, we have found progressive changes in serum enzymes and oxidative stress markers, suggesting a subclinical hepatic injury, as well as a progressive change of blood thyroxin (T_4), triiodothyronine (T_3) and thyroid-stimulating hormone (*TSH*) concentrations, indicating either a thyroid or a hepatic malfunction. Although the mean changes are not exceeding the upper or bottom normal limits of the considered biochemical parameters, that means they do not constitute a clinical alarm signal, they can lead to long term health effects which are to be considered in the interest of this workers category. The individual responses of the divers group, which showed an enzymes concentration increase which exceeded the upper normal values in blood, can signalize an increased hyperbaric stress and it is advisable to follow more rigorously the health state of these individuals. The cumulative effect of the sub-clinical microlesions has to be considered as a potential risk of this professional category and has to be studied in order to decide if a professional disease is to be established.

Key words: saturation diving, hepatic enzymes, thyroid hormones, oxidative stress, platelets.

INTRODUCTION

Decompression sickness (the so-called "bends") is the main danger to which divers are exposed after a work at great depth in the sea, during their lifting to the surface. When pressure decreases, the large amount of inert gas dissolved in the body tissues forms bubbles which may block blood vessels, may physically damage surrounding cells or may denaturate serum proteic molecules at the gas-blood interface. The technique of saturation diving allows a reduction of this risk. In this case the divers live under pressure in a hyperbaric environment, for the whole duration of the work of several days to weeks and are decompressed to the surface pressure only once, at the end of their work. Therefore, the risk of "bends" is significantly reduced [3, 4].

Received: May 2012.

ROMANIAN J. BIOPHYS., Vol. 22, No. 2, P. 73–81, BUCHAREST, 2012

Nevertheless performing a retrospective survey of the records of blood biochemistry for a group of 16 professional divers in the “Divers Centrum of Constanța”, over a 10 years period (2001–2010), we have found progressive changes in serum enzymes and oxidative stress markers, suggesting a sub-clinical hepatic injury, as well as a progressive change of blood thyroxin (T_4), triiodothyronine (T_3) and thyroid-stimulating hormone (*TSH*) concentrations, indicating either a thyroid or a hepatic malfunction. Although the changes are not exceeding the upper or bottom normal limits of the considered biochemical parameters, that means they do not constitute a clinical alarm signal, they can lead to long term health effects which are to be considered in the interest of this workers category.

MATERIALS AND METHODS

MATERIALS

The 16 divers were between 21–50 years old, with a mean of 35 years.

METHODS

Serum enzymes determinations included alanine transaminase (*ALT*), aspartate transaminase (*AST*), lactate dehydrogenase (*LDH*), alkaline phosphatase (*ALP*), gamma-glutamyl transferase (*GGT*). Determinations were performed photometrically, in a Dade-Dimension-AR analyzer, using commercial diagnostic test kits from Siemens. The thyroid hormones and thyroid-stimulating hormone (*TSH*) were measured with a VIDAS® immunoanalyser from BioMerieux, using the enzyme-linked fluorescence (ELFA) assay. The free oxygen radicals test (FORT) was used to assess circulating ROS in order to characterize photometrically the oxidative stress. Platelets were counted in the Horiba ABX Pentra80 Hematology Analyzer, using ABX Cleaner, ABX Basolyse, ABX Eosinofix, ABX Diluent, and ABX Lysebio. The prothrombine time was measured in a Sysmex CA-50 coagulation analyzer. The used reagents were Thromborel® S, Owren’s Veronal Buffer and Ca Clean. The result (in seconds) for a prothrombin time performed on a normal individual will vary according to the type of analytical system employed. The International normalized ratio (*INR*) was devised to standardize the results. The *INR* is the ratio of a patient's prothrombin time to a normal (control) sample, raised to the power of the International Sensitivity Index (*ISI*) value for the analytical system used.

$$INR = \left(\frac{PT_{\text{test}}}{PT_{\text{normal}}} \right)^{ISI} \tag{1}$$

STATISTICAL ANALYSIS

Student t-test was used to compare the data between the 2 groups, and one-way analysis of variance was used for multiple comparisons. Values are shown as means ± SEM, *p* values <0.05 were considered significant.

RESULTS

The dive in saturation reached 50 meter see water (msw). The compression was applied at a speed of 10 msw/min. The dive duration was of 5 days, the divers remained at the life level of 10 msw for a period of 53–69 hours, and the decompression duration was 29 hours. The duration of the working periods was at least one hour.

PLASMA ENZYMES ACTIVITIES

A significant elevation of serum aspartate (*AST*) and alanine (*ALT*) transaminases activities was found after the saturation dive (Fig. 1). The same significant increase was recorded for alkaline phosphatase (*ALP*), for gamma glutamyl transpeptidase (*GGT*) and for lactate dehydrogenase (*LDH*) (Fig. 2). A notable fact is the increasing trend of these enzymes concentrations in the blood, over the 10 years. However, the biological response of the 16 subjects was different and two groups could be formed, considering the amplitude of the after diving response: a first group which contained 60% of the divers and which showed an enzyme concentration increase which exceeded the upper normal values in plasma, and a second group of 40% whose plasma enzymes activities remained in the normal range.

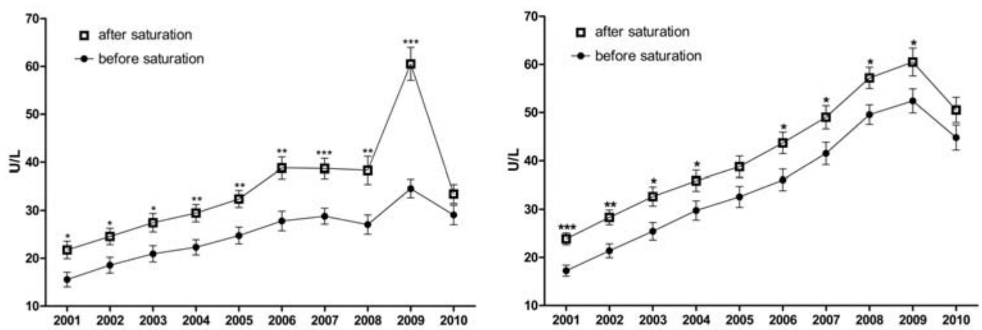

Fig. 1. Mean *AST* (left) and *ALT* (right) values before and after diving in saturation, recorded during 10 years, for 16 professional divers. ***$p < 0.001$, **$p < 0.01$ and *$p < 0.05$ indicate the level of significant difference as calculated by ANOVA test between the two conditions. Error bars are SEM. The normal range for *AST* is 5–40 U/L, and for *ALT* is 5–65 U/L.

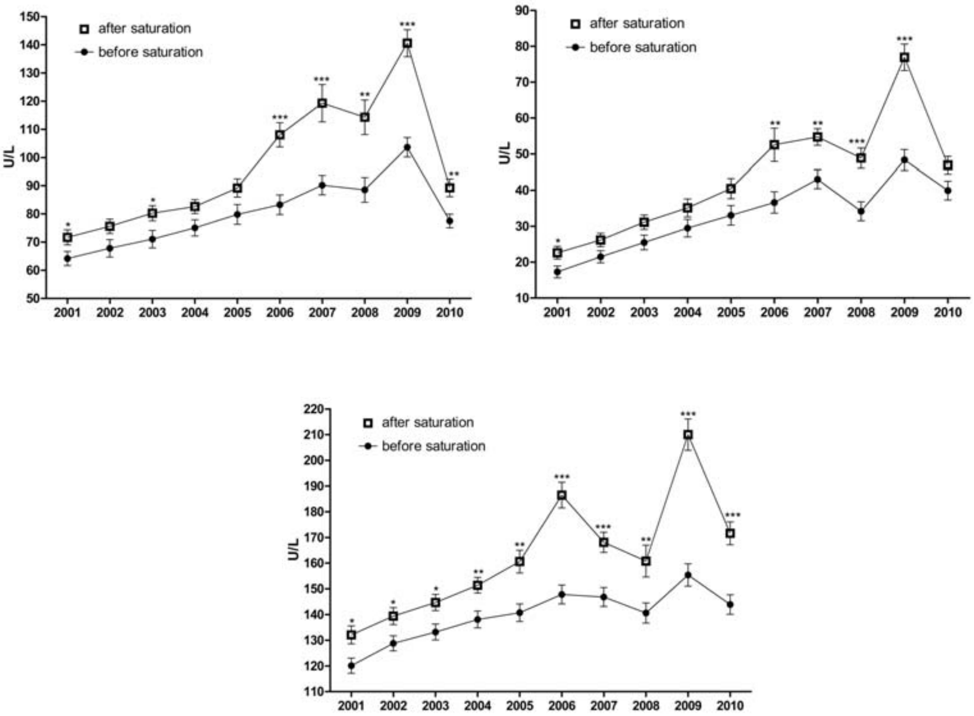

Fig. 2. Mean *ALP* (left), *GGT* (right) and *LDH* (down) values before and after diving in saturation, recorded during 10 years, for 16 professional divers. ***p < 0.001, **p < 0.01 and *p < 0.05 indicate the level of significant difference as calculated by ANOVA test between the two conditions. Error bars are SEM. The normal range for *ALP* is 50–136 U/L, for *GGT* is 5–85 U/L and for *LDH* is 5–85 U/L.

BLOOD THYROID HORMONE LEVELS

Thyroxine (T_4) and triiodothyronine (T_3) (Fig. 3), as their free forms (FT_4 and FT_3) (Fig. 4) decrease were consistent, although at no time did the values approach the lower limits of normal. *TSH* was significantly increased after the saturation diving (Fig. 5).

A notable fact is the decreasing trend of the four thyroid hormones levels in the blood, over the 5 years and the parallel increasing trend of the *TSH* level for the same period of time.

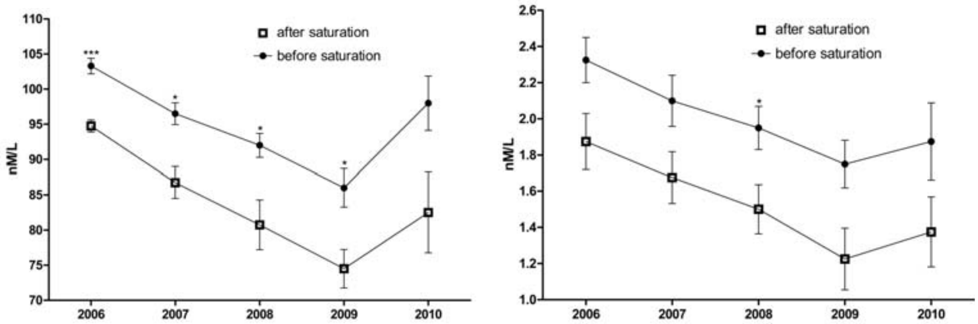

Fig. 3. Mean T_4 (left) and T_3 (right) values before and after diving in saturation, recorded during 5 years, for 16 professional divers. ***$p < 0.001$, **$p < 0.01$ and *$p < 0.05$ indicate the level of significant difference as calculated by ANOVA test between the two conditions. Error bars are SEM. The normal range for T_4 is 70–120 nM/L, and for T_3 is 0.9–2.5 nM/L.

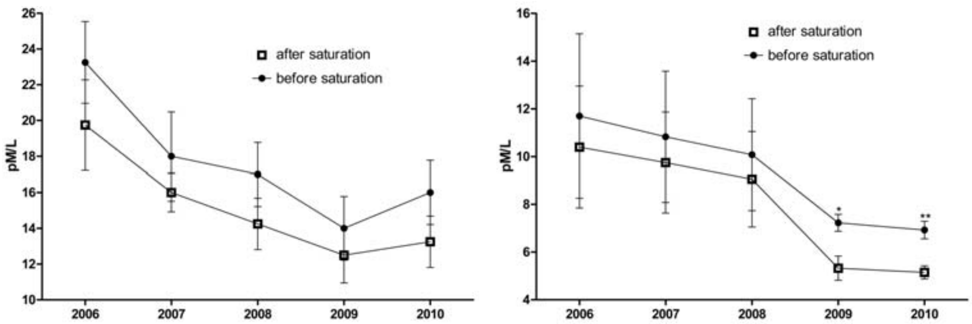

Fig. 4. Mean FT_4 (left) and FT_3 (right) values before and after diving in saturation, recorded during 5 years, for 16 professional divers. **$p < 0.01$ and *$p < 0.05$ indicate the level of significant difference as calculated by ANOVA test between the two conditions. Error bars are SEM. The normal range for FT_4 is 9–20 pM/L, and for FT_3 is 4–8.3 pM/L.

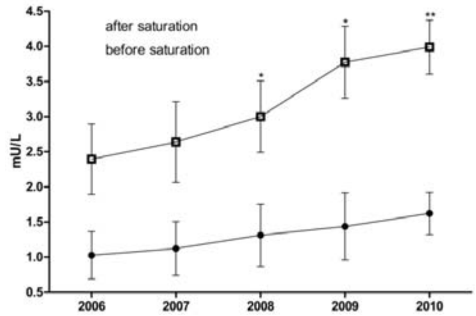

Fig. 5. Mean *TSH* values before and after diving in saturation, recorded during 5 years, for 16 professional divers. **$p < 0.01$ and *$p < 0.05$ indicate the level of significant difference as calculated by ANOVA test between the two conditions. Error bars are SEM. The normal range for *TSH* is 0.25–5 μU/L.

INDEX OF OXIDATIVE STRESS

In the hyperbaric environment the elevated O_2 partial pressure generates reactive oxygen species, which mediate O_2 toxicity which encompass pulmonary injuries, central nervous system effects manifested by seizures, and ocular effects such as reversible myopia [7]. But the reactive oxygen species also serve as signaling molecules in transduction cascades, or pathways, for a variety of growth factors, cytokines and hormones. As such, reactive species can generate either “positive” or “negative” effects depending on their concentration and intracellular localization. We have measured hydroperoxides as markers of oxidative stress before and after saturation diving. This index of oxidative stress showed a marked increase after the saturation diving, indicating a medium oxidative stress in the hyperbaric condition.

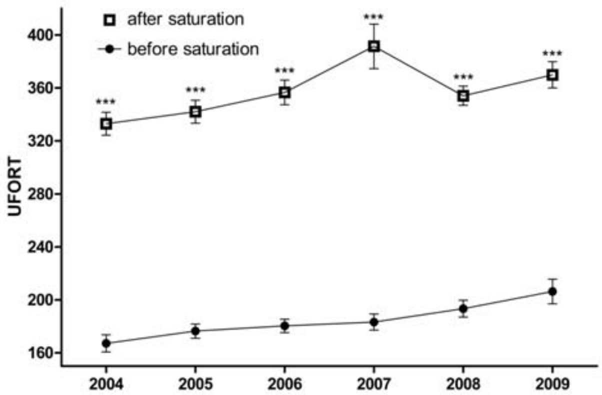

Fig. 6. Mean oxidative stress values before and after diving in saturation, recorded during 6 years, for 16 professional divers. ***$p < 0.001$, **$p < 0.01$ and *$p < 0.05$ indicate the level of significant difference as calculated by ANOVA test between the two conditions. Error bars are SEM. The free oxygen radicals test (FORT) is a simple tool to assess circulating ROS in routine clinical practice. The normal value is > than 250 FORT units. A value in the range 311–340 FORT units is considered as a low oxidative stress, a value in the range 341–400 FORT units is considered as a medium oxidative stress, and a value in the range 401–500 FORT units is considered as a high oxidative stress.

PLATELETS COUNTS AND PROTHROMBIN TIME

The platelets number was found to be significantly increased after the saturation diving. An increasing trend of the platelet number in blood was recorded over the 7 years, both before and after diving (Fig. 7). The prothrombin time, as well as the standard *INR*, were decreased after diving (Fig. 8).

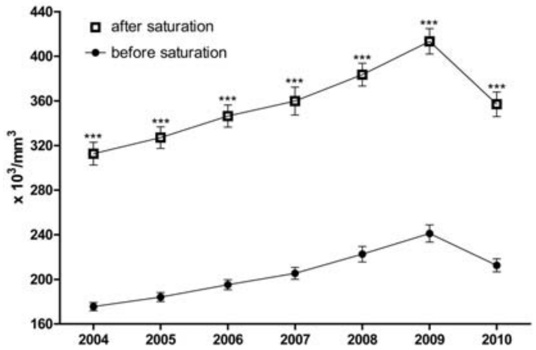

Fig. 7. Mean platelets number before and after diving in saturation, recorded during 7 years, for 16 professional divers. The normal range of platelets number is 150–350 × $10^3/mm^2$. ***$p < 0.001$, **$p < 0.01$ and *$p < 0.05$ indicate the level of significant difference as calculated by ANOVA test between the two conditions. Error bars are SEM.

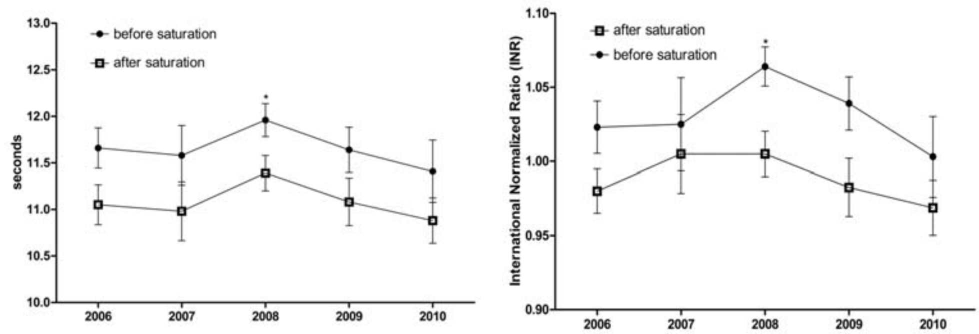

Fig. 8. Mean prothrombin time (left). The normal range is 9.8–12.7 s. International normalized ratio (right). The normal range is 0.9–1.3. *$p < 0.05$ indicates the level of significant difference as calculated by ANOVA test between the two conditions. Error bars are SEM.

DISCUSSION

Our records show a post-dive increase in the blood enzymes levels: *AST*, *ALT*, *ALP*, *GGT*, *LDH*, which however exceeded the upper normal limits in only 60% of the divers. This increase, presumably, reflects a widespread systemic embolism with secondary damage to the endothelium of embolised organ systems [4, 6]. The two transaminases (*AST*, *ALT*) and the transpeptidase (*GGT*) were found to surpass the upper normal limit of blood concentration, unlike *LDH* and *ALP*. The new fact that we have found is that over the years an increasing trend of the levels of these enzymes, both before and after diving, was detected. The transaminases are present in hepatic and muscular cells, and their increased presence in the blood indicate cytolysis in these tissues. Elevated serum gamma-glutamyl transpeptidase (*GGT*) activity can be found in diseases of the liver, biliary system, and pancreas. In this respect, it is similar to alkaline phosphatase (*ALP*) in detecting disease of the biliary tract, associated with digestive disorders produced in hyperbaric conditions. *GGT* plays a key role in the gamma-glutamyl cycle, a pathway for the synthesis and degradation of glutathione and for drug and xenobiotic detoxification. Tissue breakdown releases *LDH*, and therefore *LDH* can be measured as a surrogate for tissue breakdown, e.g. hemolysis.

A post-dive decrease was found for the thyroid hormones: T_4, T_3, FT_4, FT_3. The *TSH* post-dive elevation was also significant. However, the decreasing trend over the 5 years of the four thyroid hormones and the parallel increasing trend of the *TSH* level suggest a feed-back response of the hypophysis to the lower blood levels of the thyroid hormones evidenced in this longitudinal study. A post-dive increase was found for the markers of oxidative stress (hydroperoxides). Concerning the oxidative stress increase, the targets of reactive oxygen species are the membrane lipids and the membrane associated proteins. Depending on the composition of the gas mixture, the relative concentrations of O_2 and inert gas, and the total barometric pressure, the net effect of a particular gas on the cell membrane will be determined by the gas lipid solubility and by its ability to oxidize lipids and proteins [1]. A change in the properties of any one membrane component is anticipated to change conductance of membrane-spanning ion channels and thus neuronal function [1]. Therefore, an antioxidant administration before diving would be advisable [3].

In contrast to other authors [5, 8] we have found an increased post-dive platelet number. The prothrombin time measures the quality of the extrinsic pathway of coagulation. Its decrease, as well as the *INR* decrease, after diving, suggests a hypercoagulable state.

These data suggests that diving in saturation over years results in a subclinical change of liver and thyroid function. We suppose that the primary cause could be the liver microlesions which arise in hyperbaric conditions and their cumulative effect produces an increased enzymes release from the affected hepatocytes. Also a direct damage of the hepatic endothelium with leakage of proteins into the blood could be the cause [6]. A hepatic disfunction can be also the reason of an increased clearance rate of the thyroid hormones from the circulation [2].

The individual responses of the divers group which showed an enzymes concentration increase which exceeded the upper normal values in the blood, can signalise an increased hyperbaric stress and it is advisable to follow more rigorously the health state of these individuals. The cumulative effect of the subclinical microlesions has to be considered as a potential risk of this professional category and has to be studied in order to decide if a professional disease is to be established.

Acknowledgements. The first author (C.D.) acknowledges the permanent support and the collaborative environment of the team of the Naval Medicine Center in Constanța.

REFERENCES

1. D'AGOSTINO, D.P., D.G. COLOMB JR., J.B. DEAN, Effects of hyperbaric gases on membrane nanostructure and function in neurons, *J. Appl. Physiol.*, 2009, **106**, 996–1003.
2. DORAN, G.R., L. CHAUDRY, A.O. BRUBAKK, M.P. GARRARD, Hyperbaric liver dysfunction in saturation divers, *Undersea Biomedical Res*., 1985, **12**, 151–164.
3. IKEDA, M., K. NAKABAYASHI, M. SHINKAI, Y. HARA, T. KIZAKI, S. OH-ISHI, H. OHNO, Supplementation of antioxidants prevents oxidative stress during a deep saturation dive, *Tohoku J. Exp. Med.*, 2004, **203**, 353–357.
4. NEUMAN, T.S., Pulmonary barotrauma, in *Bove and Davis'Diving Medicine*, A.A. Bove ed., Saunders, Elsevier Science, 2004, pp. 185–194.
5. PHILP, R.B., P.B. BENNETT, J.C. ANDERSEN, G.N. FIELDS, B.A. McINTYRE, J. FRANCEY, W. BRINER, Effects of aspirin and dipyridamole on platelet function, hematology, and blood chemistry of saturation divers, *Undersea Biomedical Res*., 1979, **6**, 127–145.
6. SMITH, R.M., T.S. NEUMAN, Abnormal serum biochemistries in association with arterial gas embolism, *J. Emergency Med.*, 1997, **15**, 285–289.
7. THOM, S.R., Oxidative stress is fundamental to hyperbaric oxygen therapy, *J. Appl. Physiol.*, 2009, **106**, 988–995.
8. THORSEN, E., H. HAAVE, D. HOFSO, R.J. ULVIK, Exposure to hyperoxia in diving and hyperbaric medicine-effects on blood cell counts and serum ferritin, *Undersea Hyper Med.*, 2001, **28**, 57–62.