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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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ABSTRACT

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 pre-apneic hyperventilation and glossopharyngeal 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. The arterial partial pressures of O_2 and CO_2 (Pa_{O_2} and Pa_{CO_2}) were 128 Torr and 20 Torr, respectively, at the start of apnea. The degree of asphyxia at the end of apnea was considerable, with Pa_{O_2} and Pa_{CO_2} reaching 28 Torr and 45 Torr, respectively. The concentration of S100B in serum transiently increased from 0.066 $\mu g*L^{-1}$ at the start of apnea to 0.083 $\mu g*L^{-1}$ 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 least, the results indicate that prolonged, voluntary apnea affects the integrity of the central nervous system, and do not preclude cumulative effects.

KEY WORDS

Hemoglobin, hypoxia, ionized calcium, lactate, glucose

INTRODUCTION

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 sec (1), and during international apnea competitions breath-hold times of 4 to 7 minutes 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 due to 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, glial-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 e.g. cerebral ischemia and brain damage (19, 44). There is a correlation between the severity of ischemic lesions and serum levels of S100B

after 24 hours or more (44). In addition to this 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 (6).

The possibility that a 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); 9 trained breath-hold divers and 6 control subjects. The trained breath-hold divers (8 males/1 female) 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 investigations current or previous national record holders in different breath-hold diving categories. The control subjects (5 males/1 female) had limited experience of breath-hold diving.

Experimental protocol for trained breath-hold divers. After the 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. The vital capacity was measured also in this position. Following local infiltration with less than 1 ml of lidocaine (Xylocain®, 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 assuming 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). They were instructed to use preparations mimicking their normal training and competition routines. In all subjects except one, this included performing 2 or 3 “warm-up apneas” of sub-maximal duration (average: 162 s). All subjects

hyperventilated before apnea by controlling their breathing. Glossopharyngeal insufflation (25, 35), also known as e.g. “lung packing” and “buccal pumping”, was used by 7 of the 9 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 O_2 and CO_2 (PET_{O_2} and PET_{CO_2}) 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 post-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 hours. 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.

Measurements. Before the test, an electrocardiogram (lead II) was recorded and checked for anomalies (Cardisuny 501, Fukuda ME Kogoyo Co., Tokyo, Japan), and the vital capacity was measured with a spirometer (Micro Plus,

Micro Medical Ltd., Rochester, England). Blood samples for analysis of S100B were kept on ice, centrifuged, frozen, and later analyzed using a monoclonal two-site immunoradiometric assay (Sangtec 100, AB Sangtec Medical, Bromma, Sweden). Blood samples for blood gas determinations were kept on ice until analyzed within 15 min (Radiometer ABL725, Radiometer, Copenhagen, Denmark). Besides arterial partial pressures of O_2 and CO_2 (Pa_{O_2} and Pa_{CO_2}), the pH and arterial hemoglobin O_2 saturation (Sa_{O_2}), as well as the concentrations of Ca^{2+}, hemoglobin (Hb), and plasma lactate and glucose were analyzed. PET_{O_2} and PET_{CO_2} were recorded with an open-circuit spirometry system (CPX/D Cardiopulmonary Exercise System, Medical Graphics, Minneapolis, MN). Temperature, barometric pressure, and humidity, used for calibration of the system against gases of known mixtures, were measured in the laboratory just prior to each experimental session. With a pulse oximeter, non-invasive arterial hemoglobin O_2 saturation (Sp_{O_2}) was recorded with an ear lobe probe (Biox 3700e, Ohmeda, Madison, WI). Heart rate and arterial blood pressure were recorded with a photoplethysmometer (Finapres 2300, Ohmeda, Madison, WI) with the cuff on the right middle finger. The hand was positioned at the same level relative to the heart throughout the entire experiment.

Data analysis. Standard descriptive analysis was performed, and data are expressed as means (SD) for the nine trained breath-hold divers and six resting control subjects, respectively, unless stated otherwise. The Sp_{O_2}, mean arterial pressure, and heart rate were analyzed as the time-averaged values during the following periods: 90-30 s before the maximal-duration apnea (control), 110-130 s into apnea (120 s), 230-250 s into apnea (240 s), and the last 20 s of the maximal-duration apnea. Comparisons were made by one-way analysis of

variance. In the event of a significant *F* ratio, Fisher's protected least significant differences (PLSD) post hoc analysis was used to identify pairwise differences. In addition, the maximum concentration of S100B in all subjects within the first 10 min after apnea was compared to the concentration at the start of the maximum-duration apnea, and the $P_{ET_{O_2}}$ and $P_{ET_{CO_2}}$ at the start and end of apnea were compared using a paired Student's t test. $P < 0.05$ was considered statistically significant.

RESULTS

Breath-holding times. The average time achieved by the 9 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 breath-holding time shorter than 5 min, and three of the divers held their breath for more than 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 to 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 pre-apneic 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).

Arterial blood samples. Many of the variables analyzed in the arterial blood samples were affected by pre-apneic hyperventilation and the maximal-duration apnea (Fig. 3). The Pa_{O_2} 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 Sa_{O_2} was 100% (SD 1) and 54% (SD 9), respectively. The Pa_{CO_2} was reduced to 20 Torr (SD 2) at the start of apnea, and during the apnea it increased to 45 Torr (SD 4). The 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^{-1} (SD 0.03), and at the end of apnea it had increased to 1.23 mmol*L^{-1} (SD 0.03). Four of the divers reported that they experienced symptoms, i.e. paresthesia, as a consequence of the hyperventilation prior to the start of the maximal-duration apnea.

The Hb concentration increased compared to baseline during the maximal-duration apnea, with a peak value of 154 g*L^{-1} at the end of apnea, an increase by 8% compared to baseline. The lactate concentration was not changed at the end of the apnea compared to at the start of apnea but 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^{-1} (SD 0.2). The glucose concentration was higher in samples collected after the pre-apneic preparations and the maximal-duration apnea than at baseline, while at 120 min after apnea the glucose concentration was not changed compared to baseline.

End-tidal P_{O_2} and P_{CO_2}. The end-tidal partial pressure of O_2 decreased from 132 Torr (SD 5) in the last expiration before apnea to 29 Torr (SD 5) in the

228 expiration ending apnea. The end-tidal partial pressure of CO_2 increased from
229 the pre-apneic 22 Torr (SD 2) to 45 Torr (SD 4) at the end of apnea.

230 *Sp_{O_2}, mean arterial pressure, and heart rate*. The pulse oximeter signal was
231 lost after 20 s of apnea in one of the divers, and therefore the continuous SpO_2
232 recording shown in Fig. 4 is based on 8 subjects. Also, during the last 30 s of
233 apnea and the early recovery period, we experienced additional problems with
234 the pulse oximeter measurements in several of the other divers, presumably
235 because of low SpO_2 values. Around the end of apnea, the pulse oximeter signal
236 was gradually lost in a total of 6 of the divers. Therefore, the nadir in Fig. 4 is
237 based on recordings in 4 divers, and the period 10-30 s into recovery represents
238 the average from 3 of the divers. However, the data from the beginning of apnea
239 until the 250-s mark are averages from 8 divers.

240 The Sp_{O_2} was 99% (SD 1) during the control period (90-30 s before apnea)
241 and had not changed compared to control at 120 s into apnea (97% (SD 2); Fig.
242 4). At 240 s into apnea, the Sp_{O_2} was reduced to 83% (SD 4). Due to the
243 recordings failures described above, no statistical analysis of Sp_{O_2} was
244 performed for the last 20 s of apnea and the nadir. The mean arterial pressure
245 was 101 mmHg (SD 15) during the control period. At 120 s it had not changed
246 compared to control, being 104 mmHg (SD 21), whereas at 240 s and the last 20
247 s of apnea the mean arterial pressure had increased to 126 mmHg (SD 22) and
248 143 mmHg (SD 26), respectively (Fig. 4). The heart rate was 75 bpm (SD 15)
249 during the control, and it was unchanged at 120 s and 240 s into apnea, 76 bpm
250 (SD 15) and 69 bpm (SD 9), respectively (Fig. 4). During the last 20 s of apnea,
251 the heart rate had been reduced to 57 bpm (SD 12).
252
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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 to before the apnea. In addition, the changes in e.g. arterial blood gases expand previous knowledge from end-tidal gases (24, 35) concerning the degrees of pre-apneic hyperventilation and post-apneic asphyxia that trained breath-hold divers can be subjected to.

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 (6, 43, 44). The 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 noticing 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 years using standard neuropsychological tests, with known sensitivity to mild brain insults. The apnea divers performed tasks within the average range compared to norms. These to some extent contrasting results enlighten 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 (5). However, in another study on sleep apnea patients, S100B in serum of blood samples collected in the morning was unchanged compared to samples collected in the evening before overnight polysomnography (17). Various types of sports have also been investigated with respect to their effect on serum S100B. Both 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 are comparable to that observed in the present study, in contrast to the much larger changes associated with e.g. ischemic stroke and hypoxic brain damage after cardiac arrest (6, 43, 44).

Pre-apneic hyperventilation. Monitoring the divers during the experiments as well as both the arterial and end-tidal P_{O_2} and P_{CO_2} revealed that the divers hyperventilated extensively before apneas, in accordance with previous studies on trained breath-hold divers (12, 24, 32). This pre-apneic hyperventilation markedly increased the arterial pH and decreased the ionized calcium

328 concentration compared to baseline. To our knowledge, no previous study has
329 shown the extent of changes in these variables induced by the type of
330 hyperventilation performed by competitive breath-hold divers before apnea
331 attempts. Only part of the calcium in blood plasma is ionized, the remainder
332 being bound mainly to protein and to a lesser degree to carbonate and
333 bicarbonate. Plasma pH regulates the ratio of ionized/bound fractions, and the
334 respiratory alkalosis associated with hyperventilation causes the observed
335 reduction in plasma ionized calcium concentration by increasing the bound
336 fraction at the expense of the ionized fraction (10). The observed average
337 ionized calcium concentration at the start of apnea (1.13 mmol*L^{-1}) reached a
338 hypocalcemic level. In this context it is interesting that four of the divers
339 reported that they experienced paresthesia during the hyperventilation. This can
340 most likely be attributed to the change in ionized calcium concentration alone or
341 in combination with the increase in pH (28, 49), which increases the excitability
342 of sensory neurons. Parestheisas are probably unrelated to any concomitant
343 decrease of cerebral blood flow caused by hypocapnia (49).

344 *End-apneic asphyxia*. There is a limited amount of data revealing the degree
345 of asphyxia experienced by competitive breath-hold divers during static apneas.
346 Overgaard et al. (35) studied divers using glossopharyngeal insufflation when
347 performing dry static apneas preceded by hyperventilation for 2 min. The
348 average duration of apnea was 346 s, i.e. both the protocol and apneic duration
349 was similar in the present study. The trained breath-hold divers in the study by
350 Overgaard et al. had end-apneic values of $P_{ET_{O_2}}$ and $P_{ET_{CO_2}}$ close to those of the
351 present study, i.e. the $P_{ET_{O_2}}$ fell to 26 Torr and the $P_{ET_{CO_2}}$ increased to 49 Torr
352 (35). Lindholm and Lundgren (24) measured end-tidal P_{O_2} and P_{CO_2} before and

353 at the end of static apneas of an average duration nearly 1 min shorter than the
354 duration of the apneas in the present study. Four of the seven divers had at least
355 one episode of loss of motor control during the experiments, with the PET_{O_2}
356 reported to be as low as 20 mmHg, while PET_{CO_2} levels were hypocapnic or
357 normocapnic at the end of apneas (24). It was not explicitly stated whether or
358 not the divers in the study by Lindholm and Lundgren used glossopharyngeal
359 insufflation to increase the volume of air in the lungs before beginning the
360 apnea, whereas seven of our divers used this technique. This and other
361 differences in the experimental protocols may explain why the averages for
362 PET_{O_2} and PET_{CO_2} are comparable even though the breath-holding times were
363 longer in the present study. In any case, the arterial and end-tidal P_{O_2} and P_{CO_2} at
364 the end of apnea in the present study reveal that the divers approached the
365 degree of asphyxia reported to be associated with a loss of motor control (24). It
366 should be noted that because of the extensive pre-apneic hyperventilation, the
367 Pa_{CO_2} at the end of apnea (45 Torr) was only slightly above the baseline value
368 (39 Torr). Observations of small increases in P_{CO_2} have been used as support for
369 the notion that trained breath-hold divers rely on the hypoxic ventilatory drive as
370 a cue to end apneas (24, 35). In fact, the old notion that hypercapnia is the most
371 important factor generating “air hunger” is challenged by observations that the
372 hypoxic ventilatory drive predicts breath-hold duration (11) and that hypoxia
373 can generate “air hunger” equivalent to that generated by hypercapnia (31). In
374 addition to the possible importance of the hypoxic ventilatory drive, some
375 competitive breath-hold divers use self-checks of cerebral function towards the
376 end of breath-holds to avoid an impending loss of consciousness (personal
377 communications, 4).

Hb, lactate and glucose concentrations. The observed increase in hemoglobin 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 increase in hemoglobin concentration after apnea as that in the present study. The apnea-induced increases in hemoglobin concentration previously reported has typically ranged between 2-4% (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 pre-apneic hyperventilation, glossopharyngeal insufflation, 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 the 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 a 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 the glucose homeostasis directly or the

secretion of e.g. insulin and glucagon (15). In fact, intermittent hypoxia may impair insulin sensitivity, glucose effectiveness, and insulin secretion (26).

Cardiovascular changes. The 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 glossopharyngeal insufflation that markedly increases the intrathoracic pressure (35), thereby impeding venous return and consequently reducing both the 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, 18). Concurrently, the 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 arterial oxygen saturation. Hypoxia is known to augment both apnea-induced peripheral vasoconstriction (21) and bradycardia (22). Therefore, the observed changes towards 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) have observed similar readjustments coinciding with the fall in arterial oxygen saturation during prolonged apneas without glossopharyngeal insufflation.

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 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 to clarify these issues.

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606

607 Table 1. *Subject characteristics.*

	Trained breath-hold divers (n = 9)	Control subjects (n = 6)
Age, yr	31 (7)	26 (4)
	21-39	21-32
Height, cm	180 (6)	183 (4)
	171-188	176-187
Weight, kg	75 (9)	78 (7)
	60-87	68-90
VC,	6.5 (0.9)	5.6 (0.9)
standing, liters	5.1-8.2	4.4-6.8
VC,	6.1 (1.0)	5.1 (0.8)
supine, liters	4.4-7.7	4.1-6.1
VC+GI,	7.2 (1.3)	
supine, liters	6.0-9.1	

608 Values are means (SD) and range. VC = Vital capacity; GI = glossopharyngeal
609 insufflation. VC+GI values are from 7 subjects.

610

611

612 **FIGURE LEGENDS**

613

614 Fig. 1. Concentration of S100B in serum, in percent of baseline, before apnea
615 and up to 120 min after the maximal-duration apnea. Values are means from the
616 9 breath-hold divers (filled circles; BHD) and 6 resting control subjects (open
617 squares; C). Bars indicating SD have been left out for sake of clarity. Vertical,
618 grey bars indicate the period for preparations and the maximal-duration apnea
619 performed by the breath-hold divers.

620

621 Fig. 2. Individual values (filled circles) and group mean (open circles; n = 9
622 breath-hold divers) for the concentration of S100B in serum at the start of the
623 apnea and the maximal value observed within the first 10 min after apnea. *P <
624 0.05 compared with Start (Student's t test).

625

626 Fig. 3. Arterial partial pressures of O_2 and CO_2 (Pa_{O_2} and Pa_{CO_2}), pH, Ca^{2+}
627 concentration ($[Ca^{2+}]$), arterial hemoglobin O_2 saturation (Sa_{O_2}), hemoglobin
628 concentration ([Hb]), lactate concentration ([La]), and glucose concentration
629 ([Glu]) at Baseline, Start of apnea, 3 min into apnea, End of apnea, and 0.5 and
630 120 min after apnea. Values are means with SD from the 9 breath-hold divers.
631 *P < 0.05 compared with Baseline, †P < 0.05 compared with Start, and ‡P <
632 0.05 compared with End (Fisher's PLSD).

633

634 Fig. 4. Mean arterial pressure (MAP), heart rate (HR), and arterial hemoglobin
635 O_2 saturation (Sp_{O_2}) in association with the maximal-duration apnea. Values are
636 means (MAP, HR: n = 9; Sp_{O_2}: n = 3-8) from before apnea (-60-0 s), during the

first 250 s of apnea (0-250 s), during the last 30 s of apnea (-30-0 s), and the first 60 s after apnea (0-60 s). For the number of divers included in the Sp_{O_2}-recordings, refer to the text (Results: *Sp_{O_2}, mean arterial pressure, and heart rate*). The breaks in the lines reflect the fact that breath-holding times varied among the divers. The vertical dashed lines indicate the start and end of apnea. *P < 0.05 compared with control, †P < 0.05 compared with 120 s (Fisher's PLSD).