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Effects of two weeks of daily apnea training on diving response, spleen contraction, and erythropoiesis in novel subjects

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Three potentially protective responses to hypoxia have been reported to be enhanced in divers: (1) the diving response, (2) the blood-boosting spleen contraction, and (3) a long-term enhancement of hemoglobin concentration (Hb). Longitudinal studies, however, have been lacking except concerning the diving response. Ten untrained subjects followed a 2-week training program with 10 maximal effort apneas per day, with pre- and posttraining measurements during three maximal duration apneas, and an additional post-training series when the apneic duration was kept identical to that before training. Cardiorespiratory parameters and venous blood samples were collected across tests, and spleen diameters were measured via ultrasound imaging. Maximal apneic duration increased by 44 s ($P < 0.05$). Diving bradycardia developed 3 s earlier and was more pronounced after training ($P < 0.05$). Spleen contraction during apneas was similar during all tests. The arterial hemoglobin desaturation (SaO_2) nadir after apnea was 84% pretraining and 89% after the duration-mimicked apneas post-training ($P < 0.05$), while it was 72% ($P < 0.05$) after maximal apneas post-training. Baseline Hb remained unchanged after training, but reticulocyte count increased by 15% ($P < 0.05$). We concluded that the attenuated SaO_2 decrease during mimic apneas was due mainly to the earlier and more pronounced diving bradycardia, as no enhancement of spleen contraction or Hb had occurred. Increased reticulocyte count suggests augmented erythropoiesis.

Apnea, whether voluntary or involuntary, represents a potentially hazardous condition that can compromise vital body functions within minutes. In mammals that voluntarily endure apnea during diving, several responses occur in order to maintain their vital physiological functions based on the oxygen stored in the lung, blood, and other tissues (Kooyman et al., 1981). At least two such responses are shared by humans; the cardiovascular diving response and the contraction of the spleen (Schagatay et al., 2007a), both of which have been shown to prolong voluntary apneic duration (Andersson & Schagatay, 1998; Schagatay et al., 2001).

The cardiovascular diving response is characterized by selective vasoconstriction, diverting blood flow primarily to the organs that are most sensitive to asphyxia, mainly the heart and the brain, while other tissue must rely to a great extent on anaerobic metabolism (Gooden, 1994; Andersson et al., 2004). The pronounced bradycardia of the diving response also reduces the oxygen (O_2) requirements of the heart (Lin, 1982; Butler & Woakes, 1987; Andersson et al., 2002). While the diving response is triggered by apnea, it can be further increased by chilling of the face, for example, by immersion (Kawakami et al., 1967; Schagatay & Holm, 1996).

The second defense mechanism to prevent asphyxia – contraction of the spleen – may lead to increased blood-gas storage capacity because of the release of stored red blood cells into circulation (Hurford et al., 1990; Schagatay et al., 2001). Some seal species store a considerable volume of high-hematocrit (Hct) blood in the spleen that can improve gas transport and buffering capacity during apnea and prolonged the aerobic dive period (Hurford et al., 1996; Qvist et al., 1996). The average human spleen contains a blood reservoir of approximately 200–250 mL (Stewart & McKenzie, 2002), which, if ejected, may typically increase the total amount of circulating red blood cells by 2–4% (Richardson, 2008). However, variability in spleen size and its ability to contract are large among individuals (Prassopoulos et al., 1997; Schagatay et al., 2005). The stimuli responsible for initiating spleen contraction have not been fully clarified, but hypoxia (Richardson et al., 2009) and hypercapnia are likely involved (Richardson, 2008; Lodin-Sundström & Schagatay, 2010).

A third line of defense against asphyxia present in diving mammals and possibly shared by humans is a high level of total hemoglobin (Hb; Kooyman et al., 1981). The responsible mechanisms may be similar to adaptation to high-altitude hypoxia observed in man (Bärtsch & Saltin, 2008). The response may be training induced as it develops when seal pups begin to dive (Lander et al., 2003). Elevated Hb levels compared with untrained individuals and other athletes have also been found in human elite-divers engaging in frequent and repeated apnea training (De Bruijn et al., 2004). Two studies suggest that this higher observed Hb in apneic divers may be a training effect: Erythropoietin (EPO) concentration was found to increase by 24% after 15 voluntary maximal apneas in untrained individuals (De Bruijn et al., 2008), while Choi et al. (2006) showed higher values of Hb in patients suffering from severe obstructive sleep apnea than in control subjects and those with moderate apneic episodes. Studies have not, however, methodologically addressed the extent to which these augmentations can be attributed to genetic differences or effects of training.

The temporal aspects of these potentially protective mechanisms are different: the diving response is rapid, producing a considerable bradycardia in 30 s (Jung & Stolle, 1981), while the blood-boosting spleen response needs several subsequent apneas to be fully established (Schagatay et al., 2005). Finally, an enhanced erythropoiesis resulting in increased levels of erythrocytes is a longer-term adaptation, requiring several weeks to be fully developed (Saunders et al., 2009).

Trained apnea divers appear to have augmented defense mechanisms against hypoxia (reviewed in Schagatay, 2009), as suggested by their longer apneic durations, although the inherent or training-derived nature of the responsible mechanisms have not been fully clarified. Several studies have confirmed the presence of an enhanced diving response in divers compared with nondivers (Irving, 1963; Schagatay & Andersson, 1998), and an enhancement of the response after apnea training has been shown in two long-term studies (Schagatay et al., 2000; Joulia et al., 2003). Larger spleen volumes have also been noted in elite apnea divers (Schagatay et al., 2007b), and the spleen contraction of apnea-trained individuals may be augmented (Bakovic et al., 2003; Prommer et al., 2007). In addition, trained apnea divers showed a more powerful spleen-derived elevation of Hb during apnea as compared with untrained individuals or cross-country skiers (Richardson et al., 2005), but no previous long-term apnea training studies have been done concerning spleen function.

The present study investigated to what extent these major defense mechanisms in untrained individuals responded to 2 weeks of daily apnea training. This period was used as earlier studies indicate that 2 weeks was a sufficient training period for cardiovascular adaptations to develop in a study using five maximal apneas per day (Schagatay et al., 2000; De Bruijn et al., 2008). To impose a significant stimulus, we used a greater daily training load of 10 maximal apneas daily during 2 weeks. Speed of onset and magnitude of the cardiovascular diving response during apnea, the magnitude of spleen contraction and its subsequent hematological response during apnea, and enhancements of baseline Hb and erythropoiesis were the main features analyzed for training effects.

Methods

Subjects

Ten volunteers (four women and six men) participated in the study. The subjects were 25 ± 6 years of age, weighted 72 ± 9 kg, and were 175 ± 11 cm high, with a vital capacity of 4.9 ± 1.0 liters. They were healthy and moderately trained, and no one used products containing nicotine. None of the subjects were participating in breath-holding sports on any regular basis. All subjects received a description of the procedures and potential risks involved and signed an informed consent document for the study, which was approved by the regional human ethics board of Umeå University, Sweden.

Apnea training

The training program consisted of 12 days of self-planned daily apneic training at home. Subjects performed a total of 10 maximal effort apneas each day, grouped in two series of five apneas at 2-min intervals, with series separated by 10-min rest. Apneas were performed at rest in a sitting or supine position. Prior to each apnea, subjects hyperventilated for 1 min in order to decrease arterial carbon dioxide tension ($PaCO_2$) and thereby extend the apneic period and to increase the level of hypoxia reached. Following hyperventilation and a full exhale, the subject took a deep, but not maximal inspiration that marked the start of the apnea. The duration of each apnea was recorded by the subject or by an observer. Subjects could monitor arterial hemoglobin desaturation (SaO_2) by a pulse oxymeter, and were instructed to interrupt apneas if SaO_2 levels approached 60%. Subjects were instructed to maintain the same level of physical exercise during the apneic training period as before the study.

Experimental procedures

All subjects were tested prior to and immediately after the training program, by performing three maximal duration apneas without preceding hyperventilation and without time cues, separated by 2 min of recovery (Fig. 1). All individual apneas were preceded by a deep but not maximal inspiration, similar to the method used for the training regimen. An earlier study has shown that instructing subjects to take "a deep but not maximal breath" results in an approximately 85% filling of total lung vital capacity (Schagatay, 1996). As the duration of the apneas before and after training would most likely differ, an additional series of three apneas mimicking the apneic duration in the test prior to training was also performed during the post-training test. The order of the time-mimicking test and the maximal-duration test was randomized, and all apneic series (both pre- and post-training) were preceded by 20 min of supine rest. Subjects were notified at 2 min and 1 min prior to each apnea, received a nose clip with 30 s remaining, and started the apnea after a 10-s countdown. If SaO_2 levels decreased below 60% during apneas, the subjects were told to resume breathing in order to avoid hypoxic syncope.
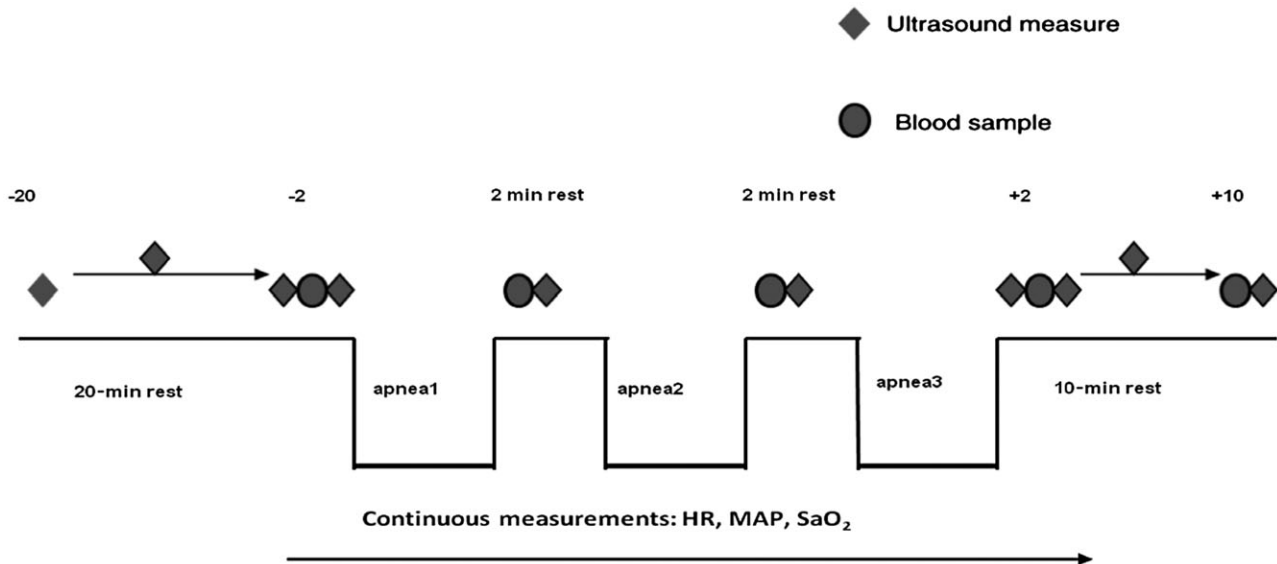
Fig. 1. The time course of the test protocol during pre- and post-test, illustrating the apnea periods and sampling points for blood and ultrasound measurements as well as the continuous measurements of heart rate (HR), mean arterial pressure (MAP), and arterial hemoglobin oxygen saturation (SaO_2).

Subjects reported to the laboratory without physical exercise at the same day, and after 1 h without any meals or liquid intake and at least 2 h without heavy meals. On arrival to the lab, height and weight were measured, and vital capacity was recorded with a spirometer (Compact 11, Vitalograph, Buckingham, England). The ambient air temperature was maintained within 21 ± 1 C. A catheter for venous blood sampling was inserted in the antecubital region of the right arm (Venflon Pro, Becton-Dickson AB, Helsingborg, Sweden); probes for recording of heart rate (HR), oxyhemoglobin saturation level (SaO_2), and mean arterial pressure (MAP) were attached to the left hand; and a chest bellows for registration of breathing movements was attached. Twenty minutes of supine rest preceded each apneic series performed.

Measurements

Hematological parameters

Venous blood samples (2 mL) were drawn 2 min prior to the first apnea, immediately following each apnea, and at 2 min and 10 min after the final apnea. Blood samples for reticulocyte measurements were drawn 2 min prior to the first apnea. The volume drawn was below 40 mL per subject including waste samples, and 20–24 mL of sterile isotonic NaCl solution was infused at 2-mL doses after each blood sample for rinsing the catheter.

Blood samples were analyzed for Hb concentration using an automated blood analysis unit (Micros 60 Analyzer, ABX Diagnostics, Montpellier, France). All stored blood samples for Hb were analyzed in duplicate, and in cases were a deviation of more than 2 units was observed, a third analysis was performed. Reticulocyte measurement was conducted via automated blood analysis unit (Cell-Din, ABBOTT, San Fransisco, California, USA).

Ultrasonic imaging

Spleen size was measured via ultrasonic imaging (Mindray DP-6600, Shenzhen Mindray Bio-Medical Electronics Co., Ltd, Shenzhen, China) every minute starting 20 min prior to the first apnea, at the same five occasions when blood samples were taken,

and every minute for 10 min after termination of the apneic series. Three different diameters were measured on the observed spleen image: maximal length, maximal thickness, and maximal width.

Cardiovascular parameters

HR and SaO_2 were recorded on the left forefinger with a pulse oximeter (Biox 3700e, Ohmeda, Madison, Wisconsin, USA), MAP was recorded on the left middle finger with the hand at heart level using a cuff photoplethysmometer (Finapres 2300, Ohmeda). Breathing movements were measured continuously using a laboratory-developed pneumatic chest bellows with pressure change output converted to a digital signal. Apneic time was marked with an analog switch. These parameters were recorded continuously starting 2 min prior to the first apnea until 10 min after each apnea series via a BioPac MH100A CE multichannel data acquisition system (BioPac Systems Inc., Goleta, California, USA).

Data analysis

All subjects served as their own controls. The third apnea was analyzed for training-induced changes, with the two preceding apneas treated as "warm up." To avoid the effects related to apneic duration, data from the pretraining apneas and time-mimicked post-training apneas were used for comparison, and to determine effects at maximal performance, maximal effort apneas before and after training were compared for the hematological parameters, apneic durations, and SaO_2. Baseline values for the various variables were obtained as specified below.

Spleen volume was calculated from the measurements of spleen length (L), thickness (T), and width (W) according to the Pilström equation: $L\pi(WT - T^2) / 3$, which has been described in greater detail by Richardson (2008). Mean pre-apneic spleen volumes were calculated from the two measurements prior to the first apnea. Volume changes arising from apneas were obtained by comparing values obtained directly after the third apnea to the pre-apneic volumes.
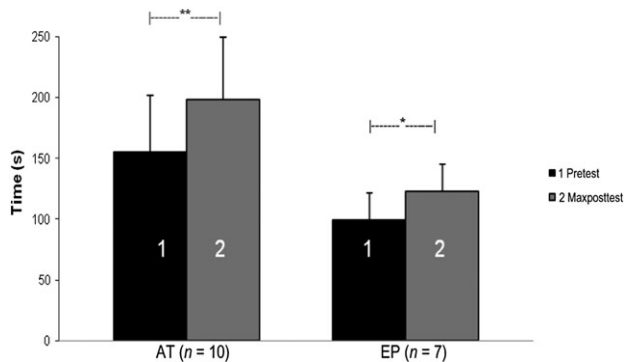
Fig. 2. Mean (SD) apneic time (AT) and duration of the easy-going phase (EP) from apnea three in pre- and maximal posttest. *$P \leq 0.05$, **$P \leq 0.01$.

Reference values used for HR and MAP were the averages of the 20-s period preceding the first apnea, to which apneic value of the last 10 s during the third apnea was compared for relative differences from their respective baseline values. To analyze the onset of HR reduction from the initial tachycardia, the time from apnea initiation to HR peak was measured by visual inspection of the individual HR/time relationship by an observer blinded to the performance results. The degree of SaO_2 was calculated as the percent change at the lowest SaO_2 after the apnea, from the 30-s period preceding the first apnea. Because of recording failure during the experimental protocol for some subjects, the HR data are based on $n = 9$ and MAP data are based on $n = 6$.

The recording of breathing movements was used to distinguish between the duration of the so-called "easy going phase," defined as the period up to the first involuntary breathing movement during apnea, and the corresponding "struggle-phase" (Dejours, 1965). At this point, a critical level of P_aCO_2 is reached, triggering involuntary breathing movements, as is referred to as the physiological breaking point (Lin et al., 1974). A clear physiological breaking point could be identified in 7 out of 10 subjects, while the remaining subjects terminated their apneas either at or before this point.

Statistics

Data are presented as means $\pm$ SD for $n = 10$ subjects unless otherwise indicated. Paired Student's t-test was used to compare values before and after training and within series, and Bonferroni corrections were used when multiple comparisons were made. One-way repeated-measures ANOVA was used to identify differences before and after training for the continuous measurements of HR and MAP, and Bonferroni-adjusted t-tests were used to identify specific differences. Statistical significance was accepted at $P \leq 0.05$.

Results
Apneic duration during training

The self-recorded mean (SD) daily apneic time increased by 32 (43) % from day 1 to day 12 across the 12-day training program ($P \leq 0.05$).

Apneic time

Total mean (SD) apneic time from the third apnea increased by 28 (31) % after training ($P \leq 0.01$; Fig. 2).

The easy going phase was prolonged by 33 (43) % after apnea training ($P \leq 0.05$; Fig. 2), while the struggle-phase increased by 35 (47) % [not significant (NS)].

Cardiovascular responses
HR

There were no differences in baseline HR before apneas between the pretraining [69 (14) beats per minute (bpm)] and post-training tests [67 (14) bpm]. During apnea, after an initial tachycardia, HR gradually decreased both in pretraining and post-training tests. HR during the time-mimicked post-training apneas started to decline 3 s earlier than pretraining apneas ($P \leq 0.05$), and the HR during the last 10 s was lower [61 (16) bpm] than in pretraining apneas [69 (16) bpm; $P \leq 0.01$]. The decrease from baseline HR during this period was 0.8 (14) % in pretraining apneas and 9 (14) % in time-mimicked post-training apneas, respectively (Fig. 3).

MAP

There were no differences in baseline MAP values between the pretraining [103 (4) mmHg] and post-training tests [96 (3) mmHg; NS]. The MAP increased during apnea in both the pretraining and post-training tests; by 34 (14) mmHg before training and by 35 (12) mmHg in the time-mimicked post-training test (change from baseline $P \leq 0.05$ for both). The magnitude of the increase was similar between tests (NS).

Arterial hemoglobin desaturation

Baseline values for SaO_2 were similar at 97.7 (1.4) % and 97.9 (0.8) % in the pretraining and post-training tests (NS). Mean SaO_2 after the pretraining apneas was 84.2 (13.1) % and it was higher, at 88.7 (11.8) %, after apneas in the time-mimicked post-training test ($P \leq 0.05$; Fig. 4). After maximal apneas after training, SaO_2 was 72.7 (29.9) %, representing a decrease from the pretraining apnea test ($P \leq 0.05$; Fig. 4).

Blood-boosting spleen response
Spleen volume

During the two last minutes before apnea, spleen volume was 225 (72) mL in the pretraining test, and 216 (75) mL in the post-training test (NS). Spleen volume reduced from baseline by 20.5 (22.4) % in the pretraining series, by 21.8 (20.2) % in the maximal post-training test, and by 14.8 (19.8) % in the time-mimicked post-training test (all $P \leq 0.05$). There was no effect of the apnea training period on the magnitude of spleen volume reduction (NS; Fig. 5). Ten minutes following the final apnea, spleen size had returned to baseline values in all tests.

Hematological response to apnea

Baseline values for Hb was 131 (12.6) g/liter in the pretraining test, and 131 (10.4) g/liter in the post-training
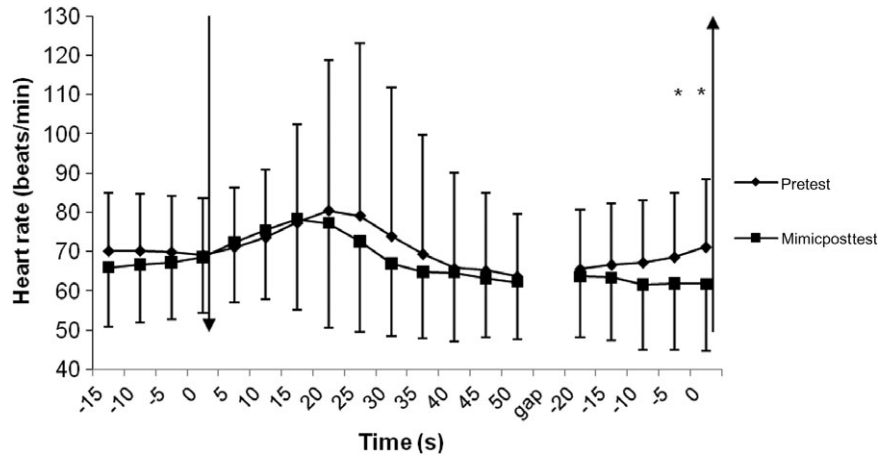
Fig. 3. Average heart rate (SD) during the third apnea for the pretest and when the apneic duration was kept identical to that before training. Arrows indicate the start and end of the third apnea. *$P \leq 0.05$ between pretest and mimicposttest ($n = 9$).
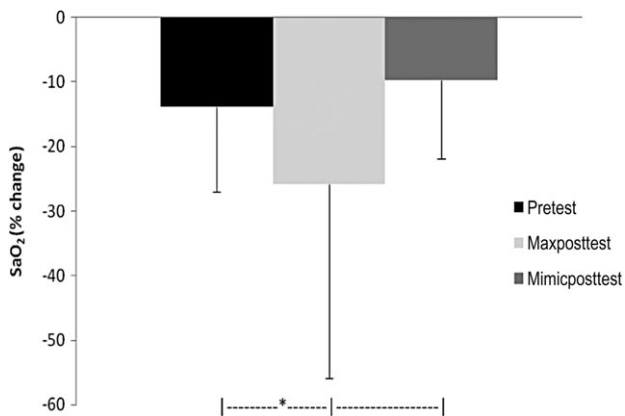

Fig. 4. Percent change in arterial hemoglobin oxygen saturation (SaO$_2$) (SD) from baseline values during the third apnea in all three tests. Values are means (SD). *$P \leq 0.05$ ($n = 9$).

Fig. 6. Mean percent (SD) change in hemoglobin (Hb) concentration from baseline values immediately following apnea three. * denotes significance at $P \leq 0.05$ for difference in Hb from baseline values.
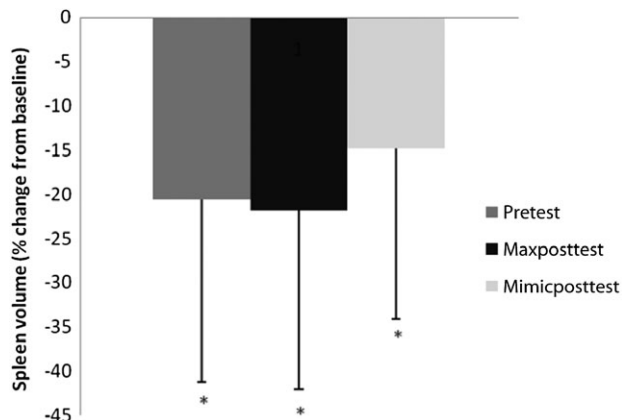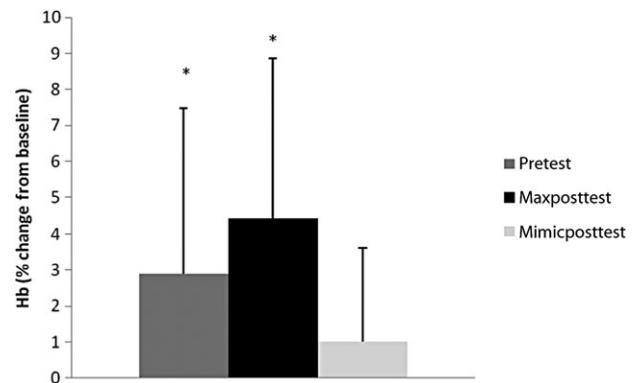

Fig. 5. Mean percent (SD) change in spleen volume from baseline values immediately following apnea three. * denotes significance at $P \leq 0.05$ for difference in spleen volume from baseline values.

test (NS). In the pretraining apnea series, Hb increased from baseline values by 2.8 (4.5) % and in the maximal post-training test by 4.4 (4.4) % (both $P \leq 0.05$; Fig. 6). These increases in Hb were not significantly different. Hb did not change from baseline in the time-mimicked post-training apnea test 1.0 (2.6) % (NS). Ten minutes after the final apnea, values were at baseline in all tests.

Long-term hematological response

Reticulocyte count had increased by 15% after training, from 42 (13) to 48 (11) $\times 10^9$/liter ($P \leq 0.05$), while the baseline Hb level remained at 131 g/liter (Fig. 7).

Discussion

Important findings of this study were that an earlier onset of the diving response contributes to the enhanced apneic performance seen after apnea training, but that neither
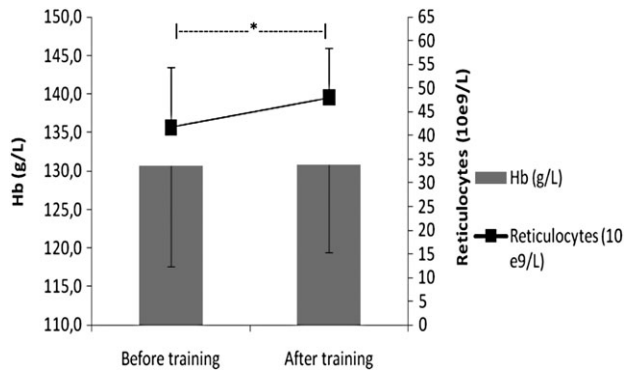
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Fig. 7. Reticulocyte count and hemoglobin (Hb) concentration (SD) before and after the apnea training period. *$P \leq 0.05$.

enhanced spleen contraction nor elevated baseline Hb – two factors identified in trained divers – is affected by a 2-week training period. The more pronounced bradycardia observed at full development of the diving response is in accordance with previous findings (Schagatay et al., 2000). In addition, the earlier response onset could contribute to an increased oxygen conserving efficiency of the diving response, and these two modifications of the diving response are likely essential factors contributing to the prolonged apneic duration seen after training. The observation that the SaO$_2$ after apneas of the same duration was higher after training further supports the conclusion that metabolic restrictions, likely by the diving response, may have been improved. The unaffected spleen contraction and baseline Hb may suggest either that previous reports of elevations in divers represent inherent genetic traits unaffected by training, or that more time is needed for training effects to develop. The increase in reticulocyte count suggests that apnea training could augment erythropoiesis.

The increase in maximal apneic duration seen after training was partly because of a prolonged "easy going phase," suggesting a main contribution of physiological adaptation, for example, involving reduced metabolism potentially via an enhanced diving response, while psychological adaptation may have a lesser role as only a slight, insignificant, prolongation of the "struggle phase" was seen. In a previous study of apneic duration before and after 2 weeks of apnea training, a control group retested after the same period of time without training revealed no enhancement of either of these phases (Schagatay et al., 2000), thus we conclude that the re-testing per se would have no significant effects. In that study, the major prolongation after apnea training was also that of the "easy going phase" (Schagatay et al., 2000).

As this enhanced diving response with an earlier onset was observed in the time-mimicked apneas post-training, it likely represents an effect of the training. Should such an effect be present only during the maximal apneas, an enhanced HR reduction could

instead be the result of the full establishment of the diving response during the extra time reached after training, or as a result of the subjects making a greater effort. Despite the clear indications of an enhanced diving bradycardia, it cannot be ruled out that other physiological responses than those controlled for in this study could have contributed to a better adaptation to apnea. The potential for a contribution from other physiological systems is intriguing, but several studies both on animals and man are consistent in the interpretation of an oxygen conserving effect of the bradycardic response (Elsner & Gooden, 1983; Schagatay, 2009). Although the efficiency of the diving response has often been determined from the magnitude of bradycardia, it should be noted that peripheral vasonstriction and redistribution of regional blood flow are important characteristics of the response, which were not measured in this study. We found no difference after training with respect to arterial blood pressure, showing that the more powerful bradycardia was compensated by further vascular constriction.

Spleen contraction and Hb increase were observed both before and after training, but to the same extent, thus training effects on these mechanisms cannot explain the prolongation of total apneic duration. As one of the identified triggers for spleen contraction is the degree of arterial O$_2$ desaturation (Richardson, 2008), less O$_2$ desaturation after equal apneic duration after training may have involved a reduced stimulus to the spleen. However, the degree of desaturation was increased during the maximal duration apneas after training, yet both the volume change of the spleen and the associated hematological changes were unaltered as compared with the pretest and time-mimic conditions, confirming that no enhancement of spleen contraction can be seen after 2 weeks of training. This also shows that O$_2$ desaturation is not the only factor responsible for initiating spleen contraction, in accord with previous results (Lodin-Sundström & Schagatay, 2010), one such factor being PaCO$_2$. However, in the present study, the longer duration maximal apneas after training would most likely have yielded a higher PaCO$_2$, which could possibly also enhance spleen contraction, yet no such effect was seen. This study therefore suggests that the previously observed large spleen volumes and augmented spleen contraction observed in trained apnea divers (Bakovic et al., 2003; Richardson et al., 2005; Prommer et al., 2007; Schagatay et al., 2007b) may either reflect predisposition, or that such effects require longer periods of training or perhaps a more significant training intensity or load. Apneic training history and apneic durations were considerably longer in previous studies of spleen function in trained divers. Further studies concerning these effects may require at least several months of intense apnea training before the possibility of a trainable spleen can be ruled out.

There were no changes in baseline Hb observed after 12 days of apneic training in this study, but an increase in

reticulocyte count suggests enhanced erythropoiesis. This corresponds with the finding by De Bruijn and associates (2008) of increased EPO levels after serial apneas, and our results support this finding that recurrent apneic episodes during training provides a sufficient hypoxic stimulus for enhanced renal EPO production. MacArthur et al. (2003) reported significant gains in Hb and Hct for muskrats after dive conditioning in laboratory settings, and prior studies of humans have established that intermittent hypoxia in hypobaric conditions (Rodriguez et al., 2000), as well as severe sleep apnea (Choi et al., 2006), may result in significant increments in Hb and Hct. Prommer et al. (2007) did not find a higher Hb nor total Hb in apnea divers compared with nondivers, while De Bruijn et al. (2004) found an elevated baseline Hb in elite divers, the difference possibly being a result of a different level of training in the divers included. Although the present study implicates an accelerated erythropoiesis, it is unknown when the increase of reticulocyte count started to be evident. Further investigations should increase the training load and/or duration in order to see if this effect develops further into an elevated Hb.

The conclusion that the enhanced diving response might be a major cause of apnea prolongation after training (Andersson & Schagatay, 1998; Schagatay & Andersson, 1998; Andersson et al., 2002) was supported in the present study by the smaller SaO_2 reduction when the apneic duration was kept identical to that before training, which has not been shown previously in long-term training studies. Several mechanisms may lead to this effect, of which only the magnitude of HR reduction was shown to be affected by training: An increased bradycardia will lower metabolic demand of the cardiac muscle (Lin, 1982), and reduce alveolar gas exchange during apneas via reduced cardiac output, while elevated plasma lactate concentration after apneas with a more pronounced diving response reflects a shift toward anaerobic metabolism in underperfused tissues (Andersson et al., 2004). Along with the HR reduction, there was a steady increase in MAP of similar magnitude both in the pre- and post-training tests in the present study, indicating an increase in systemic resistance because of peripheral vasoconstriction (Bjertnaes et al., 1984). The similar MAP despite lowered HR after training suggests an enhanced overall vasoconstriction after training, elevating total peripheral resistance. In a previous long-term study, the decrease in skin blood flow was unaffected by apnea training despite an increase in MAP and a decrease in HR, suggesting that other capillary beds than those in the skin were involved in increasing the total peripheral resistance (Schagatay et al., 2000).

Enhanced tolerance to hypoxia and hypercapnia may also be important for enduring prolonged apneas. The lowered SaO_2 at nadir after maximal duration apneas post-training suggests that the training has increased tolerance to hypoxia, a major determining factor of apneic duration in trained divers, and certainly the most important factor for maintaining conscious apnea (Feiner et al., 1994). It is also possible that a decreased sensitivity to P_aCO_2 may have contributed to the prolonged duration and subsequently more pronounced desaturation during maximal duration apneas post-training (Schaefer, 1965). Previous studies have reported a blunted hypercapnic ventilatory response in various groups of divers such as submarine escape training instructors (Schaefer, 1965), Ama divers (Masuda et al., 1982), and underwater hockey players (Davis et al., 1987; Lemaître et al., 2007). A change in set point and a reduced sensitivity of the central chemoreceptors have been proposed (Delapille et al., 2001; Ferreti & Costa, 2003), thereby reducing the respiratory drive. Also, diving mammals that are regularly exposed to extreme asphyxia exhibit increased tolerance of hypoxia (Meir et al., 2009) and a blunted ventilatory response to CO_2 compared with non-diving mammals (Dejours, 1988).

This study shows that humans respond with a number of physiological changes during apnea and that some of these factors are affected by 2 weeks of daily apnea training leading to enhanced apneic performance. The total apneic time increased mainly by a delay of the physiological breaking point after training, indicating that mainly physiological factors were affected. The apnea training enhanced both speed of onset and magnitude of the diving bradycardia, but not spleen contraction or Hb elevation, suggesting that a more efficient diving response might contribute to the apnea prolongation. The unaffected spleen contraction and baseline Hb suggest either that longer time may be needed for such changes, or that previous observations of enhanced effects in divers are because of predisposition. Apneas with identical durations resulted in less arterial desaturation after training, indicating an enhanced O_2 conservation by the enhanced diving response. This study further shows that the cardiovascular diving response and the spleen contraction are two separate adjustments, representing two different lines of defense against hypoxia. The lower SaO_2 after maximal apneas after training suggests an increased tolerance to asphyxia. In addition, the increase in reticulocyte count shows that longer-term adjustments to hypoxic exposure may induce elevated erythropoiesis, although the unchanged baseline Hb suggests that more time may be needed for an elevation of Hb to occur.

Perspectives

The present results show that responses restricting metabolism, most likely the diving response, are modified after 2 weeks of apnea training, while the acute increase of blood storages induced by spleen contraction is not modified by long-term training. Changes caused by hypoxic exposure could not only be of interest in sports where oxygen availability is a limiting factor for

performance, but also in order to better understand pathological conditions such as sleep apnea. A direction for further research could be to extend the training period from several weeks to months in order to investigate the possible trainability of spleen contraction and the potential adaptive changes of hemoglobin levels. In addition, studies of the spleen contraction during chronic exposure to hypoxia, such as during high altitude training, would be of interest in order to understand the adaptive potential in greater detail.

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