Increased serum levels of the brain damage marker S100B after apnea in trained breath-hold divers: a study including respiratory and cardiovascular observations

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Andersson JP, Linér MH, Jönsson 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 107: 809–815, 2009. First published July 2, 2009; doi:10.1152/japplphysiol.91434.2008.—The concentration of the protein S100B in serum is used as a brain damage marker in various conditions. We wanted to investigate whether a voluntary, prolonged apnea in trained breath-hold divers resulted in an increase of S100B in serum. Nine trained breath-hold divers performed a protocol mimicking the procedures they use during breath-hold training and competition, including extensive preapneic hyperventilation and glossophrayngeal insufflation, in order to perform a maximum-duration apnea, i.e., “static apnea” (average: 335 s, range: 281–403 s). Arterial blood samples were collected and cardiovascular variables recorded. Arterial partial pressures of O2 and CO2 (PaO2 and PaCO2) were 128 Torr and 20 Torr, respectively, at the start of apnea. The degree of asphyxia at the end of apnea was considerable, with PaO2 and PaCO2 reaching 28 Torr and 45 Torr, respectively. The concentration of S100B in serum transiently increased from 0.066 μg/l at the start of apnea to 0.083 μg/l after the apnea (P < 0.05). The increase in S100B is attributed to the asphyxia or to other physiological responses to apnea, for example, increased blood pressure, and probably indicates a temporary opening of the blood-brain barrier. It is not possible to conclude that the observed increase in S100B levels in serum after a maximal-duration apnea reflects a serious injury to the brain, although the results raise concerns considering negative long-term effects. At the least, the results indicate that prolonged, voluntary apnea affects the integrity of the central nervous system and do not preclude cumulative effects.

hemoglobin; hypoxia; ionized calcium; lactate; glucose

In recent years, breath-hold diving as a competitive sport has developed tremendously (1, 27). In different categories, divers are competing for maximum underwater time, distance, or depth. Elite breath-hold divers can perform “static apnea,” i.e., maximal-duration apnea during rest at the surface of a pool, of astonishing duration. The current world record is 10 min 12 s (1), and during international apnea competitions breath hold times of 4–7 min are common in the static apnea event (13). These apneas are performed after hyperventilation and are known to be associated with considerable hypoxia. As a consequence of hypoxia, symptoms and signs can range from relative minor “loss of motor control” to complete “loss of consciousness” during both competitions and training sessions (13, 23). In six international apnea competitions between 1998 and 2004, the frequency of static apnea performances being disqualified because of loss of motor control or loss of consciousness was 10% (23). Whether such hypoxic episodes are associated with a risk for brain damage in these athletes remains to be established. Studying the changes in established biochemical markers of brain damage after such performances offers the possibility to address this question.

S100 is a family of acidic, calcium-binding proteins, first purified from bovine brain (30). The relatively brain-specific, glia-derived protein S100B has several intra- and extracellular functions (8). The concentration of S100B in serum increases after many types of brain damage, and it is used as a serum marker of, for example, cerebral ischemia and brain damage (17, 44). There is a correlation between the severity of ischemic lesions and serum levels of S100B after 24 h or more (44). In addition to a late release, an early release has been observed. This probably reflects a release of extracellular S100B from the brain tissue to the blood in situations associated with a disruption of the blood-brain barrier (20, 51). Although the usefulness of changes in serum S100B as a brain damage marker in the late phase has been studied extensively, the early release immediately after an event with global hypoxia has only been investigated to a limited extent (5).

The possibility that maximal-duration apnea results in a release of S100B from the brain to the blood has not been studied previously. Therefore, we wanted to investigate whether a voluntary, prolonged apnea in trained breath-hold divers resulted in an increase of S100B in serum. It could be argued that an increase in S100B in serum may indicate that brain damage is a feasible consequence of maximal-duration apneas. We hypothesized that maximal-duration apneas leading to profound asphyxia would be associated with increased S100B levels in serum in trained breath-hold divers. In addition to measuring the S100B levels, we also collected samples for arterial blood gas analysis and recorded other cardiovascular and respiratory variables in the trained breath-hold divers to be able to further elucidate the responses to prolonged apnea in this group of athletes.

METHODS

The study was conducted in accordance with the Declaration of Helsinki and was approved by the research ethics committee at Lund University. After receiving a description of the procedures and an explanation of potential risks involved, all subjects gave their informed consent.

Subjects. Two groups of healthy subjects volunteered for the study (Table 1): nine trained breath-hold divers and six control subjects. The trained breath-hold divers (8 men/1 woman) were recruited among competitive breath-hold divers. All except one had participated in...
international breath-hold diving competitions, and five of the divers were at the time of the investigations current or previous national record holders in different breath-hold diving categories. The control subjects (5 men/1 woman) had limited experience of breath-hold diving.

**Experimental protocol for trained breath-hold divers.** After vital capacity had been measured in the standing position, the subject assumed a supine position on a mattress and stayed in this position for the remainder of the experiment. Vital capacity was measured also in this position. After local infiltration with <1 ml of lidocaine (Xylocaine, 10 mg/ml, Astra, Södertälje, Sweden), an arterial catheter was inserted in the radial artery at the left wrist. Thereafter, the probes of the instruments were attached. When stable cardiovascular data were observed, recordings of cardiovascular and respiratory variables began, at least 25 min after the subject assumed the horizontal position. After two more minutes of rest, the subject began preparing according to a manner of his/her own choice, in order to be able to perform a maximum-duration apnea (corresponding to the competitive category “static apnea,” with the exception that this protocol was conducted dry, i.e., without whole body immersion). Subjects were instructed to use preparations mimicking their normal training and competition routines. In all subjects except one, this included performing two or three “warm-up apneas” of submaximal duration (average: 162 s). All subjects hyperventilated before apnea by controlling their breathing. Glossopharyngeal insufflation (25, 35), also known as, for example, “lung packing” and “buccal pumping,” was used by seven of the nine trained breath-hold divers to increase the lung volume at the start of apnea (Table 1).

During the last expiration before the maximum-duration apnea, the subject exhaled deeply through an open-circuit spirometry mouthpiece and inhaled ambient air. The apneas were terminated at the subject’s own decision with a maximal exhalation through the mouthpiece. This allowed the measurement of end-tidal partial pressures of O2 and CO2 (PETO2 and PETCO2) at the start and end of apnea. Arterial blood samples for determination of serum levels of S100B were collected at the beginning of recordings (baseline), at the start of the maximal-duration apnea, at the end of apnea, and at fixed intervals up to 120 min after apnea (5, 10, 15, 30, 60, and 120 min after apnea). Blood samples for arterial blood gas analysis were collected at baseline, during the last expiration before the start of the maximal-duration apnea, 3 min into apnea, during the expiration ending the apnea, and 0.5 and 120 min after apnea.

**Experimental protocol for control subjects.** The control subjects did not perform any apneas and instead just rested in the supine position for 2.5–3 h. Blood samples for determination of serum levels of S100B and arterial blood gases were collected at times corresponding to the sampling times for the trained breath-hold divers.

**Results.**

**Breathing-hold times.** The average time achieved by the nine breath-hold divers during the maximal-duration apneas was 335 s (SD 38), with a range from 281 to 403 s. Only one diver had a breathing-hold time shorter than 5 min, and three of the divers held their breath for >6 min.

**S100B.** As shown in Fig. 1, the concentration of S100B in serum transiently increased after the maximal-duration apneas in the breath-hold divers. An increase in S100B after apnea was observed in seven of the nine divers. However, not all these divers had the peak right at the end of apnea as indicated by the group mean shown in Fig. 1. Instead, the peak in S100B was observed 5 min after apnea in one diver and 10 min after apnea in three divers. For all nine divers, the average change in S100B concentration within the first 10 min after the end of the maximal-duration apnea was 37% compared with the S100B concentration at the start of apnea (Fig. 2). The individual maximal change ranged from −17% to 167%. Within 120 min, the S100B concentration was back to preapnea levels in the breath-hold divers (Fig. 1). It should be noted that in resting control subjects the concentration of S100B in serum never increased above the baseline level (Fig. 1).

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Table 1. **Subject characteristics**

<table>
<thead>
<tr>
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<th>Trained Breath-Hold Divers (n = 9)</th>
<th>Control Subjects (n = 6)</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>31 (7) 21–39</td>
<td>26 (4) 21–32</td>
</tr>
<tr>
<td>Height, cm</td>
<td>180 (6) 171–188</td>
<td>183 (4) 176–187</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>75 (9) 60–87</td>
<td>78 (7) 68–90</td>
</tr>
<tr>
<td>VC, standing, liters</td>
<td>6.5 (0.9) 5.1–8.2</td>
<td>5.6 (0.9) 4.4–6.8</td>
</tr>
<tr>
<td>VC, supine, liters</td>
<td>6.1 (1.0)</td>
<td>5.1 (0.8)</td>
</tr>
<tr>
<td>VC+GI, supine, liters</td>
<td>7.2 (1.3)</td>
<td>4.4–7.7</td>
</tr>
<tr>
<td></td>
<td>6.0–9.1</td>
<td>4.1–6.1</td>
</tr>
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Values are means (SD) and ranges. VC, vital capacity; GI, glossopharyngeal insufflation. VC+GI values are from 7 subjects.
Arterial blood samples. Many of the variables analyzed in the arterial blood samples were affected by preapneic hyperventilation and the maximal-duration apnea (Fig. 3). PaO2 was increased to 128 Torr (SD 9) at the start of apnea and fell to 28 Torr (SD 4) at the end of apnea, while the corresponding values for SArO, were 100% (SD 1) and 54% (SD 9), respectively. PaCO2 was reduced to 20 Torr (SD 2) at the start of apnea, and during the apnea it increased to 45 Torr (SD 4). pH decreased from 7.64 (SD 0.04) to 7.40 (SD 0.03) during the apnea. The ionized calcium concentration at the start of apnea was 1.13 mmol/l (SD 0.03), and at the end of apnea it had increased to 1.23 mmol/l (SD 0.03). Four of the divers reported that they experienced symptoms, i.e., paresthesia, as a consequence of the hyperventilation before the start of the maximal-duration apnea.

Hb concentration increased compared with baseline during the maximal-duration apnea, with a peak value of 154 g/l at the end of apnea, an increase by 8% compared with baseline. Lactate concentration was not changed at the end of the apnea compared with at the start of apnea but was higher than at baseline. An even higher lactate concentration was observed 30 s after the end of apnea, when it reached 1.3 mmol/l (SD 0.2). Glucose concentration was higher in samples collected after the preapneic preparations and the maximal-duration apnea than at baseline, while at 120 min after apnea the glucose concentration was not changed compared with baseline.

End-tidal PO2 and PCO2. PETCO2 decreased from 132 Torr (SD 5) in the last expiration before apnea to 29 Torr (SD 5) in the expiration ending apnea. PETCO2 increased from the preapneic 22 Torr (SD 2) to 45 Torr (SD 4) at the end of apnea.

SPO2, mean arterial pressure, and heart rate. The pulse oximeter signal was lost after 20 s of apnea in one of the divers, and therefore the continuous SPO2 recording shown in Fig. 4 is based on eight subjects. Also, during the last 30 s of apnea and the early recovery period, we experienced additional problems with the pulse oximeter measurements in several of the other divers, presumably because of low SPO2 values. Around the end of apnea, the pulse oximeter signal was gradually lost in a total of six of the divers. Therefore, the nadir in Fig. 4 is based on recordings in four divers, and the period 10–30 s into recovery represents the average from three of the divers. However, the data from the beginning of apnea until the 250-s mark are averages from eight divers.

SPO2 was 99% (SD 1) during the control period (90–30 s before apnea) and had not changed compared with control at 120 s into apnea [97% (SD 2); Fig. 4]. At 240 s into apnea, SPO2 was reduced to 83% (SD 4). Because of the recording failures described above, no statistical analysis of SPO2 was performed for the last 20 s of apnea and the nadir. Mean arterial pressure was 101 mmHg (SD 15) during the control period. At 120 s it had not changed compared with control, being 104 mmHg (SD 21), whereas at 240 s and the last 20 s of apnea mean arterial pressure had increased to 126 mmHg (SD 22) and 143 mmHg (SD 26), respectively (Fig. 4). The heart rate was 75 beats per minute (bpm) (SD 15) during the control, and it was unchanged at 120 s and 240 s into apnea, 76 bpm (SD 15) and 69 bpm (SD 9), respectively (Fig. 4). During the last 20 s of apnea, the heart rate had been reduced to 57 bpm (SD 12).

DISCUSSION

In this study we examined the changes in serum levels of the brain damage marker S100B after apnea in trained breath-hold divers. The most important finding was that in the early recovery period after a maximal-duration apnea, i.e., within 10 min after apneas with an average duration longer than 5.5 min, the concentration of S100B was increased compared with before the apnea. In addition, the changes in, for example, arterial blood gases expand previous knowledge from end-tidal gases (24, 35) concerning the degrees of preapneic hyperventilation and postapneic asphyxia to which trained breath-hold divers can be subjected.

S100B. We attribute the observed increase in S100B to the asphyxia that developed during apnea or to other physiological responses to apnea, for example, the increased blood pressure. The precise mechanism(s) behind the increase in S100B is not established and could involve both neuronal damage and a temporary opening of the blood-brain barrier. The quick and transient nature of the increase probably indicates that it is primarily an opening of the blood-brain barrier that is involved, allowing S100B from the extracellular fluid of the brain to
escape into the circulation (29). Of concern regarding conclusions from the observations is the variability in S100B values, even in the control subjects. Nevertheless, it should be noted that the concentration of S100B never increased above the baseline level in the control subjects, whereas the divers had a peak in the early recovery period after the apnea, supporting the conclusion that there were effects from the apnea on the S100B concentration.

The clinical significance of the increase in S100B under the conditions of the present study is uncertain. First, even though the increase may indicate that the integrity of the central nervous system (e.g., the blood-brain barrier) was affected, it does not reveal the risk for neuronal damage. Second, the S100B levels in the present study are well below those reported after, for example, ischemic stroke and hypoxic brain damage after cardiac arrest (5, 43, 44). S100B can increase by several hundred percent in patients affected by such conditions. However, it should be taken into consideration that many repetitive exposures to severe hypoxia such as that experienced by individuals training and competing in static apnea, each episode not being severe enough to cause any acute noticeable effects, possibly could accumulate damage. In this context, it is...
worth noting that hypoxia due to obstructive sleep apnea can result in neuropathological changes and neuropsychological impairments such as impairments in attention, short-term memory, and general intellectual functioning (14). Furthermore, Potkin and Uszler (40) performed brain imaging on five asymptomatic elite breath-hold divers. While magnetic resonance images were normal, abnormal single photon emission computed tomographic scans were observed in all five divers, reflecting brain function abnormalities. On the other hand, Ridgway and McFarland (42) tested 21 elite apnea divers with a breath-hold diving history of 1–20 yr, using standard neuro-psychological tests with known sensitivity to mild brain insults. The apnea divers performed tasks within the average range compared with norms. These to some extent contrasting results reveal that the risk for hypoxic brain damage in competitive apnea divers needs further evaluation. In addition, it should be noted that none of the divers in the present study suffered a loss of consciousness at the end of the apnea, and it is therefore uncertain whether or not such an episode is associated with a larger increase in S100B.

A comparison with other situations associated with elevated levels of S100B is of interest here. Obstructive sleep apnea patients have been reported to have higher morning levels of S100B than control subjects (6). However, in another study on sleep apnea patients, S100B in serum of blood samples collected in the morning was unchanged compared with samples collected in the evening before overnight polysomnography (18). Various types of sports have also been investigated with respect to their effect on serum S100B. Boxing, headings in soccer, and running have been shown to increase S100B (34, 50), showing the effect of direct head trauma and accelerations/decelerations of the body without head trauma on the release of S100B. Also, cycling in a warm environment (51) and long-distance swimming (7) are reported to have caused increased serum S100B levels, indicating changes in the blood-brain barrier function under these conditions (51). In fact, it has been suggested that the permeability of the blood-brain barrier can be increased in a variety of conditions characterized by physiological or psychological stress (16, 45, 51). The magnitude of changes in S100B reported in the above-mentioned studies is comparable to that observed in the present study, in contrast to the much larger changes associated with, for example, ischemic stroke and hypoxic brain damage after cardiac arrest (5, 43, 44).

**Preapneic hyperventilation.** Monitoring the divers during the experiments as well as both arterial and end-tidal PO2 and PCO2 revealed that the divers hyperventilated extensively before apneas, in accordance with previous studies on trained breath-hold divers (12, 24, 32). This preapneic hyperventilation markedly increased arterial pH and decreased ionized calcium concentration compared with baseline. To our knowledge, no previous study has shown the extent of changes in these variables induced by the type of hyperventilation performed by competitive breath-hold divers before apnea attempts. Only part of the calcium in blood plasma is ionized, the remainder being bound mainly to protein and to a lesser degree to carbonate and bicarbonate. Plasma pH regulates the ratio of ionized to bound fractions, and the respiratory alkalosis associated with hyperventilation causes the observed reduction in plasma ionized calcium concentration by increasing the bound fraction at the expense of the ionized fraction (10). The observed average ionized calcium concentration at the start of apnea (1.13 mmol/l) reached a hypocalcemic level. In this context it is interesting that four of the divers reported that they experienced paresthesia during the hyperventilation. This can most likely be attributed to the change in ionized calcium concentration alone or in combination with the increase in pH (28, 49), which increases the excitability of sensory neurons. Paresthesias are probably unrelated to any concomitant decrease of cerebral blood flow caused by hypocapnia (49).

**End-apneic asphyxia.** There is a limited amount of data revealing the degree of asphyxia experienced by competitive breath-hold divers during static apneas. Overgaard et al. (35) studied divers using glossohyparyngeal insufflation when performing dry static apneas preceded by hyperventilation for 2 min. The average duration of apnea was 346 s, i.e., both the protocol and apneic duration were similar to the present study. The trained breath-hold divers in the study by Overgaard et al. had end-apneic values of PETO2 and PETCO2 close to those of the present study, i.e., PETO2 fell to 26 Torr and PETCO2 increased to 49 Torr (35). Lindholm and Lundgren (24) measured PETO2 and PETCO2 before and at the end of static apneas of an average duration nearly 1 min shorter than the duration of the apneas in the present study. Four of the seven divers had at least one episode of loss of motor control during the experiments, with PETO2 reported to be as low as 20 mmHg, while PETCO2 levels were hypocapnic or normocapnic at the end of the apneas (24). It was not explicitly stated whether or not the divers in the study by Lindholm and Lundgren used glossohyparyngeal insufflation to increase the volume of air in the lungs before beginning the apnea, whereas seven of our divers used this technique. This and other differences in the experimental protocols may explain why the averages for PETO2 and PETCO2 are comparable even though the breath-holding times were longer in the present study. In any case, the arterial and end-tidal PO2 and PCO2 at the end of apnea in the present study reveal that the divers approached the degree of asphyxia reported to be associated with a loss of motor control (24). It should be noted that because of the extensive preapneic hyperventilation, PaCO2 at the end of apnea (45 Torr) was only...
slightly above the baseline value (39 Torr). Observations of small increases in P\textsubscript{CO\textsubscript{2}} have been used as support for the notion that trained breath-hold divers rely on the hypoxic ventilatory drive as a cue to end apneas (24, 35). In fact, the old notion that hypercapnia is the most important factor generating “air hunger” is challenged by observations that the hypoxic ventilatory drive predicts breath-hold duration (11) and that hypoxia can generate “air hunger” equivalent to that generated by hypercapnia (31). In addition to the possible importance of the hypoxic ventilatory drive, some competitive breath-hold divers use self-checks of cerebral function toward the end of breath-holds to avoid an impending loss of consciousness (personal communications; Ref. 4).

**Hb, lactate, and glucose concentrations.** The observed increase in Hb concentration is in accordance with earlier studies ascribing similar increases to an apnea-induced splenic contraction and consequent release of stored erythrocytes (3, 37, 46, 48). However, there has been no previous study reporting as great an increase in Hb concentration after apnea as that in the present study. The apnea-induced increases in Hb concentration previously reported have typically ranged between 2% and 4% (9, 46–48), whereas we observed an increase by 8%. This could be related to the fact that the apneas of the present study were performed according to a protocol mimicking the competitive and training procedures of these divers (e.g., extensive preapneic hyperventilation, glossoopharyngeal inflation, and long duration of apnea), whereas apneas in the previous studies have been of a less extreme type. Our observation could also lend support for the notion that splenic contraction and release of erythrocytes can be augmented by apnea training (41).

The increase in lactate concentration could indicate that peripheral tissues were deprived of oxygen during apnea, increasing anaerobic metabolism (12). The fact that the peak in lactate concentration was observed in the blood sample collected 30 s after the end of apnea could reflect that the peripheral tissues were affected by vasoconstriction as part of the diving response, delaying the release of lactate into the general circulation. In addition, we observed increased glucose concentrations in association with the preparations and the maximal-duration apnea. The cause of the higher glucose concentrations is uncertain but may relate to an increased sympathetic activity during the experimental protocol, affecting glucose homeostasis directly or the secretion of, for example, insulin and glucagon (15). In fact, intermittent hypoxia may impair insulin sensitivity, glucose effectiveness, and insulin secretion (26).

**Cardiovascular changes.** Cardiovascular responses to apnea have been studied extensively in both novice and experienced subjects. The diving response is usually characterized by a bradycardia developing during the initial 30 s of apnea, a reduction in cardiac output, and a marked increase in blood pressure due to peripheral vasoconstriction (2, 36, 38). However, the pattern of changes observed in the present study differs to some extent from these normal responses. Probably, this can be attributed to the use of glossoopharyngeal inflation that markedly increases the intrathoracic pressure (35), thereby impeding venous return and consequently reducing stroke volume, cardiac output, and arterial pressure (33, 39). Most likely to compensate for a dramatic reduction in stroke volume, the bradycardic response is attenuated. This is in accordance with studies showing differences in bradycardic responses during apnea ascribed to differences in cardiac filling (2, 19). Concurrently, blood pressure did not increase markedly during the initial 3 min of apnea. The increase in blood pressure and reduction in heart rate coincided with the beginning of fall in \( \text{SaO}_2 \). Hypoxia is known to augment both apnea-induced peripheral vasoconstriction (21) and bradycardia (22). Therefore, the observed changes toward the end of apnea probably reflect an increasing influence of the arterial chemoreceptors on the cardiovascular responses. Palada et al. (36) and Perini et al. (38) observed similar readjustments coinciding with the fall in \( \text{SaO}_2 \) during prolonged apneas without glossoopharyngeal inflation.

In conclusion, it should be stated that it is not possible to establish that the observed increase in S100B levels in serum after a maximal-duration apnea reflects a serious injury to the brain, although the results raise concerns considering negative, cumulative long-term effects. At the least, the results indicate that prolonged, voluntary apnea affects the integrity of the central nervous system. A long-term follow-up study on individuals at the beginning of their careers as competitive breath-hold divers and after some years of apnea diving would be of great interest in clarifying these issues.

**ACKNOWLEDGMENTS**

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**GRANTS**

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