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

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6 Johan P. A. Andersson¹, Mats H. Linér², and Henrik Jönsson³

7

8 ¹Department of Cell and Organism Biology, Lund University, Lund, Sweden

9 ²Department of Clinical Sciences, Section of Anesthesiology and Intensive Care,

10 Lund University, Lund, Sweden

11 ³Department of Clinical Sciences, Section of Thoracic Surgery, Lund

12 University, Lund, Sweden

13

14 Running head: Brain damage marker after apnea in breath-hold divers

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16 **Address for correspondence:**

17 Johan Andersson

18 Department of Cell and Organism Biology

19 Lund University

20 Helgonav. 3 B, SE-223 62 Lund

21 Sweden.

22 Phone: +46 46 222 93 48.

23 Fax: +46 46 222 45 39.

24 E-mail: johan.andersson@cob.lu.se

25

26 **ABSTRACT**

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28 The concentration of the protein S100B in serum is used as a brain damage
29 marker in various conditions. We wanted to investigate whether a voluntary,
30 prolonged apnea in trained breath-hold divers resulted in an increase of S100B
31 in serum. Nine trained breath-hold divers performed a protocol mimicking the
32 procedures they use during breath-hold training and competition, including
33 extensive pre-apneic hyperventilation and glossopharyngeal insufflation, in
34 order to perform a maximum-duration apnea, i.e. “static apnea” (average: 335 s,
35 range: 281-403 s). Arterial blood samples were collected and cardiovascular
36 variables recorded. The arterial partial pressures of O₂ and CO₂ (PaO₂ and PaCO₂)
37 were 128 Torr and 20 Torr, respectively, at the start of apnea. The degree of
38 asphyxia at the end of apnea was considerable, with PaO₂ and PaCO₂ reaching 28
39 Torr and 45 Torr, respectively. The concentration of S100B in serum transiently
40 increased from 0.066 μg*L⁻¹ at the start of apnea to 0.083 μg*L⁻¹ after the apnea
41 (P < 0.05). The increase in S100B is attributed to the asphyxia or to other
42 physiological responses to apnea, for example increased blood pressure, and
43 probably indicates a temporary opening of the blood-brain barrier. It is not
44 possible to conclude that the observed increase in S100B levels in serum after a
45 maximal-duration apnea reflects a serious injury to the brain, although the
46 results raise concerns considering negative long-term effects. At least, the results
47 indicate that prolonged, voluntary apnea affects the integrity of the central
48 nervous system, and do not preclude cumulative effects.

49

50 **KEY WORDS**

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52 Hemoglobin, hypoxia, ionized calcium, lactate, glucose

53

54 INTRODUCTION

55

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

73 S100 is a family of acidic, calcium-binding proteins, first purified from
74 bovine brain (30). The relatively brain specific, glial-derived protein S100B has
75 several intra- and extracellular functions (8). The concentration of S100B in
76 serum increases after many types of brain damage, and it is used as a serum
77 marker of e.g. cerebral ischemia and brain damage (19, 44). There is a
78 correlation between the severity of ischemic lesions and serum levels of S100B

79 after 24 hours or more (44). In addition to this late release, an early release has
80 been observed. This probably reflects a release of extracellular S100B from the
81 brain tissue to the blood in situations associated with a disruption of the blood-
82 brain barrier (20, 51). Although the usefulness of changes in serum S100B as a
83 brain-damage marker in the late phase has been studied extensively, the early
84 release immediately after an event with global hypoxia has only been
85 investigated to a limited extent (6).

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

98

99 **METHODS**

100

101 The study was conducted in accordance with the Declaration of Helsinki and
102 was approved by the research ethics committee at Lund University. After

103 receiving a description of the procedures and an explanation of potential risks
104 involved all subjects gave their informed consent.

105 *Subjects.* Two groups of healthy subjects volunteered for the study (Table 1);
106 9 trained breath-hold divers and 6 control subjects. The trained breath-hold
107 divers (8 males/1 female) were recruited among competitive breath-hold divers.
108 All except one had participated in international breath-hold diving competitions,
109 and five of the divers were at the time of investigations current or previous
110 national record holders in different breath-hold diving categories. The control
111 subjects (5 males/1 female) had limited experience of breath-hold diving.

112 *Experimental protocol for trained breath-hold divers.* After the vital
113 capacity had been measured in the standing position, the subject assumed a
114 supine position on a mattress and stayed in this position for the remainder of the
115 experiment. The vital capacity was measured also in this position. Following
116 local infiltration with less than 1 ml of lidocaine (Xylocain®, 10 mg/ml, Astra,
117 Södertälje, Sweden), an arterial catheter was inserted in the radial artery at the
118 left wrist. Thereafter, the probes of the instruments were attached. When stable
119 cardiovascular data were observed, recordings of cardiovascular and respiratory
120 variables began, at least 25 min after assuming the horizontal position. After two
121 more minutes of rest, the subject began preparing according to a manner of
122 his/her own choice, in order to be able to perform a maximum-duration apnea
123 (corresponding to the competitive category “static apnea”, with the exception
124 that this protocol was conducted dry, i.e. without whole-body immersion). They
125 were instructed to use preparations mimicking their normal training and
126 competition routines. In all subjects except one, this included performing 2 or 3
127 “warm-up apneas” of sub-maximal duration (average: 162 s). All subjects

128 hyperventilated before apnea by controlling their breathing. Glossopharyngeal
129 insufflation (25, 35), also known as e.g. “lung packing” and “buccal pumping”,
130 was used by 7 of the 9 trained breath-hold divers to increase the lung volume at
131 the start of apnea (Table 1).

132 During the last expiration before the maximum-duration apnea, the subject
133 exhaled deeply through an open-circuit spirometry mouthpiece and inhaled
134 ambient air. The apneas were terminated at the subject’s own decision with a
135 maximal exhalation through the mouthpiece. This allowed the measurement of
136 end-tidal partial pressures of O₂ and CO₂ (PET_{O₂} and PET_{CO₂}) at the start and end
137 of apnea.

138 Arterial blood samples for determination of serum levels of S100B were
139 collected at the beginning of recordings (baseline), at the start of the maximal-
140 duration apnea, at the end of apnea, and at fixed intervals up to 120 min after
141 apnea (5, 10, 15, 30, 60, and 120 min post-apnea). Blood samples for arterial
142 blood gas analysis were collected at baseline, during the last expiration before
143 the start of the maximal-duration apnea, 3 min into apnea, during the expiration
144 ending the apnea, and 0.5 and 120 min after apnea.

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

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

153 Micro Medical Ltd., Rochester, England). Blood samples for analysis of S100B
154 were kept on ice, centrifuged, frozen, and later analyzed using a monoclonal
155 two-site immunoradiometric assay (Sangtec 100, AB Sangtec Medical,
156 Bromma, Sweden). Blood samples for blood gas determinations were kept on
157 ice until analyzed within 15 min (Radiometer ABL725, Radiometer,
158 Copenhagen, Denmark). Besides arterial partial pressures of O₂ and CO₂ (P_{aO₂}
159 and P_{aCO₂}), the pH and arterial hemoglobin O₂ saturation (S_{aO₂}), as well as the
160 concentrations of Ca²⁺, hemoglobin (Hb), and plasma lactate and glucose were
161 analyzed. PET_{O₂} and PET_{CO₂} were recorded with an open-circuit spirometry
162 system (CPX/D Cardiopulmonary Exercise System, Medical Graphics,
163 Minneapolis, MN). Temperature, barometric pressure, and humidity, used for
164 calibration of the system against gases of known mixtures, were measured in the
165 laboratory just prior to each experimental session. With a pulse oximeter, non-
166 invasive arterial hemoglobin O₂ saturation (Sp_{O₂}) was recorded with an ear lobe
167 probe (Biox 3700e, Ohmeda, Madison, WI). Heart rate and arterial blood
168 pressure were recorded with a photoplethysmometer (Finapres 2300, Ohmeda,
169 Madison, WI) with the cuff on the right middle finger. The hand was positioned
170 at the same level relative to the heart throughout the entire experiment.

171 *Data analysis.* Standard descriptive analysis was performed, and data are
172 expressed as means (SD) for the nine trained breath-hold divers and six resting
173 control subjects, respectively, unless stated otherwise. The Sp_{O₂}, mean arterial
174 pressure, and heart rate were analyzed as the time-averaged values during the
175 following periods: 90-30 s before the maximal-duration apnea (control), 110-
176 130 s into apnea (120 s), 230-250 s into apnea (240 s), and the last 20 s of the
177 maximal-duration apnea. Comparisons were made by one-way analysis of

178 variance. In the event of a significant F ratio, Fisher's protected least significant
179 differences (PLSD) post hoc analysis was used to identify pairwise differences.
180 In addition, the maximum concentration of S100B in all subjects within the first
181 10 min after apnea was compared to the concentration at the start of the
182 maximum-duration apnea, and the PET_{O_2} and PET_{CO_2} at the start and end of
183 apnea were compared using a paired Student's t test. $P < 0.05$ was considered
184 statistically significant.

185

186 **RESULTS**

187

188 *Breath-holding times.* The average time achieved by the 9 breath-hold divers
189 during the maximal-duration apneas was 335 s (SD 38), with a range from 281
190 to 403 s. Only one diver had a breath-holding time shorter than 5 min, and three
191 of the divers held their breath for more than 6 min.

192 *S100B.* As shown in Fig. 1, the concentration of S100B in serum transiently
193 increased after the maximal-duration apneas in the breath-hold divers. An
194 increase in S100B after apnea was observed in seven of the nine divers.
195 However, not all these divers had the peak right at the end of apnea as indicated
196 by the group mean shown in Fig. 1. Instead the peak in S100B was observed 5
197 min after apnea in one diver and 10 min after apnea in three divers. For all nine
198 divers, the average change in S100B concentration within the first 10 min after
199 the end of the maximal-duration apnea was 37% compared to the S100B
200 concentration at the start of apnea (Fig. 2). The individual maximal change
201 ranged from -17% to 167%. Within 120 min, the S100B concentration was back
202 to pre-apneic levels in the breath-hold divers (Fig. 1). It should be noted that in

203 resting control subjects, the concentration of S100B in serum never increased
204 above the baseline level (Fig. 1).

205 *Arterial blood samples.* Many of the variables analyzed in the arterial blood
206 samples were affected by pre-apneic hyperventilation and the maximal-duration
207 apnea (Fig. 3). The P_{aO_2} was increased to 128 Torr (SD 9) at the start of apnea
208 and fell to 28 Torr (SD 4) at the end of apnea, while the corresponding values
209 for Sa_{O_2} was 100% (SD 1) and 54% (SD 9), respectively. The P_{aCO_2} was
210 reduced to 20 Torr (SD 2) at the start of apnea, and during the apnea it increased
211 to 45 Torr (SD 4). The pH decreased from 7.64 (SD 0.04) to 7.40 (SD 0.03)
212 during the apnea. The ionized calcium concentration at the start of apnea was
213 $1.13 \text{ mmol}\cdot\text{L}^{-1}$ (SD 0.03), and at the end of apnea it had increased to 1.23
214 $\text{mmol}\cdot\text{L}^{-1}$ (SD 0.03). Four of the divers reported that they experienced
215 symptoms, i.e. paresthesia, as a consequence of the hyperventilation prior to the
216 start of the maximal-duration apnea.

217 The Hb concentration increased compared to baseline during the maximal-
218 duration apnea, with a peak value of $154 \text{ g}\cdot\text{L}^{-1}$ at the end of apnea, an increase
219 by 8% compared to baseline. The lactate concentration was not changed at the
220 end of the apnea compared to at the start of apnea but higher than at baseline.
221 An even higher lactate concentration was observed 30 s after the end of apnea,
222 when it reached $1.3 \text{ mmol}\cdot\text{L}^{-1}$ (SD 0.2). The glucose concentration was higher
223 in samples collected after the pre-apneic preparations and the maximal-duration
224 apnea than at baseline, while at 120 min after apnea the glucose concentration
225 was not changed compared to baseline.

226 *End-tidal P_{O_2} and P_{CO_2} .* The end-tidal partial pressure of O_2 decreased from
227 132 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 CO₂ increased from
229 the pre-apneic 22 Torr (SD 2) to 45 Torr (SD 4) at the end of apnea.

230 *SpO₂, 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 SpO₂
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 SpO₂ 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 SpO₂ 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 SpO₂ was reduced to 83% (SD 4). Due to the
243 recordings failures described above, no statistical analysis of SpO₂ 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

253

254 **DISCUSSION**

255

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

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

278 The clinical significance of the increase in S100B under the conditions of the
279 present study is uncertain. First, even though the increase may indicate that the
280 integrity of the central nervous system (e.g., the blood-brain barrier) was
281 affected, it does not reveal the risk for neuronal damage. Second, the S100B
282 levels in the present study are well below those reported after, for example,
283 ischemic stroke and hypoxic brain damage after cardiac arrest (6, 43, 44). The
284 S100B can increase by several hundred percent in patients affected by such
285 conditions. However, it should be taken into consideration that many repetitive
286 exposures to severe hypoxia such as that experienced by individuals training and
287 competing in static apnea, each episode not being severe enough to cause any
288 acute noticeable effects, possibly could accumulate damage. In this context, it is
289 worth noticing that hypoxia due to obstructive sleep apnea can result in
290 neuropathological changes and neuropsychological impairments such as
291 impairments in attention, short-term memory, and general intellectual
292 functioning (14). Furthermore, Potkin and Uszler (40) performed brain imaging
293 on five asymptomatic elite breath-hold divers. While magnetic resonance images
294 were normal, abnormal single photon emission computed tomographic scans
295 were observed in all five divers, reflecting brain function abnormalities. On the
296 other hand, Ridgway and McFarland (42) tested 21 elite apnea divers with a
297 breath-hold diving history of 1-20 years using standard neuropsychological tests,
298 with known sensitivity to mild brain insults. The apnea divers performed tasks
299 within the average range compared to norms. These to some extent contrasting
300 results enlighten that the risk for hypoxic brain damage in competitive apnea
301 divers needs further evaluation. In addition, it should be noted that none of the
302 divers in the present study suffered a loss of consciousness at the end of the

303 apnea, and it is therefore uncertain whether or not such an episode is associated
304 with a larger increase in S100B.

305 A comparison with other situations associated with elevated levels of S100B
306 is of interest here. Obstructive sleep apnea patients have been reported to have
307 higher morning levels of S100B than control subjects (5). However, in another
308 study on sleep apnea patients, S100B in serum of blood samples collected in the
309 morning was unchanged compared to samples collected in the evening before
310 overnight polysomnography (17). Various types of sports have also been
311 investigated with respect to their effect on serum S100B. Both boxing, headings
312 in soccer, and running have been shown to increase S100B (34, 50), showing the
313 effect of direct head trauma and accelerations/decelerations of the body without
314 head trauma on the release of S100B. Also cycling in a warm environment (51)
315 and long-distance swimming (7) are reported to have caused increased serum
316 S100B levels, indicating changes in the blood-brain barrier function under these
317 conditions (51). In fact, it has been suggested that the permeability of the blood-
318 brain barrier can be increased in a variety of conditions characterized by
319 physiological or psychological stress (16, 45, 51). The magnitude of changes in
320 S100B reported in the above-mentioned studies are comparable to that observed
321 in the present study, in contrast to the much larger changes associated with e.g.
322 ischemic stroke and hypoxic brain damage after cardiac arrest (6, 43, 44).

323 *Pre-apneic hyperventilation.* Monitoring the divers during the experiments
324 as well as both the arterial and end-tidal P_{O_2} and P_{CO_2} revealed that the divers
325 hyperventilated extensively before apneas, in accordance with previous studies
326 on trained breath-hold divers (12, 24, 32). This pre-apneic hyperventilation
327 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 ($1.13 \text{ mmol}\cdot\text{L}^{-1}$) 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. Paresthesias 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 PET_{O_2} and PET_{CO_2} close to those of the
351 present study, i.e. the PET_{O_2} fell to 26 Torr and the PET_{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).

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

392 The increase in lactate concentration could indicate that peripheral tissues
393 were deprived of oxygen during apnea, increasing the anaerobic metabolism
394 (12). The fact that the peak in lactate concentration was observed in the blood
395 sample collected 30 s after the end of apnea could reflect that the peripheral
396 tissues were affected by vasoconstriction as a part of the diving response,
397 delaying the release of lactate into the general circulation. In addition, we
398 observed increased glucose concentrations in association with the preparations
399 and the maximal-duration apnea. The cause of the higher glucose concentrations
400 is uncertain, but may relate to an increased sympathetic activity during the
401 experimental protocol, affecting the glucose homeostasis directly or the

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

404 *Cardiovascular changes.* The cardiovascular responses to apnea have been
405 studied extensively in both novice and experienced subjects. The diving
406 response is usually characterized by a bradycardia developing during the initial
407 30 s of apnea, a reduction in cardiac output, and a marked increase in blood
408 pressure due to peripheral vasoconstriction (2, 36, 38). However, the pattern of
409 changes observed in the present study differs to some extent from these normal
410 responses. Probably, this can be attributed to the use of glossopharyngeal
411 insufflation that markedly increases the intrathoracic pressure (35), thereby
412 impeding venous return and consequently reducing both the stroke volume,
413 cardiac output, and arterial pressure (33, 39). Most likely to compensate for a
414 dramatic reduction in stroke volume, the bradycardic response is attenuated.
415 This is in accordance with studies showing differences in bradycardic responses
416 during apnea ascribed to differences in cardiac filling (2, 18). Concurrently, the
417 blood pressure did not increase markedly during the initial 3 min of apnea. The
418 increase in blood pressure and reduction in heart rate coincided with the
419 beginning of fall in arterial oxygen saturation. Hypoxia is known to augment
420 both apnea-induced peripheral vasoconstriction (21) and bradycardia (22).
421 Therefore, the observed changes towards the end of apnea probably reflect an
422 increasing influence of the arterial chemoreceptors on the cardiovascular
423 responses. Palada et al. (36) and Perini et al. (38) have observed similar
424 readjustments coinciding with the fall in arterial oxygen saturation during
425 prolonged apneas without glossopharyngeal insufflation.

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

434

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436

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606

607 Table 1. *Subject characteristics.*

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

637 first 250 s of apnea (0-250 s), during the last 30 s of apnea (-30-0 s), and the first
638 60 s after apnea (0-60 s). For the number of divers included in the SpO_2 -
639 recordings, refer to the text (Results: *SpO₂, mean arterial pressure, and heart*
640 *rate*). The breaks in the lines reflect the fact that breath-holding times varied
641 among the divers. The vertical dashed lines indicate the start and end of apnea.
642 *P < 0.05 compared with control, †P < 0.05 compared with 120 s (Fisher's
643 PLSD).

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