Review Article • DOI: 10.2478/s13380-013-0130-5 • Translational Neuroscience • 4(3) • 2013 • 302-313

VERSITA

Translational Neuroscience

# BREATH-HOLD DIVING AS A BRAIN SURVIVAL RESPONSE

Zeljko Dujic*,
Toni Breskovic,
Darija Bakovic

*Department of Integrative Physiology, University of Split School of Medicine, Split, Croatia*

**Abstract**

Elite breath-hold divers are unique athletes challenged with compression induced by hydrostatic pressure and extreme hypoxia/hypercapnia during maximal field dives. The current world records for men are 214 meters for depth (Herbert Nitsch, No-Limits Apnea discipline), 11:35 minutes for duration (Stephane Mifsud, Static Apnea discipline), and 281 meters for distance (Goran Čolak, Dynamic Apnea with Fins discipline). The major physiological adaptations that allow breath-hold divers to achieve such depths and duration are called the "diving response" that is comprised of peripheral vasoconstriction and increased blood pressure, bradycardia, decreased cardiac output, increased cerebral and myocardial blood flow, splenic contraction, and preserved $O_2$ delivery to the brain and heart. This complex of physiological adaptations is not unique to humans, but can be found in all diving mammals. Despite these profound physiological adaptations, divers may frequently show hypoxic loss of consciousness. The breath-hold starts with an easy-going phase in which respiratory muscles are inactive, whereas during the second so-called "struggle" phase, involuntary breathing movements start. These contractions increase cerebral blood flow by facilitating left stroke volume, cardiac output, and arterial pressure. The analysis of the compensatory mechanisms involved in maximal breath-holds can improve brain survival during conditions involving profound brain hypoperfusion and deoxygenation.

**Keywords**

• Apnea • Breath-hold diving • Brain perfusion • Brain oxygenation

Received 13 July 2013
accepted 25 July 2013



## Past and the modern history of free-diving (breath-hold diving)

Men and women have been breath-hold diving for thousands of years to harvest a variety of items (such as sponges, pearls, fish), to conduct rescue and military operations, and for exploration. The oldest group of breath-hold divers (BHD) is the women Ama divers from Japan and diving women of Korea (*Cachido Ama*) who dive primarily to obtain food. They have continued their diving routines for the past 2000 years fairly unchanged, except for the recent decades, when they started to use wetsuits for protection against cold. During their daily diving practice, Ama divers usually dive to a depth of up to 25 meters of seawater (msw), and the duration of dives is relatively short (up to 1 min). In 1911, one of the first free-diving competitions was held when a Greek fisherman, Yorgos Haggi Statti, was offered a few dollars to dive and to rescue the anchor of an Italian ship sunk at depth of 77 msw. The Italian ship was set free and Yorgos became known as the "father of free-diving". After World War II, breath-hold diving became an international sport where athletes test the limits of human diving in depth, time, and distance. The depth records set by elite divers have increased several-fold during the second half of the 20th century.

Exposure to excessive depths can be very dangerous and cause serious acute health problems such a collapse of the lungs, barotrauma during descent and ascent, pulmonary edema and alveolar hemorrhage, cardiac arrest, blackouts, nitrogen narcosis, decompression sickness and even death. Moreover, long-term health risks of frequent maximal apneas are not currently known, but should be addressed in future research. Additionally, shallow but frequent apnea dives are made not only by BHD, but also by underwater hockey and rugby players and synchronized and spring swimmers. Such healthy athletes in fact practice voluntary apnea on a regular basis. Thus, apnea divers are only an extreme example of voluntary apnea. In spite of the variety of physiological adaptations during breath-hold diving, a large number of neurological events appear regularly with apnea training and competitions. The most frequent are loss of consciousness, restrictions of cognitive efficiency, and loss of motor control [1]. The loss of motor control is also called "*samba*" by the divers, because, despite full consciousness, tremor develops on both sides of the body and the head undergoes tremor-like movements ("*head bobbing*"). More detailed information regarding the effects of breath-holding on cardiovascular, respiratory, and cerebrovascular health can be found elsewhere [2].

## Apnea disciplines and current records

Breath-hold divers compete in different disciplines such as Static Apnea, Dynamic Apnea, Constant Weight, and No-Limits Apnea. During static apnea, divers float motionless face down in a pool, while during dynamic

* *E-mail: zeljko.dujic@mefst.hr*

apnea, the goal is to attain maximal underwater swimming distances. During constant weight, divers swim down as deeply as possible along a vertically suspended rope using fins while in the no limit discipline the dive is performed by descending using a weight and ascending with help of flotation device (balloon). While performing static and dynamic apnea, divers are exposed to progressively increasing hypercapnic hypoxia. During constant weight diving, divers are exposed during descent and at the bottom to progressive hyperoxic hypercapnia due to hydrostatic pressure-induced compression of the chest wall [3], while the hypoxic hypercapnia is experienced only at the end of the dive just below the surface (due to chest wall expansion). Current world records for men and women in different apnea disciplines are presented in Table 1.

This review describes physiological adaptations (primarily cardiovascular and cerebrovascular) to breath-holding, taking special consideration that the majority of the experimental data in this field was collected in dry laboratory conditions and some of them may not apply to real situations during field diving (immersion, cold, hydrostatic pressure, psychological stress, etc). Further studies should be directed to monitoring physiological data during underwater immersion at rest or during swimming, made possible by using recently produced portable physiological monitors [4-6].

## Challenging the traditional concepts

Breath-hold diving to depths up to 200 msw or deeper causes extreme lung compression according to Boyle's law. Elite divers have achieved record depths that exceed the maximal depth predicted from the physiological ratio of total lung capacity (TLC) to residual volume (RV) [7]. Due to hydrostatic pressure-induced compression, injuries or pathologies after deep dives include pulmonary edema, alveolar hemorrhage, and hemoptysis. To prevent lung collapse and compression during descent, divers try to achieve lung volumes above TLC at the start of breath-hold. Having large lung volumes enables better equilibration of the inner ear by extra volume and increases oxygen stores, which all improve apnea performance. One technique used to achieve lung volume above TLC is called glossopharhyngeal insufflation (GI), by which, after maximal inspiration to TLC, the diver fills his or her mouth with air and, after opening of the glottis, forces this air into the lung. This respiratory technique results in a significant increase of lung volume above TLC, up to almost 50% [8]. However, this maneuver performed in laboratory conditions increases intrathoracic pressure, decreases venous return, compromises cardiac pumping by changing the geometry of both ventricles, and decreases arterial blood pressure, which may lead to symptomatic hypotension or syncope called 'packing blackout' [9,10]. Thus, apnea divers provide an excellent model for cardiac and hemodynamic studies on lung hyperinflation. Lindholm and Nyren [7] investigated the morphological cardiovascular consequences of GI with magnetic resonance imaging (MRI) and found extreme reduction in thoracic blood volume with a small heart and compressed thoracic vessels. In addition, Potkin *et al.* [10] employed transthoracic echocardiography during GI and reported acute biventricular systolic dysfunction (right consistent with acute pressure overload and left possibly due to ventricular interdependence). A recent cardiac MRI study by Batinic and collaborators [11] has found that submaximal GI further decreased cardiac output in comparison to maximal inspiratory apnea; however, there was no evidence of severe biventricular dysfunction as reported by Potkin *et al.* [10]. The extent of heart compression during TLC or functional residual capacity (FRC) apnea is shown in Figure 1. During a maximal breath-hold in dry conditions, mean arterial pressure (MAP) is substantially reduced at the start of the attempt, followed by renormalization and finally with significant hypertension [12,13]. Thus during few minutes the cardiovascular system of the elite diver is stressed to the maximum, starting from extreme hypotension and ending with marked hypertension. These previously described hemodynamic consequences of the GI maneuver are attenuated in the immersed conditions due to redistribution of approximately 1 l of blood from the periphery to the intrathoracic vascular compartment, due to the increased surrounding hydrostatic pressure.

Table 1. Current world records in different apnea disciplines (www.aidainternational.org).

| | World records | |
|---|---|---|
| **Discipline** | **Men** | **Women** |
| Static Apnea | 11 min 35 s | 8 min 23 s |
| Dynamic Apnea | 281 m | 225 m |
| Constant Weight | 126 m | 101 m |
| No-Limits | 214 m | 160 m |

Divers are adapted to extreme hypoxia/hypercapnia due to ventilatory, cardiovascular, and cerebrovascular adjustments, such as decreased ventilatory sensitivity to $CO_2$, increased lung volume, enhanced peripheral sympathetic and parasympathetic activation, and increased lactate production, among others [13-15]. Furthermore, they show reduced post-apnea as well as post-exercise blood acidosis and oxidative stress, mimicking the responses of diving animals [16].

## Physiological diving response

The success of breath-hold diving depends on how well a diver tolerates the physiological and psychological stress related to the depth and duration of the dive. The human body has several adaptations under diving conditions, which originate from the mammalian diving reflex. These adaptations enable the human body to endure depth and lack of oxygen far beyond what would be possible without the reflex. Human diving response involves bradycardia, vasoconstriction of selected vascular beds, with an increase in blood pressure, changes in cardiac output and spleen contraction [17]. It is composed of a unique combination of trained sympathetic and parasympathetic components of

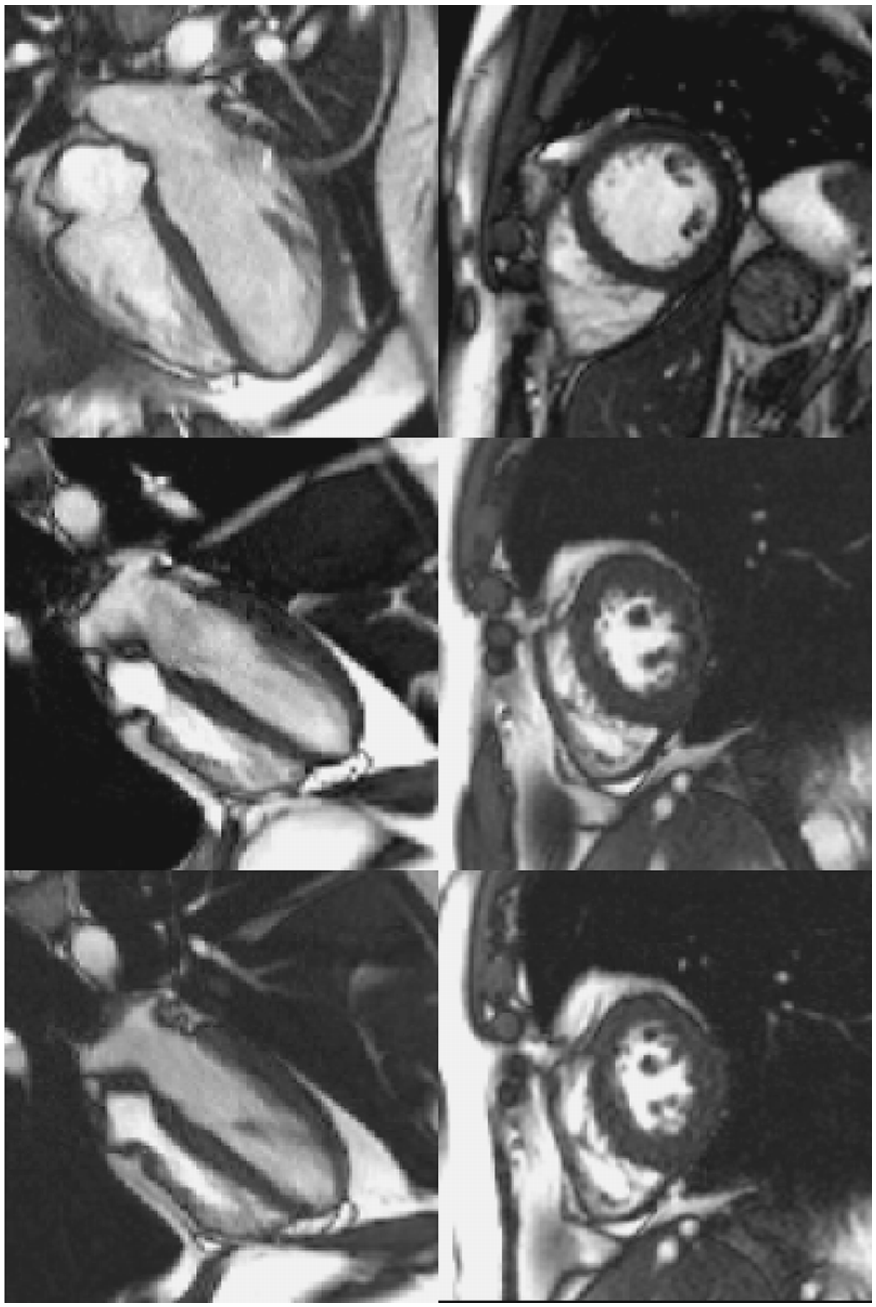

Figure 1. Representative end-diastolic MR images in the LV horizontal long axis (*left*) and midventricular short axis (*right*) during baseline (FRC, top), 1 minute of apnea (TLC, *middle*), and 1 minute of apnea with glossopharyngeal insufflation (*bottom*). Lung volume increased during glossopharyngeal insufflation by 1.16 l in this diver. Note the profound change of shape and diameters of both ventricles after lung inflation with and without glossopharyngeal insufflation. Reproduced by the kind permission of The American College of Sports Medicine from Batinic *et al.* [11].

the autonomic nervous system. These mechanisms are aimed to reduce the oxygen uptake in order to prolong the dive duration and are accentuated in breath-hold divers [18]. Bradycardia is caused by an increased vagal activity, whereas the peripheral vasoconstriction is related to increased sympathetic discharge [19]. A recent heart rate variability study by Kiviniemi *et al.* [20] evaluated sympathetic/vagal balance during static and dynamic apnea while submersed. It was found that apnea blunts the effects of exercise on cardiac vagal activity at the end of dynamic apnea. However, larger heart rate during dynamic apnea when compared to static apnea indicates larger sympathetic activity. In addition, we and others have suggested that splenic contraction is also a part of diving response [14,21] and that it occurs even with very short breath-holds lasting only 15 s, without the presence of chemical stimuli like hypoxia and/or hypercapnia [22].

During simulated breath-holding cardiac output is decreased predominantly due to bradycardia (increased vagal activity) and an increased intrathoracic pressure, which reduces venous return and consequently stroke volume [9,17]. Palada *et al.* [13] have showed that cardiac output is substantially reduced during the initial phase of the maximal dry breath-hold due to reduced inferior vena cava flow. During the later (struggle) phase of breath-hold, cardiac output is normalized because of an increase in the inferior vena cava venous return due to occurrence of involuntary breathing movements, with consequent increases in stroke volume (SV) [12]. After initial hypotension, mean arterial pressure is progressively increased during the breath-hold due to sympathetically-mediated vasoconstriction and later due to involuntary breathing movements [12,23] (Figure 2).

Blood pressure (BP) changes during maximal end-inspiratory apneas have been recorded in the past with different measuring devices and under different experimental conditions (supine apnea at rest or during exercise, with or without subject's face immersion in cold or thermo-neutral water). In all these circumstances, moderate increases in BP were found with an augmented response to face immersion. The only study that measured invasive BP during deep breath-hold dives was the study of Ferrigno *et al.* [24]. They measured BP responses in two elite divers that were compressed in the wet compartment of the chamber to 50 meters of freshwater (mfw). The alarming values of BP

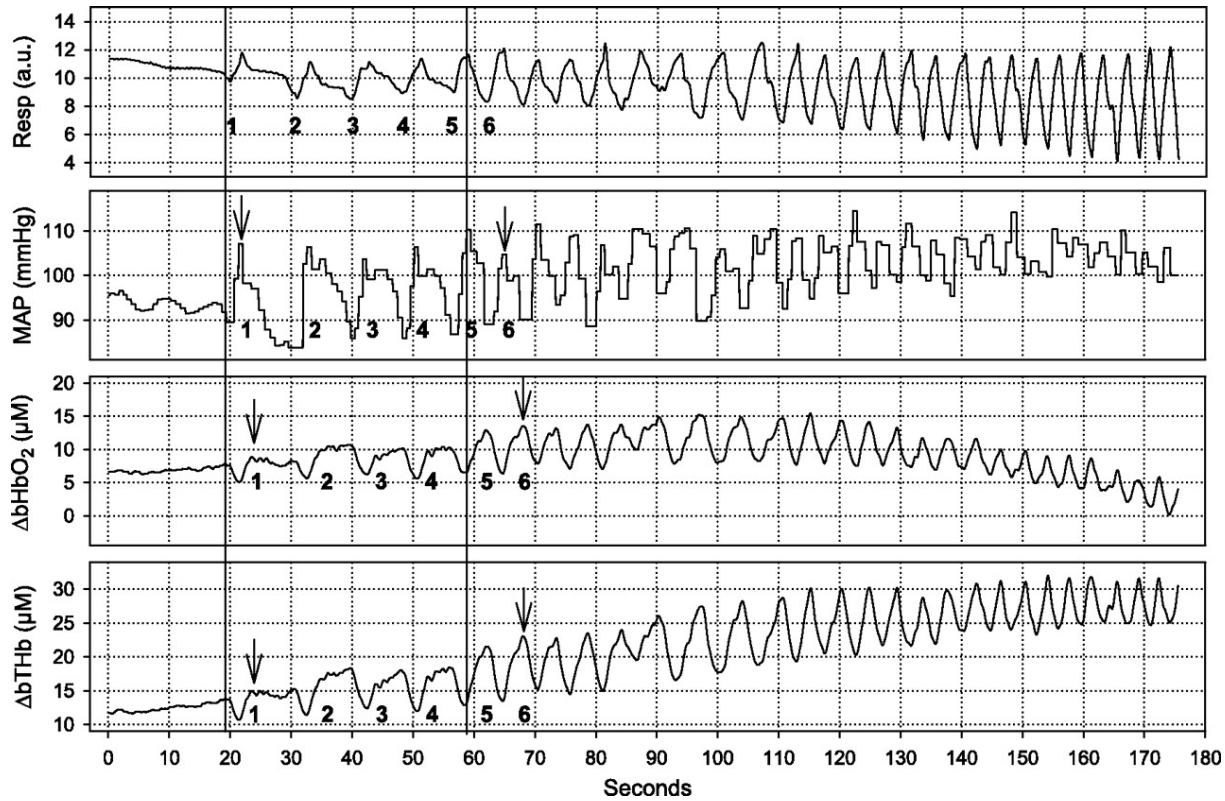


Figure 2. Individual response of various measured parameters during the struggle phase of apnea in one breath-hold diver. Respiratory movements of the chest wall recorded by a respiratory belt; MAP, mean arterial pressure; $\Delta bO_2Hb$, change in concentration of oxygenated hemoglobin in brain; ΔbTHb, change in concentration of total hemoglobin in brain. Vertical lines represent the onset of individual involuntary breathing movements (beginning of downward deflection). Arrows point at the peaks in MAP, $\Delta bO_2Hb$ and ΔbTHb tracings, which follow certain involuntary breathing movements (labeled by vertical lines). Numbers mark consecutive involuntary breathing movements and respective oscillations in MAP, $\Delta bO_2Hb$ and ΔbTHb. Note the gradual increase in ΔbTHb, indicating enhanced cerebral blood perfusion. Reproduced by the kind permission of The American Physiological Society from Dujic *et al.* [23].

(systolic 280-300 mmHg and diastolic 150-200 mmHg) were measured during 10-20 mfw descent. Contrary to the study of Ferrigno *et al.*, Sieber *et al.* [25] have recently measured BP at 10 mfw with a novel noninvasive subaquatic sphygmomanometer in elite breath-hold divers (BHD) during the 2nd min of static apnea. They have reported unchanged BP values when compared with values obtained at the surface. Recently, Perini *et al.* [26] reported continuous BP changes during prolonged immersed static apnea below the water surface. They reported significantly increased systolic (193 mmHg) and diastolic (127 mmHg) BP at the end of static apnea. Thus, reported BP changes during immersed SA are controversial, ranging from unchanged to very high values. No data exists for underwater BP measurement during dynamic apnea. Recently, we measured BP changes during static and dynamic apnea under immersed conditions and found moderate increases in BP during static and dynamic apnea that are in agreement with those measured during dry static apnea [4].

Changes of cardiac rhythm in addition to bradycardia include initial anticipatory tachycardia (stimulation of the lung mechanoreceptors, hyperventilation, excitement) usually at the start of breath hold. Increased parasympathetic input to the sino-atrial node is potentiated by immersion of the face in the cold water and enlargement of venous inflow and distention of heart cavities [27]. Frequently, arrhythmias (bradyarrhythmia and extra beats) have been reported [24] during field dives. These rhythm disturbances include not only inhibitory type arrhythmias, which may be expected from increased vagal tone, but also premature contractions. Arrhythmias are more frequent when diving in cold water [28].

## Recruitment of sympathetic neurons during maximal inspiratory apnea; Henneman's principle revisited for sympathetic activation

Breath-holds starting at different lung volumes elicit increases in sympathetic neural traffic through different mechanisms. The sympathetic response to a breath-hold starting at FRC of the lungs appears to be controlled principally by arterial oxygen desaturation and increasing levels of blood $CO_2$ [29], representing a dominant influence of chemoreflex stress. The reduction in alveolar oxygen partial pressure can be as low as 20-30 mmHg, with arterial oxygen saturation around 50% [30,31]. However, the increase in sympathetic tone during TLC breath-hold is driven by diverse stimuli present at different phases of the TLC breath-hold, causing a biphasic response in muscle sympathetic neural activity (MSNA)

(Figure 3). During the initial 30 s of a TLC breath-hold, the sympathetic neural activity response resembles that observed during a Valsalva maneuver [15]. This neural response is likely due to the high intrathoracic pressure that, in turn, reduces venous return and cardiac output, which elicits unloading of low and high-pressure baroreceptors [9,32]. After the initial phase of the TLC breath-hold, blood pressure stabilizes, but MSNA continues to increase linearly towards the end of the breath-hold. The underlying mechanisms for the increase in MSNA during the latter phase of the breath-hold must include an increase of chemoreflex stress [15,29,33] and the lack of ventilatory MSNA inhibition [29]. In all circumstances, the aforementioned increase in sympathetic neural outflow leads to augmentation of the tone of peripheral vasculature. Such pronounced peripheral vasoconstriction helps to maintain adequate oxygen supply to the brain and heart under asphyxic conditions.

Heusser *et al.* [15] showed that, when compared to baseline, the overall increase in MSNA during breath-holding in trained BHD is larger than 20-fold, a level that is approximately five times higher than observed in untrained control subjects. However, if control subjects are brought to the comparable level of chemoreflex stress as BHD by performing a prolonged breath-hold of at least several minutes, a similar level of sympathetic activation occurs, thus suggesting that chemoreflex sensitivity is unchanged in BHD compared to controls [34-38]. Under such conditions, increases in the action potential (AP) firing frequency and/or the recruitment of postganglionic sympathetic neurons are necessary in order to enable such a large augmentation in MSNA.

To address the issue of postganglionic recruitment strategies, previous studies examined single-unit recordings and showed that sympathetic neurons, when active, fire predominantly once with a given burst of activity (~70% of occurrences) [39] with the probability of multiple firings of the same neuron within a burst increasing during voluntary apnea [39] and with certain pathologies [40]. The aforementioned studies, although valuable for understanding properties of sympathetic neural activation, are hampered by the inability to record activity of multiple neural fibers simultaneously.

To expand on this single neuron approach and examine how the multi-unit signal changes with reflex activation, a new spike detection algorithm was developed. It uses the continuous wavelet transform approach that enables determination of the number of sympathetic APs contributing to the multi-unit MSNA [41]. Steinback *et al.* [42] applied this technique to analyze the sympathetic activity in BHD during prolonged, maximal end-inspiratory breath-holds. The study suggested that large sympathetic neurons, which generate larger APs and faster conduction

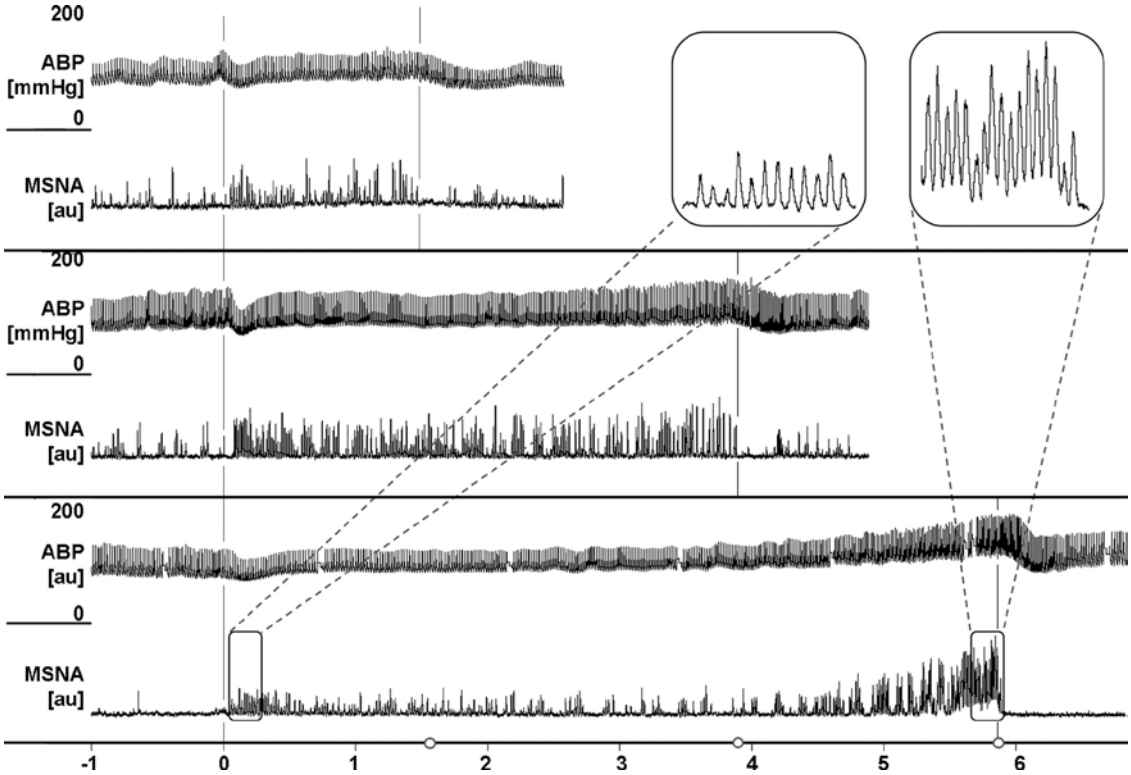


Figure 3. Original recordings of beat-to-beat arterial blood pressure (ABP; Finometer, Finapres Medical Systems, Amsterdam, The Netherlands) and muscle sympathetic neural activity (MSNA) in three subjects. Upper trace: control subject. Lower traces: apnea divers. Note the sympathetic activation during the first 15 to 20 s of apnea. The activation is a baroreflex triggered by the blood pressure drop immediately after starting the breath-hold. The lowest trace demonstrates that early sympathetic activation is achieved by an increase in burst frequency (bursts per minute). During the last minute of apnea, a rise in spike frequency dominates, as reflected by the increasing amplitude of the bursts. Reproduced by the kind permission of The American Heart Association from Heusser *et al.* [15].

velocities, are silent at rest but may be recruited during chemoreceptor-induced increases in sympathetic drive at the end of maximal end-inspiratory apneas. In conclusion, the sympathetic neural activation resembles a similar pattern of activation already described in motor neurons [43].

By utilizing the above mentioned spike detection algorithm, Breskovic *et al.* [36] were able to distinguish different properties of sympathetic neuron activity depending whether the stimulus was baroreceptor unloading or an increase in chemoreflex stress. Throughout the FRC breath-hold and later phase of TLC breath-hold (both phases are characterized by the rise in chemoreceptor stress) the increase in total AP frequency was achieved by an increase in burst frequency and the number of APs/burst, which implies recruitment of a previously inactive neural fibers combined with possible repeated firing of the same neurons. Additionally, with the increase in burst content, the increase in spike amplitude was observed, suggesting that these neurons have larger diameter and thus are faster conducting.

On the other hand, the initial 30 s of the TLC breath-hold is characterized by low BP, SV, and cardiac output, thus representing a baroreceptor unloading stimulus. Although being a potent provocation for the increase in sympathetic neural activity, different patterns of activation were observed in this phase. There was a marked increase in burst frequency accompanied by a small increase in the content of APs within single burst, suggesting no or minimal neuron recruitment. Salmanpour *et al.* [44] have utilized the same technique for AP detection during lower-body negative pressure (up to -60 mmHg) to provoke baroreceptor unloading. Their study also showed that the increase in sympathetic neural activity caused by baroreflex activation was attained by changes in the burst frequency and burst incidence alone, without change in AP content or apparent recruitment of additional axons. The cause for such different pattern of sympathetic activation is unclear but may involve a relatively higher "threshold" for the onset of the recruitment of new sympathetic neurons for baroreflex unloading than during increased chemoreflex stress (Figure 4).

At this point it is important to mention that the aforementioned pattern of sympathetic activation was observed in both groups without significant differences. However, further studies will be necessary to further elucidate why varying sympathoexcitatory stimuli cause a different pattern of sympathetic outflow, and voluntary breath-holding has proved to be an excellent model for investigation of sympathetic neural outflow.

## Cerebral blood flow and oxygenation

Cerebral blood flow changes during breath-holds were analyzed in the past during shorter and longer static apneas. Pan *et al.* [45] found

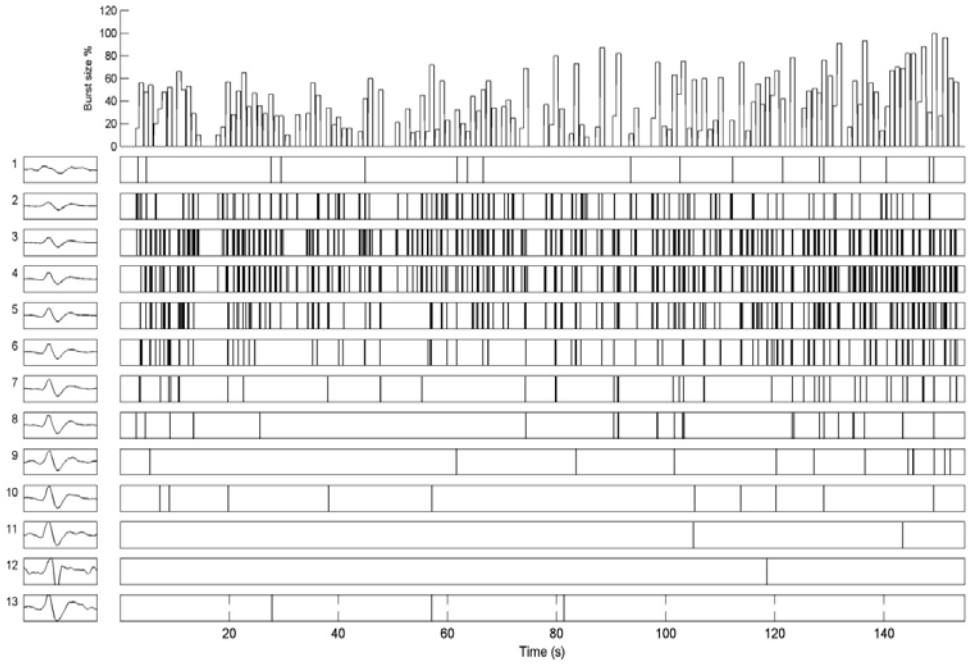


Figure 4. Identification of single APs using continuous wavelet transform algorithm in a single diver during end-inspiratory breath-hold (total of 2 min 34 s in duration). It can be seen that towards the end of the breath-hold the burst size (*i.e.* burst content) increases. The detected APs are divided in 13 clusters depending on their amplitude size. Note the increase in firing probability of larger APs (*i.e.* neurons of larger diameter) at the very beginning of the breath-hold and towards the end of the breath-hold. This suggests neuronal recruitment of previously "silent", larger, faster conducting neural fibers. At the same time we see an increase in firing frequency of smaller units (*i.e.* lower clusters).

increased carotid artery blood flow during short static apnea lasting one minute in non-divers during submersion to 2 m. Przybylowski *et al.* [46] reported increased flow velocity in the middle cerebral artery (MCAV) 20 s from the start of apnea and have suggested that apnea-induced fluctuations in cerebral blood flow were caused primarily due to changes in arterial partial pressure of $CO_2$ ($PaCO_2$), and that the sympathetic nervous system plays a lesser role. In BHD with maximal static apneas lasting 4-6 minutes, Palada *et al.* [13] observed more than a 100% increase in MCAV, which was significantly larger than in controls (~50%). As reported previously, at the end of the breath-hold in divers, a large increase in mean arterial pressure was found as a part of the adaptive autonomic mechanisms serving to protective vital organs (brain and heart). Since long breath-holds are associated with hypercapnia, our results of are in line with recent study of Vantanajal *et al.* [47] and Ainslie *et al.* [48], who concluded that the vasculature of the brain is more sensitive to changes in arterial partial pressure of carbon dioxide ($PCO_2$) and less sensitive to sympathetic stimulation than the vasculature in the forearm [47]. Our study showed moderate reduction in cerebral oxygenation during the maximal static breath-holds in trained divers, despite larger increases in cerebral blood velocity and similar cerebral oxygen delivery [13].

Despite the significant increase in cerebral blood flow during a breath-hold, is there evidence for neuronal damage during such extreme hypoxia/hypercapnia in elite breath-hold divers? Recently, Andersson *et al.* found a moderate increase in serum level of glial-specific S100 calcium binding protein B (S100B) (expressed primarily by a subtype of mature astrocytes that ensheat blood vessels) after breath-hold attempts, a marker of disruption of blood-brain barrier [49]. However, this finding should be complemented by measurement of other central nervous system (CNS) damage, such as glial fibrillary acidic protein and neuron-specific enolase, as extracranial sources of S100B from skeletal muscle and fat cells has been recently proposed [50]. Still, the caution about the acute or chronic negative effects of breath-hold diving on CNS morphologic or functional changes is warranted due to a report using MRI. The study by Kohshi *et al.* [51] showed multiple T2-weighted hyperintensities in apnea divers with neurological symptoms after a competition. Furthermore Potkin *et al.* [52] used single photon computed tomography and have reported large focal and/or diffuse areas of hypoperfusion in the frontal and temporal lobes and the cerebellar hemispheres.

## Involuntary breathing movements improve cerebral oxygenation during the apnea struggle phase

The human physiological response to maximal voluntary apnea can be characterized by two distinct phases: the initial, easy-going phase that resembles a quiescent period during which no significant movement of the thorax occurs and that lasts until the physiological breaking point when the accumulated $CO_2$ stimulates the respiratory drive [53], and the struggle phase during which the subject feels a growing urge to breathe and shows progressive involuntary breathing movements [54]. Divers continue to "struggle" for the remainder of apnea until the break point is reached when the glottis is reopened and inspiration occurs. Recently, we analyzed the pattern of respiratory pressure development of the diaphragm, rib cage and abdominal muscles during the struggle phase of maximal breath-holding in elite breath-hold divers [55]. Respiratory muscle contractions were assessed via measurement of esophageal, gastric, and transdiaphragmatic pressures. We found the inspiratory rib cage pressure development during struggle phase of the breath-hold increased at a rate exceeding that of the diaphragm, with proportionally rising expiratory pressures generated by the rib cage/abdominal muscles [55]. At the end of the breath-hold, developed respiratory pressures approached potentially fatigue levels, suggesting the extent of respiratory neuromuscular activation by these maneuvers.

The influence of varying fractions of $O_2$and $CO_2$ on the initiation of involuntary breathing movements was recently investigated by Breskovic *et al.* [56]. Intra-arterial $PaO_2$ and $PaCO_2$ at involuntary breathing movements onset and apnea cessation were measured during voluntary apneas after breathing normoxic, hyperoxic, hypoxic, and hypercapnic gas mixtures. It was concluded that there is no single threshold in $PaO_2$ and $PaCO_2$, but rather the chemoreceptor threshold is dependent on an interaction between $PaO_2$ and $PaCO_2$. The modulatory effect of $PaO_2$ on the level of $PaCO_2$ at which involuntary breathing movements start occurs below 18 kPa (135 mmHg), whereas above that value a relatively constant value of $PaCO_2$ of approximately 6.3 kPa (49 mmHg) is found.

Recently, we reported that the involuntary breathing movements are involved in the restoration of venous return during the struggle phase of maximal inspiratory apneas by improving the inferior vena cava blood flow, which leads to augmentation of SV and normalization of cardiac output [12]. Furthermore, we showed that involuntary breathing movements are followed by the simultaneous phasic fluctuations of cerebral oxygenated hemoglobin [23,57]. The brain oxygenation fluctuations occurred in parallel with the fluctuations of mean arterial pressure, heart rate, and SV, indicating that involuntary breathing movements may influence central hemodynamics by maintaining cardiac output (Figure 2). This shows that involuntary breathing movements, together with peripheral vasoconstriction-induced centralization of the blood volume and progressive hypercapnia-induced cerebral vasodilatation, likely act to maintain cerebral oxygenation throughout the struggle phase. Future research about the precise mechanism(s) for beneficial effects of involuntary breathing movements on cerebral perfusion is needed.

## Fainting during breath-hold

As previously mentioned, breath-hold divers may experience loss of consciousness either during dry attempts or during field dives ("deep or shallow water blackouts"). The cardiovascular and autonomic mechanisms leading to fainting during maximal inspiratory apnea with GI were unknown until recently. We found that divers fainted with different mechanisms [58]. In one diver, BP and cardiac output decreased even though both the

heart rate and sympathetic vasomotor tone were well maintained, suggesting reduced responsiveness between efferent sympathetic nerve activity and vascular smooth muscle. Our study suggested that syncope with increased intrathoracic pressure is mainly induced by parasympathetic activation and bradycardia (four out of five divers), in contrast to the sympathetic vasomotor reduction with or without bradycardia mostly seen in neurally mediated ("vasovagal") syncope.

## The role of the spleen in the diving response and its role during sympathetic activation

In many mammals, the spleen contains a significant volume of a thick blood, which is partially released to the active circulation during increased physical activity or diving [59]. The spleen of the Weddell seal (*Leptonychotes weddelli*) may contract and inject red blood cells into the peripheral circulation during diving. Approximately, 20.1 l of red blood cells were sequestered at rest, presumably in the spleen, and released either on epinephrine injection or during diving. Catecholamine release and splenic contraction appear to be an integral part of the voluntary diving response of Weddell seals [59]. Spleen emptying has also been observed in humans during exercise and apnea diving, with a reduction in its volume and a two-thirds decrease in splenic erythrocyte content [60] and increase in blood oxygen conserving capacity [21,61]. Moreover, lately, spleen emptying has been recognized as part of the diving response. It was recently suggested that spleen volume changes after an increase in sympathetic activity represent a passive collapse, rather than active contraction. The assumption was that only splenic arterial vessels are sufficiently sympathetically innervated. However, Bakovic *et al.* [14] provided, for the first time, results regarding active spleen contraction in response to repetitive apneas. These authors showed that in simulated apnea diving, reduction of the spleen volume is fast and in the presence of conserved flow in the splenic artery. This ruled out the possibility of passive collapse and showed that in apnea diving, the spleen is not part of the periphery with reduced blood flow secondary to elevated sympathetic tone. The spleen contracted immediately upon the onset of apnea in parallel with a simultaneous increase in heart rate, when arterial blood gases are still unaffected (Figure 5). The rapidity of the splenic response to apnea diving argues against peripheral triggers and indicated the existence of the centrally mediated feed-forward mechanism. Palada *et al.* [22] came to similar conclusions that the spleen contraction is present at the very beginning of large lung volume apnea, and probably facilitated by baroreflex inhibition in conditions of decreased blood pressure and cardiac output. Moreover, unpublished observations from Bakovic *et al.* show that low doses of epinephrine (which predominately stimulates β-adrenoreceptors) trigger a rapid splenic contraction with concomitant increase in MSNA. The spleen contracted at the onset of epinephrine infusion, in parallel with a simultaneous decrease in total peripheral resistance and mean arterial pressure and increase in heart rate, SV and MSNA. This suggests unloading of baroreceptors and the existence of a central sympathetic mechanism that initiates early splenic contraction.

On the other hand, the human spleen normally retains about one-third of the body's platelets in an exchangeable pool, which can be released into the circulation by adrenergic stimulation [62,63]. For example, Schmidt and Rasmussen [64] reported that 98% of the rise in circulating $^{111}$In-labelled platelets after exercise could be accounted for by the loss of labeled platelets from the spleen. It was also previously shown that mean platelet volume (MPV) of the splenic platelet population was about 20% greater than MPV of the circulating platelets [65]. However, platelet size correlates with their reactivity [66]. Large platelets are metabolically and enzymatically more active than small platelets and produce more thromboxane $A_2$. Thus, the spleen could be an important source of large platelets, mobilized by adrenergic stimulation, e.g. breath-hold diving, thereby increasing the risk of sudden thrombotic

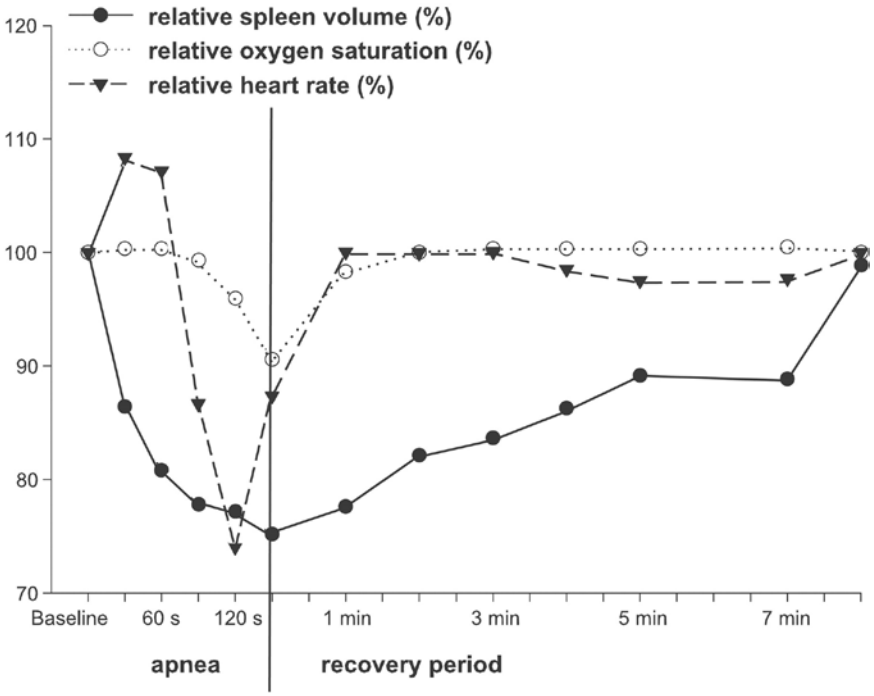


Figure 5. Intra-apneic and post-apneic sampling of the spleen volume (assessed ultrasonographically), transcutaneous arterial oxygen saturation and heart rate in three trained apnea divers who performed maximal simulated apnea dives with face immersion in cold water. The values are expressed as the percent of baseline value and averaged over the three divers. The duration of apnea was 150 s in all divers. Note that the spleen starts to contract immediately on the apnea onset, decreases in size steadily throughout apnea duration, and slowly recovers after the end of apnea diving. In contrast, arterial oxygen saturation and heart rate have different intra-apneic dynamics and recover quickly after cessation of apnea. Reproduced by the kind permission of The American Physiological Society from Bakovic *et al.* [14].

incidents. In the study of Bakovic *et al.* [61], spleen contraction in response to repetitive breath-hold resulted in an increase in red blood cell volume and leukocytes but without changes in the total platelet count. This data suggested that the spleen did not have an important role in regulation of platelet concentration, at least in response to breath-hold stimulation, despite changes in spleen size. Ojiri *et al.* [67] came to the same conclusion by analyzing changes in circulating blood cell counts with adrenergic stimulation of the canine spleen. However, several studies in humans reported epinephrine-induced increases in circulating platelet count [68-70]. To further elucidate this problem, Bakovic *et al.* [71] provided the results of changes in both MPV and total platelets in response to repetitive breath-hold dives in three groups of subjects: trained apnea divers, untrained intact, and splenectomized subjects. They showed that the series of successive breath-holds with face-immersion in cold water result in a fast and sustained increase in MPV in systemic venous blood, both in trained divers and untrained subjects, but without changes in the total platelet count. They suggested that breath-holding triggered the exchange of platelets between the splenic and circulating blood pools. This hypothesis is substantiated by unchanged MPV in splenectomized persons. Furthermore, since the platelet concentration did not change in any of the three studied groups, there must have been the splenic retention of smaller platelets, in addition to ejection of the larger ones, in response to series of apneas in intact persons. Thus, the ejection of the platelet population with larger MPV during contraction of the spleen could have been followed by splenic recovery and sequestration of the platelet population with smaller MPV from the active circulation in the periods between successive apnea attempts.

A few recent studies have shown a strong connection between high MPV and thrombotic events like acute coronary incidents and stroke [72-74]. If we knew whether these conditions were associated with a high level of sympathetic activation, then we could reach the conclusion that the centrally-mediated splenic contraction is an important source of large platelets, thereby increasing the risk of sudden coronary incidents [71]. In our recent study [75], we found an increase in MPV in response to splenic contraction induced by low dose epinephrine infusion in conditions of decreased blood pressure. Thus, the spleen is a dynamic reservoir of large platelets, the recognized prothrombotic factors.

Additionally, in patients with obstructive sleep apnea (OSA), MPV was significantly higher when compared with control subjects [76]. OSA is a common disorder associated with systemic hypertension, myocardial infarction, stroke, and premature death [77,78]. Elevated sympathetic tone has been documented previously in OSA [79] and possibly may contribute to the increased cardiovascular risk, perhaps through spleen contraction and an increase in peripheral MPV. Moreover, Sahota *et al.* [80] found splenic contraction after ischemic stroke followed by a re-expansion. Characterization of the splenic response after stroke and its contribution to cerebral ischemic injury has the potential to provide new opportunities for the development of novel stroke therapies [80].

## Concluding remarks and future perspective

Currently, we are working on different approaches related to the further characterization and analysis of involuntary breathing movements, especially related to their beneficial effects on cerebral blood flow, as well as to visualize with dynamic MRI diaphragmatic contribution in their development. We are proposing that involuntary breathing movements are part of the brain survival reflex that is initiated during extreme brain hypoxia/hypercapnia and that elite divers may serve as *in vivo* human models for studying brain survival mechanisms [81]. In the near future, we will use knowledge about the discharge properties of the sympathetic nervous system from BHD and controls to the benefit of patients that show sympathetic overactivity (heart failure patients, metabolic syndrome). By doing so, we hope to gain knowledge how acute or chronic noninvasive interventions such as changes in breathing patterns could help patients with severe heart failure (NYHA class II-IV) in reducing symptoms and improving long-term prognosis. Ultimately, our goal is to investigate whole body human integrative physiological responses complemented with cellular data (such as mitochondrial function) in order to formulate better predictions for disease prevention and treatment.

## Acknowledgments

Authors wish to declare they don't have any conflicts of interests that could have influenced the reporting of the experimental data or conclusions in this paper. This study was supported by the Croatian Ministry of Science, Education and Sports Grant No. 216-2160133-0130. All authors participated in writing and the critical review of the manuscript.

## References

[1] Lindholm P., Loss of motor control and/or loss of consciousness during breath-hold competitions, Int. J. Sports Med., 2007, 28, 295-299

[2] Dujic Z., Breskovic T., Impact of breath holding on cardiovascular respiratory and cerebrovascular health, Sports Med., 2012, 42, 459-472

[3] Muth C.M., Radermacher P., Pittner A., Steinacker J., Schabana R., Hamich S., et al., Arterial blood gases during diving in elite apnea divers, Int. J. Sports Med., 2003, 24, 104-107

[4] Breskovic T., Uglesic L., Zubin P., Kuch B., Kraljevic J., Zanchi J., et al., Cardiovascular changes during underwater static and dynamic breath-hold dives in trained divers, J. Appl. Physiol, 2011, 111, 673-678

[5] Kuch B., Koss B., Dujic Z., Buttazzo G., Sieber A., A novel wearable apnea dive computer for continuous plethysmographic monitoring of oxygen saturation and heart rate, Diving. Hyperb. Med., 2010, 40, 34-40

[6] Tocco F., Marongiu E., Pinna M., Roberto S., Pusceddu M., Angius L., et al., Assessment of circulatory adjustments during underwater apnoea in elite divers by means of a portable device, Acta. Physiol. (Oxf), 2013, 207, 290-298
[7] Lindholm P., Nyren S., Studies on inspiratory and expiratory glossopharyngeal breathing in breath-hold divers employing magnetic resonance imaging and spirometry, Eur. J. Appl. Physiol, 2005, 94, 646-651
[8] Novalija J., Lindholm P., Loring S.H., Diaz E., Fox J.A., Ferrigno M., Cardiovascular aspects of glossopharyngeal insufflation and exsufflation, Undersea Hyperb. Med., 2007, 34, 415-423
[9] Ferrigno M., Hickey D.D., Liner M.H., Lundgren C.E., Cardiac performance in humans during breath holding, J. Appl. Physiol, 1986, 60, 1871-1877
[10] Potkin R., Cheng V., Siegel R., Effects of glossopharyngeal insufflation on cardiac function: an echocardiographic study in elite breath-hold divers, J. Appl. Physiol, 2007, 103, 823-827
[11] Batinic T., Utz W., Breskovic T., Jordan J., Schulz-Menger J., Jankovic S., et al., Cardiac magnetic resonance imaging during pulmonary hyperinflation in apnea divers, Med. Sci. Sports Exerc., 2011, 43, 2095-2101
[12] Palada I., Bakovic D., Valic Z., Obad A., Ivancev V., Eterovic D., et al., Restoration of hemodynamics in apnea struggle phase in association with involuntary breathing movements, Respir. Physiol. Neurobiol., 2008, 161, 174-181
[13] Palada I., Obad A., Bakovic D., Valic Z., Ivancev V., Dujic Z., Cerebral and peripheral hemodynamics and oxygenation during maximal dry breath-holds, Respir. Physiol. Neurobiol., 2007, 157, 374-381
[14] Bakovic D., Valic Z., Eterovic D., Vukovic I., Obad A., Marinovic-Terzic I., et al., Spleen volume and blood flow response to repeated breath-hold apneas, J. Appl. Physiol., 2003, 95, 1460-1466
[15] Heusser K., Dzamonja G., Tank J., Palada I., Valic Z., Bakovic D., et al., Cardiovascular regulation during apnea in elite divers, Hypertension, 2009, 53, 719-724
[16] Joulia F., Steinberg J.G., Wolff F., Gavarry O., Jammes Y., Reduced oxidative stress and blood lactic acidosis in trained breath-hold human divers, Respir. Physiol. Neurobiol., 2002, 133, 121-130
[17] Liner M.H., Ferrigno M., Lundgren C.E., Alveolar gas exchange during simulated breath-hold diving to 20 m, Undersea Hyperb. Med., 1993, 20, 27-38
[18] Ferretti G., Extreme human breath-hold diving, Eur. J. Appl. Physiol., 2001, 84, 254-271
[19] Fagius J., Sundlof G., The diving response in man: effects on sympathetic activity in muscle and skin nerve fascicles, J. Physiol., 1986, 377, 429-443
[20] Kiviniemi A.M., Breskovic T., Uglesic L., Kuch B., Maslov P.Z., Sieber A., et al., Heart rate variability during static and dynamic breath-hold dives in elite divers, Auton. Neurosci., 2012, 169, 95-101
[21] Schagatay E., Andersson J.P., Hallen M., Palsson B., Selected contribution: role of spleen emptying in prolonging apneas in humans, J. Appl. Physiol., 2001, 90, 1623-1629
[22] Palada I., Eterovic D., Obad A., Bakovic D., Valic Z., Ivancev V., et al., Spleen and cardiovascular function during short apneas in divers, J. Appl. Physiol., 2007, 103, 1958-1963
[23] Dujic Z., Uglesic L., Breskovic T., Valic Z., Heusser K., Marinovic J., et al., Involuntary breathing movements improve cerebral oxygenation during apnea struggle phase in elite divers, J. Appl. Physiol., 2009, 107, 1840-1846
[24] Ferrigno M., Ferretti G., Ellis A., Warkander D., Costa M., Cerretelli P., et al., Cardiovascular changes during deep breath-hold dives in a pressure chamber, J. Appl. Physiol., 1997, 83, 1282-1290
[25] Sieber A., L'abbate A., Passera M., Garbella E., Benassi A., Bedini R., Underwater study of arterial blood pressure in breath-hold divers, J. Appl. Physiol., 2009, 107, 1526-1531
[26] Perini R., Gheza A., Moia C., Sponsiello N., Ferretti G., Cardiovascular time courses during prolonged immersed static apnoea, Eur. J. Appl. Physiol., 2010, 110, 277-283
[27] Irving L., Bradycardia in Human Divers, J. Appl. Physiol., 1963, 18, 489-491
[28] Hong S.K., Song S.H., Kim P.K., Suh C.S., Seasonal observations on the cardiac rhythm during diving in the Korean ama, J. Appl. Physiol., 1967, 23, 18-22
[29] Somers V.K., Mark A.L., Zavala D.C., Abboud F.M., Contrasting effects of hypoxia and hypercapnia on ventilation and sympathetic activity in humans, J. Appl. Physiol., 1989, 67, 2101-2106
[30] Lindholm P., Lundgren C.E., Alveolar gas composition before and after maximal breath-holds in competitive divers, Undersea Hyperb. Med., 2006, 33, 463-467
[31] Overgaard K., Friis S., Pedersen R.B., Lykkeboe G., Influence of lung volume, glossopharyngeal inhalation and P(ET) O2 and P(ET) CO2 on apnea performance in trained breath-hold divers, Eur. J. Appl. Physiol., 2006, 97, 158-164
[32] Macefield V.G., Wallin B.G., Effects of static lung inflation on sympathetic activity in human muscle nerves at rest and during asphyxia, J. Auton. Nerv. Syst., 1995, 53, 148-156
[33] Morgan B.J., Denahan T., Ebert T.J., Neurocirculatory consequences of negative intrathoracic pressure vs. asphyxia during voluntary apnea, J. Appl. Physiol., 1993, 74, 2969-2975
[34] Breskovic T., Ivancev V., Banic I., Jordan J., Dujic Z., Peripheral chemoreflex sensitivity and sympathetic nerve activity are normal in apnea divers during training season, Auton. Neurosci., 2010, 154, 42-47
[35] Breskovic T., Valic Z., Lipp A., Heusser K., Ivancev V., Tank J., et al., Peripheral chemoreflex regulation of sympathetic vasomotor tone in apnea divers, Clin. Auton. Res., 2010, 20, 57-63
[36] Breskovic T., Steinback C.D., Salmanpour A., Shoemaker J.K., Dujic Z., Recruitment pattern of sympathetic neurons during breath-holding at different lung volumes in apnea divers and controls, Auton. Neurosci., 2011, 164, 74-81
[37] Steinback C.D., Breskovic T., Banic I., Dujic Z., Shoemaker J.K., Autonomic and cardiovascular responses to chemoreflex stress in apnoea divers, Auton. Neurosci., 2010, 156, 138-143

[38] Dujic Z., Ivancev V., Heusser K., Dzamonja G., Palada I., Valic Z., et al., Central chemoreflex sensitivity and sympathetic neural outflow in elite breath-hold divers, J. Appl. Physiol., 2008, 104, 205-211

[39] Macefield V.G., Wallin B.G., Firing properties of single vasoconstrictor neurones in human subjects with high levels of muscle sympathetic activity, J. Physiol., 1999, 516, 293-301

[40] Elam M., Sverrisdottir Y.B., Rundqvist B., McKenzie D., Wallin B.G., Macefield V.G., Pathological sympathoexcitation: how is it achieved?, Acta Physiol. Scand., 2003, 177, 405-411

[41] Salmanpour A., Brown L.J., Shoemaker J.K., Spike detection in human muscle sympathetic nerve activity using a matched wavelet approach, J. Neurosci. Methods, 2010, 193, 343-355

[42] Steinback C.D., Salmanpour A., Breskovic T., Dujic Z., Shoemaker J.K., Sympathetic neural activation: an ordered affair, J. Physiol., 2010, 588, 4825-4836

[43] Henneman E., Somjen G., Carpenter D.O., Functional siginifcance of cell size in spinal motoneurons, J. Neurophysiol., 1965, 28, 560-580

[44] Salmanpour A., Brown L.J., Steinback C.D., Usselman C.W., Goswami R., Shoemaker J.K., Relationship between size and latency of action potentials in human muscle sympathetic nerve activity, J. Neurophysiol., 2011, 105, 2830-2842

[45] Pan A.W., He J., Kinouchi Y., Yamaguchi H., Miyamoto H., Blood flow in the carotid artery during breath-holding in relation to diving bradycardia, Eur. J. Appl. Physiol. Occup. Physiol., 1997, 75, 388-395

[46] Przybylowski T., Bangash M.F., Reichmuth K., Morgan B.J., Skatrud J.B., Dempsey J.A., Mechanisms of the cerebrovascular response to apnoea in humans, J. Physiol., 2003, 548, 323-332

[47] Vantanajal J.S., Ashmead J.C., Anderson T.J., Hepple R.T., Poulin M.J., Differential sensitivities of cerebral and brachial blood flow to hypercapnia in humans, J. Appl. Physiol., 2007, 102, 87-93

[48] Ainslie P.N., Barach A., Murrell C., Hamlin M., Hellemans J., Ogoh S., Alterations in cerebral autoregulation and cerebral blood flow velocity during acute hypoxia: rest and exercise, Am. J. Physiol. Heart Circ. Physiol., 2007, 292, H976-H983

[49] Andersson J.P., Liner M.H., Jonsson H., Increased serum levels of the brain damage marker S100B after apnea in trained breath-hold divers: a study including respiratory and cardiovascular observations, J. Appl. Physiol., 2009, 107, 809-815

[50] Riuzzi F., Sorci G., Beccafico S., Donato R., S100B engages RAGE or bFGF/FGFR1 in myoblasts depending on its own concentration and myoblast density. Implications for muscle regeneration, PLoS One, 2012, 7, e28700

[51] Kohshi K., Katoh T., Abe H., Okudera T., Neurological accidents caused by repetitive breath-hold dives: two case reports, J. Neurol. Sci., 2000, 178, 66-69

[52] Potkin R., Uszler J.M., Brain function imaging in asymptomatic elite breath-hold divers, In: Lindholm P., Pollock N.W., Lundgren C.E., eds., Breath-hold diving. Proceedings of the Undersea and Hyperbaric Medical Society/Divers Alert Network, June 20-21 Workshop, NC: Divers Alert Network, Durham, 2006, 135-137

[53] Lin Y.C., Breath-hold diving in terrestrial mammals, Exerc. Sport Sci. Rev., 1982, 10, 270-307

[54] Dejours P., Hazards of hypoxia during diving, In: Rahn H., ed., Physiology of breath-hold diving and the Ama of Japan papers, National Academy of Sciences - National Research Council, Washington, 1965, 183-193

[55] Cross T.J., Breskovic T., Sabapathy S., Zubin M.P., Johnson B.D., Dujic Z., Respiratory muscle pressure development during breath holding in apnea divers, Med. Sci. Sports Exerc., 2013, 45, 93-101

[56] Breskovic T., Lojpur M., Maslov P.Z., Cross T.J., Kraljevic J., Ljubkovic M., et al., The influence of varying inspired fractions of O(2) and CO(2) on the development of involuntary breathing movements during maximal apnoea, Respir. Physiol. Neurobiol., 2012, 181, 228-233

[57] Cross T.J., Kavanagh J.J., Breskovic T., Zubin M.P., Lojpur M., Johnson B.D., et al., The effects of involuntary respiratory contractions on cerebral blood flow during maximal apnoea in trained divers, PLoS One, 2013, 8, e66950

[58] Dzamonja G., Tank J., Heusser K., Palada I., Valic Z., Bakovic D., et al., Glossopharyngeal insufflation induces cardioinhibitory syncope in apnea divers, Clin. Auton. Res., 2010, 20, 381-384

[59] Hurford W.E., Hochachka P.W., Schneider R.C., Guyton G.P., Stanek K.S., Zapol D.G., et al., Splenic contraction, catecholamine release, and blood volume redistribution during diving in the Weddell seal, J. Appl. Physiol., 1996, 80, 298-306

[60] Laub M., Hvid-Jacobsen K., Hovind P., Kanstrup I.L., Christensen N.J., Nielsen S.L., Spleen emptying and venous hematocrit in humans during exercise, J. Appl. Physiol., 1993, 74, 1024-1026

[61] Bakovic D., Eterovic D., Saratlija-Novakovic Z., Palada I., Valic Z., Bilopavlovic N., et al., Effect of human splenic contraction on variation in circulating blood cell counts, Clin. Exp. Pharmacol. Physiol., 2005, 32, 944-951

[62] Aster R.H., Pooling of platelets in the spleen: role in the pathogenesis of "hypersplenic" thrombocytopenia, J. Clin. Invest., 1966, 45, 645-657

[63] Branehog I., Weinfeld A., Roos B., The exchangeable splenic platelet pool studied with epinephrine infusion in idiopathic thrombocytopenic purpura and in patients with splenomegaly, Br. J. Haematol., 1973, 25, 239-248

[64] Schmidt K.G., Rasmussen J.W., Are young platelets released in excess from the spleen in response to short-term physical exercise?, Scand. J. Haematol., 1984, 32, 207-214

[65] Chamberlain K.G., Tong M., Penington D.G., Properties of the exchangeable splenic platelets released into the circulation during exercise-induced thrombocytosis, Am. J. Hematol., 1990, 34, 161-168

[66] van der Loo B., Martin J.F., A role for changes in platelet production in the cause of acute coronary syndromes, Arterioscler. Thromb. Vasc. Biol., 1999, 19, 672-679

[67] Ojiri Y., Noguchi K., Shiroma N., Matsuzaki T., Sakanashi M., Sakanashi M., Uneven changes in circulating blood cell counts with adrenergic stimulation to the canine spleen, Clin. Exp. Pharmacol. Physiol., 2002, 29, 53-59

[68] Kjeldsen S.E., Weder A.B., Egan B., Neubig R., Zweifler A.J., Julius S., Effect of circulating epinephrine on platelet function and hematocrit, Hypertension, 1995, 25, 1096-1105

[69] Sloand J.A., Hooper M., Izzo J.L.Jr., Effects of circulating norepinephrine on platelet, leukocyte and red blood cell counts by alpha 1-adrenergic stimulation, Am. J. Cardiol., 1989, 63, 1140-1142

[70] Wadenvik H., Kutti J., The effect of an adrenaline infusion on the splenic blood flow and intrasplenic platelet kinetics, Br. J. Haematol., 1987, 67, 187-192

[71] Bakovic D., Eterovic D., Palada I., Valic Z., Dujic Z., Does breath-holding increase the risk of a thrombotic event?, Platelets, 2008, 19, 314-315

[72] Butterworth R.J., Bath P.M., The relationship between mean platelet volume, stroke subtype and clinical outcome, Platelets, 1998, 9, 359-364

[73] Khandekar M.M., Khurana A.S., Deshmukh S.D., Kakrani A.L., Katdare A.D., Inamdar A.K., Platelet volume indices in patients with coronary artery disease and acute myocardial infarction: an Indian scenario, J. Clin. Pathol., 2006, 59, 146-149

[74] Greisenegger S., Endler G., Hsieh K., Tentschert S., Mannhalter C., Lalouschek W., Is elevated mean platelet volume associated with a worse outcome in patients with acute ischemic cerebrovascular events?, Stroke, 2004, 35, 1688-1691

[75] Bakovic D., Pivac N., Eterovic D., Breskovic T., Zubin P., Obad A., et al., The effects of low-dose epinephrine infusion on spleen size, central and hepatic circulation and circulating platelets, Clin. Physiol. Funct. Imaging, 2013, 33, 30-37

[76] Varol E., Ozturk O., Gonca T., Has M., Ozaydin M., Erdogan D., et al., Mean platelet volume is increased in patients with severe obstructive sleep apnea, Scand. J. Clin. Lab. Invest., 2010, 70, 497-502

[77] Parish J.M., Somers V.K., Obstructive sleep apnea and cardiovascular disease, Mayo Clin. Proc., 2004, 79, 1036-1046

[78] Kohli P., Balachandran J.S., Malhotra A., Obstructive sleep apnea and the risk for cardiovascular disease, Curr. Atheroscler. Rep., 2011, 13, 138-146

[79] Waradekar N.V., Sinoway L.I., Zwillich C.W., Leuenberger U.A., Influence of treatment on muscle sympathetic nerve activity in sleep apnea, Am. J. Respir. Crit. Care Med., 1996, 153, 1333-1338

[80] Sahota P., Vahidy F., Nguyen C., Bui T.T., Yang B., Parsha K., et al., Changes in spleen size in patients with acute ischemic stroke: a pilot observational study, Int. J. Stroke, 2013, 8, 60-67

[81] Dujic Z., Breskovic T., Ljubkovic M., Breath hold diving: in vivo model of the brain survival response in man?, Med. Hypotheses, 2011, 76, 737-740