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Eight weeks of static apnea training increases spleen volume but not acute spleen contraction

Janne Bouten[a], Kevin Caen[a], Jan Stautemas[a], Filip Lefevere[a], Wim Derave[a], Leen Lootens[b], Peter Van Eenoo[b], Jan G. Bourgois[a,c], Jan Boone[a,*]

[a] Department of Movement and Sports Sciences, Ghent University, Watersportlaan 2, 9000 Ghent, Belgium
[b] Doping Control Laboratory, Department of Clinical Chemistry, Microbiology and Immunology, Ghent University, Technologiepark Zwijnaarde 30, 9050 Ghent, Belgium
[c] Centre of Sports Medicine, Ghent University, Ghent, Belgium

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ABSTRACT

Splenic contraction is an important response to acute apnea causing the release of red blood cells into blood circulation. Current literature shows higher spleen volumes and greater spleen contractions in trained apnea divers compared to untrained individuals, but the influence of training is presently unknown.

Thirteen subjects daily performed five static apneas for 8 weeks. Before, halfway through and after the apnea training period, subjects performed five maximal breath-holds at the laboratory. Baseline values for and changes in splenic volume and hemoglobin ([Hb]) were assessed. Although baseline spleen volume had increased (from 241 ± 55 mL PRE to 299 ± 51 mL POST training, $p = 0.007$), the absolute spleen contraction (142 ± 52 mL PRE and 139 ± 34 mL POST training, $p = 0.868$) and the acute increase in [Hb] remained unchanged.

The present study shows that apnea training can increase the size of the spleen but that eight weeks of training is not sufficient to elicit significant training adaptations on the acute response.

1. Introduction

Diving mammals possess specific responses to breath-holding (apnea) enabling them to remain submerged for long periods of time. These responses also occur in terrestrial mammals such as humans, albeit to a lesser extent and focus on acute reduction in oxygen usage and acute and chronic improvements in oxygen storage capacity.

The physiological responses to apnea are predominantly based on three different mechanisms, being the cardiovascular diving response, the contraction of the spleen and the production of erythropoietin (EPO) (Lemaître et al., 2010). All three mechanisms are identified as a defensive response to apnea-induced hypoxia in order to maintain vital functions. First, the mammalian diving response is mainly characterized by peripheral vasoconstriction and bradycardia (Elsner and Gooden, 1983). This mechanism provokes an oxygen conserving effect enabling prolonged apnea (Andersson and Schagatay, 1998) and has already been extensively examined and reviewed in literature (Gooden, 1994; Schagatay et al., 2000; Foster and Sheel, 2005; Costalat et al., 2016). Contraction of the spleen is a second line of defense against hypoxia. The human spleen contains approximately 200–250 mL densely packed red blood cells (RBCs) (Rushmer, 1989; Stewart and McKenzie, 2002), which correspond to approximately 8% of the total amount of body RBC (Koga, 1979). In response to maximal apnea, splenic contraction in humans causes the release of stored RBCs into circulation, thereby augmenting blood oxygen storage and transport capacity (Schagatay et al., 2001). This results in an immediate increase in Hct and [Hb] ranging between 2 and 5% on average and up to 10% individually (Schagatay et al., 2001; Bakovic et al., 2005; Richardson et al., 2005). Finally, the production of EPO might protect against hypoxia on the long term. de Bruijn et al. (2008) showed that [EPO] increased in untrained individuals up to 5 h after maximal apneas with a peak increase of 24% two to three hours post apnea. This mechanism was confirmed by Kjeld et al. (2015) in elite freedivers after one single static and dynamic apnea.

Cross-sectional data show significant differences between apnea trained and non-apnea trained subjects for all three mechanisms. First, enhanced bradycardia in trained subjects has been reported since the early sixties (Irving, 1963). Additionally, Ilardo et al., 1963, Lemaitre et al., 2010 and Schagatay and Andersson, 1998, Ilardo et al., 2018Ilardo et al. (2018) reported enhanced heart rate reductions and peripheral vasoconstriction in trained as compared to untrained individuals. Second, Bakovic et al. (2003) evidenced greater spleen volume reductions in trained breath-hold divers than in untrained subjects, while Richardson et al. (2005) demonstrated a higher acute

* Corresponding author.
E-mail address: jan.boone@ugent.be (J. Boone).

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increase in [Hb] following maximal apnea in elite apneic divers compared to elite cross-country skiers and untrained subjects. Third, breath-hold divers are found to have higher spleen volumes (Schagatay, 2009) and 3.5% higher baseline [Hb] compared to untrained subjects (Richardson et al., 2005). Longitudinal studies examining the influence of training on these parameters, on the other hand, are scarce. Only for the cardiovascular diving response, previous research shows an apnea-specific training effect in the form of stronger heart rate reductions after respectively 2 weeks (Schagatay et al., 2000; Engan et al., 2013) and three months (Joulia et al., 2003) of apnea training. Additionally, Engan et al. (2013) did not observe differences in splenic contraction, baseline spleen volume and [Hb] after only two weeks of training. Except for this study, the impact of training on the magnitude of both the acute splenic contraction and concomitant increase in [Hb] and baseline levels for spleen volume and [Hb] has thus far not been addressed. As raised by both Schagatay (2009) and Engan et al. (2013), no statement can be made on the inherent or training induced nature of the differences in baseline values and acute responses for spleen and blood values between apnea trained and non-apnea trained individuals.

Therefore, the aim of this study was to investigate the effect of an 8-week apnea training period on the acute physiological responses to apnea (i.e., splenic contraction and [Hb] and Hct increases). It was hypothesized that [1] acute responses to apnea would be apparent on each test day and [2] these acute responses would be more pronounced after training, meaning a more pronounced spleen contraction and acute increase in [Hb] and Hct. Additionally, baseline values for spleen volume and [Hb] will be monitored. Enhanced baseline levels for spleen volume are expected [3].

2. Materials and methods

2.1. Ethical approval

All protocols and procedures conform the *Declaration of Helsinki* and approved by the ethical committee of the Ghent University Hospital (EC UZG 2016/0033). Each participant was informed about the procedure and the aim of the study and informed consent was obtained from all subjects. Both a medical history questionnaire and medical clinical examination were completed prior to the start of the experiment. All volunteers were declared to be in good health.

2.2. Subjects

Thirteen physically active male students volunteered to take part in this study. Inclusion criteria for selection were: age (18–30 years old), sex (male), physical activity level (exercising on average twice a week) and good general health. Exclusion criteria were: smoking, blood donation within three months before the study, experience with breath-hold related sports (i.e., synchronized swimming, breath-hold diving, waterpolo etc…) and residing at altitude in the three months prior to the study. All participants were well trained and practiced recreational sport activities. Subjects were instructed not to serve as blood donor nor participate in other studies and to maintain the same level of physical exercise during the intervention period as before the study.

2.3. Experimental procedure

Overview: A visual overview of the entire protocol is presented in Fig. 1A. Subjects attended the laboratory on four different occasions. During the first visit, all participants underwent a medical examination and performed a ramp incremental exercise test (25 W min^{-1}) to exhaustion on an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands) to determine $\dot{V}_{O_2}$ peak. This was scheduled at least two days and maximal a week before the first apnea test. During the second visit, subjects completed the first apnea test (PRE) which consisted of five maximal breath-holds. Subsequently, all participants were subjected to an 8-week intervention of daily apnea training at home. Both after four (MID) and eight (POST) weeks of training, the apnea test was repeated identically. All test sessions took place in the 'Sport Science Laboratory Jacques Rogge' (Ghent University) at a constant ambient air temperature of 18 °C and humidity of 48%. Prior to each visit, subjects were instructed to avoid strenuous exercise and to maintain a similar diet for 24 h.

2.3.1. Apnea tests

A visual overview of the apnea test protocol (PRE, MID, POST) is presented in Fig. 1B. Participants were instructed to hold their breath five times in a seated position for as long as possible. Each bout was separated by 2 min of recovery. Subjects were notified 30 s prior to each apnea and started the bout after a 10-second count down. All apneas were preceded by a deep, but not maximal inspiration. During the breath-holds, participants were motivated with verbal time cues and strong verbal encouragement. Apneic times were recorded using a time-clock. Venous blood draws and assessment of splenic volume were carried out at different time points throughout the test. Splenic volume was measured at baseline (BL, 5 min prior to the first apnea), and after the first and fourth breath-hold, using ultrasonography (UGEO HM70A Ultrasound System, Samsung Medison, Seoul, South Korea). The probe was a curved (60 mm radius) linear array transducer operating at a frequency of 3.2 MHz (SC1-6 Abdominal S-Vue Transducer, Samsung Medison, Seoul, South Korea). Images were obtained with the subject placed in the right lateral decubitus position. Three different diameters were measured on the observed spleen images: maximal length (*L*), thickness (*T*) and width (*W*). Thickness (*T*) and width (*W*) were measured on transversal images. The longest overall dimension was seen as width (*W*), the shortest distance between the hilum and the outer convex border of the spleen was considered as thickness (*T*). Maximal length (*L*) was measured as the greatest distance in the longitudinal plane (Yetter et al., 2003). Venous blood samples (K3 EDTA 4 mL, Vacutest Kima, Arzergrande PD, Italy) were drawn from an antecubital vein at baseline (BL), immediately after and 5, 10 and 20 min after the last apnea bout. Prior to the first blood draw, subjects sat down for approximately 15 min and moreover, they were instructed to sit down between the other blood collections in order to minimize the possible effects of body posture on the blood values. Blood samples were analyzed for [Hb] and RET%, using an automated hematology analyzer (SYSMEX XT2000i, Sysmex Corporations, Kobe, Japan). All EDTA tubes were stored cool (2–12 °C) during storage and transport and subsequently, analyzed within 48 h after sample collection.

2.3.2. Training program

The intervention period consisted of eight weeks of self-planned daily apnea training at home. Subjects performed a total of five static breath-hold apneas in a seated position every day, each separated by a 2-min rest interval. The first four bouts were completed at 80% of the maximal apneic duration, as calculated from the PRE test, while the last bout was a maximal apnea. Eighty percent time targets were chosen because preliminary data suggested that this would lead to better adherence to the training regimen. Target times were adjusted after four weeks of training based on the apnea durations on the MID test. Prior to each apnea bout, subjects took a deep, but not maximal breath that marked the start of the apnea. Participants were instructed to record the duration of the last maximal apnea using a time-clock. Moreover, after every training session a digital form had to be completed, allowing the researchers to monitor progress and compliance of each individual. Subjects were instructed to interrupt apneas if they felt uncomfortable, in order to avoid hypoxic syncope.

2.4. Data analysis

Maximal apneic time on test days (PRE, MID, POST) was defined as the duration of the longest of all five apnea bouts for each individual.

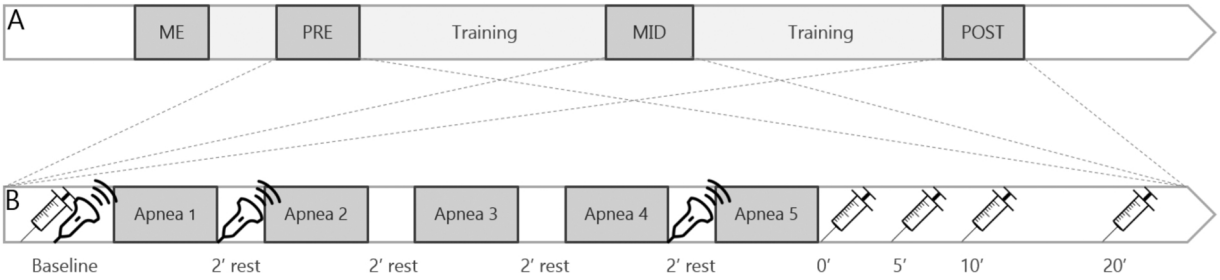

Fig. 1. Experimental timeline. The upper line (A) represents the separate test days (medical examination (ME), PRE, MID, POST) during the training period. The bottom line (B) illustrates the apnea test protocols executed on each test day: baseline (BL) followed by five maximal apneas with two minutes of rest in between. Needles illustrate venous blood sampling, probes indicate spleen echo.

Apneic time gain was the difference between the duration of the longest and the first apnea for each test day. Splenic volume was determined for each subject according to the formula for a prolate ellipse ($V = 0.524 \times W \times T \times L$), which has been compared to CT measures and shown to be an accurate and valid method for the calculation of splenic volume (Yetter et al., 2003). Unpublished data from our laboratory revealed an ICC of 0.992 and CV of 5.9% for determining spleen volume. Absolute change in spleen volume was calculated by subtracting the spleen volume measured at either the first or fourth apnea from the baseline value. Relative change in spleen contraction was determined similarly by using baseline values as reference (100%). Acute changes in [Hb] (ΔHb) were calculated by subtracting baseline values for [Hb] immediately before apnea from the value immediately after apnea.

2.5. Statistical analysis

Statistical analyses were conducted using IBM SPSS statistics 24. Shapiro Wilk tests indicated that all variables were normally distributed. Descriptive statistics were calculated as mean values ± SD for $n = 13$ subjects unless otherwise indicated. Statistical significance was accepted at $p < 0.05$.

For baseline values, a Repeated Measures ANOVA was executed. Test day (PRE, MID, POST) was used as within subject factor for the measures spleen volume, [Hb], Hct and RET%.

For the acute response, two ([Hb] and spleen volume) one way Repeated Measures ANOVA (Test day × Time point) were conducted. Test day (PRE, MID, POST) and time point (BL, 0, 5, 10 and 20 min for [Hb]; BL, 1 and 4 for spleen volume) discriminated within experimental differences. For [Hb], the interaction effect was examined to determine the influence of the apnea training period on the acute response. For absolute and relative spleen contraction, a Repeated Measures ANOVA was used to investigate differences in volume reduction between test days. When the RM ANOVA indicated significant differences, pairwise comparisons were used for further analysis.

Pearson correlation was used to correlate apnea duration (first apnea, maximal apnea and gain in duration), spleen parameters (absolute volume, absolute and relative contraction) and blood values (baseline [Hb], ΔHb) both before and after training.

3. Results

3.1. Subjects

Subjects were 22.3 ± 1.4 years of age, had a mean height of 1.79 ± 0.05 m and a mean body mass of 71.1 ± 7.4 pre and 70.9 ± 7.49 kg post training. Body mass did not change throughout the study ($p = 0.543$). Mean peak oxygen uptake ($\dot{V}_{O_2}$ peak) before training was 55.3 ± 5.6 mL min^{-1} kg^{-1}.

3.2. Apnea duration

Subjects completed 82 ± 11% of their training sessions and reached target times in 88 ± 12% of all submaximal apnea bouts at home. Apnea durations during the laboratory tests increased from bout to bout. Apneic duration increased by 25% from 93 ± 20 s for the first, to 116 ± 15 s ($p < 0.001$) for the fifth apnea on the pre test while duration increased by 44% during the post test from 124 ± 12 s to 178 ± 45 s ($p < 0.001$). Furthermore, post training each bout lasted significantly longer than the corresponding bout on the pre test ($p < 0.001$). Personal bests were attained at either the third, fourth or fifth apnea bout and were improved on average by 32% on the mid and to 41% on the post test ($p < 0.001$; Fig. 2A). Personal bests increased by 7% between mid and post ($p < 0.001$).

3.3. Impact of training on the spleen values

Baseline splenic volume was 20% larger after four and 24% after eight weeks of apnea training ($F = 9.202$, $p = 0.001$), increasing from 241 ± 55 mL on the pre test to 288 ± 47 mL ($p = 0.001$) on the mid test, and 299 ± 51 mL ($p = 0.007$) on the post test (Fig. 2B). The values for mid and post did not differ significantly ($p = 0.443$).

Acute responses were apparent on each test day ($F = 152{,}773$, $p < 0.001$, Fig. 3A). Absolute splenic volume decreased on average

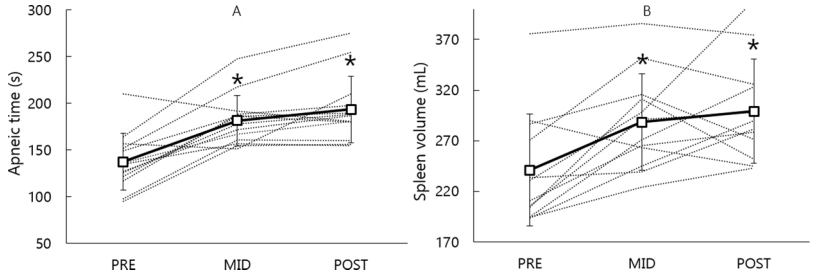

Fig. 2. Individual (dotted lines) and average ± SD (solid lines) values for maximal apneic time ($n = 13$, panel A) and baseline spleen volume ($n = 12$, panel B) before (PRE), after four (MID) and eight weeks (POST) of training.
*Significantly different from PRE test at $p < 0.05$.

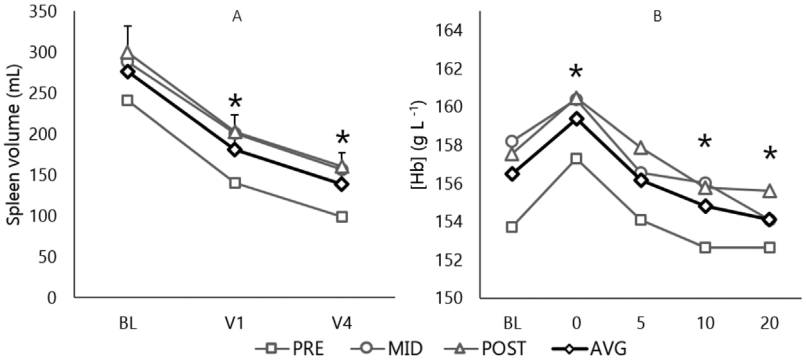

Fig. 3. Average ± SD absolute spleen volume before apnea (BL) and after the first (V1) and the fourth (V4) bout of apnea (n = 12, panel A) and average acute response in [Hb] at baseline (BL), immediately, 5, 10 and 20 min after apnea (panel B) on average for all three test days (AVG) and before (PRE), after four (MID) and eight (POST) weeks of training.
*AVG significantly different from BL at $p < 0.05$.

from 276.2 ± 56 mL before apnea to 181.2 ± 42 mL after the first and 138.4 ± 39 mL after the fourth apnea ($p < 0.001$). Absolute spleen contraction did not differ between test days for both the measurement after the first (101 ± 53 mL pre, 88 ± 32 mL mid and 97 ± 36 mL post $p = 0.559$) and fourth bout of apnea (142 ± 54 mL pre, 132 ± 35 mL mid and 138 ± 34 mL post training, $p = 0.796$), while significant differences in relative values were seen after both the first ($p = 0.025$) and fourth bout of apnea ($p = 0.004$). Relative spleen contraction was less pronounced at the mid (a volume reduction of 30 ± 6% after one, $p = 0.028$ and 46 ± 7% after four apneas, $p = 0.05$) and post test (a reduction of 32 ± 7% after one, $p = 0.043$ and 46 ± 8% after four apneas, $p = 0.027$) as compared to the pre test (41 ± 16% after one and 58 ± 9% after four apneas). Relative spleen contraction was not different between mid and post test after the first ($p = 0.707$) and fourth ($p = 0.827$) apnea.

3.4. Impact of training on the blood values

After four weeks, baseline [Hb] had already increased by 3.3%, from 153 ± 10 to 158 ± 9 g L^{-1} ($p = 0.004$). [Hb] remained elevated at the post test (158 ± 11 g L^{-1}, $p = 0.047$) but did not improve compared to the mid test ($p = 0.736$). The apnea training period had no impact on RET%, ($p = 0.302$).

No significant interaction effects (Test day × Time point) could be observed for [Hb] ($F = 0.959$, $p = 0.474$, Fig. 3B), indicating that there was no effect of training on the acute response. Main effects showed that [Hb] increased during all three test days on average from 156 ± 11 g L^{-1} to 159 ± 9 g L^{-1} ($p = 0.019$) immediately after the last apnea, and had already returned to baseline 5 min post apnea (156 ± 8 g L^{-1}, $p = 0.864$). After 10 and 20 min, [Hb] values had decreased even further with values significantly lower than baseline (153 ± 9 g L^{-1}, $p = 0.049$ and 154 ± 8 g L^{-1}, $p = 0.016$).

3.5. Correlations

Before training (pre), no significant correlations were found between parameters for apneic time (first apnea, maximal apnea, apneic time gain), spleen (baseline volume, absolute and relative contraction) and blood (baseline [Hb], ΔHb). Post training however, acute gains in apneic time were positively correlated to both baseline spleen volume (Fig. 4A, $R = 0.604$, $p = 0.037$) and absolute spleen contraction (Fig. 4B, $R = 0.615$, $p = 0.033$). Acute ΔHb was positively correlated to relative spleen contraction (Fig. 4C, $R = 0.651$, $p = 0.022$), but to absolute spleen contraction, only a trend toward significance was seen ($R = 0.504$, $p = 0.095$). Post training, ΔHb was also negatively correlated to baseline [Hb] (Fig. 4D, $R = -0.657$, $p = 0.015$).

4. Discussion

This study was the first to examine the effects of a prolonged period of daily apnea training on splenic volume and hematological values. The main findings were that, although an acute spleen contraction and an acute increase in [Hb] occurred following serial apneas on each test day, eight weeks of apnea training did not alter this response. Additionally, baseline spleen volume and [Hb] had increased post training.

In this study, apnea duration increased in general from the first to the fifth apnea bout on each test day, with a more pronounced increase between the first and the fifth on the POST test. These data confirm the results of Schagatay et al. (2000), who showed that apneic times improved throughout a series of repeated apneas. In line with previous research (Schagatay et al., 2001, Joulia et al., 2003), subject's personal bests were augmented by 32% after four weeks of training and by 41% after eight weeks of training. It is thus presumable that apneic times improve faster at the start and develop toward a plateau as the training period further evolves in non-apnea trained individuals. According to Schagatay (2009), maximal static apnea duration depends on three major determinants, being gas storage, tolerance to hypoxia and metabolic rate. Improvements in apneic duration are therefore likely the consequence of an improvement in one or more of these factors. The increased spleen volume (although not reflected in an enhanced contraction) and resting [Hb] following training support the notion that improved oxygen storage capacity can contribute to increased duration of maximal static apnea. Furthermore, after training, apneic duration correlated positively with spleen volume, whereas it did not before training, indicating an enforced mutual relationship between these factors. However, whether or not this improved storage capacity is the principal determinant, remains to be elucidated. Enhanced cardiovascular diving responses following training have already been reported and will reduce the metabolic rate (Schagatay et al., 2000; Joulia et al., 2003; Engan et al., 2013). Finally, also the tolerance to hypoxia might improve following apnea training since it has been shown that extreme breath-hold divers demonstrate higher alveolar pCO_2 and lower alveolar pO_2 at the end of apnea and thus appear to tolerate more severe levels of both hypoxia and hypercapnia than untrained individuals (Ferretti, 2001).

In agreement with our first hypothesis, acute responses were observed during all apnea tests for spleen contraction and [Hb]. Similar to Lödin-Sundström and Schagatay (2010), spleen volume decreased on average by 34% after the first apnea and 50% after the fourth apnea in this study. Furthermore, the reported volume changes are comparable to those observed during maximal exercise (Stewart et al., 2003). It is, however, noteworthy that studies using only 2 dimensions instead of 3 for volume calculations, reported less pronounced contractions (Espersen et al., 2002; Bakovic et al., 2003; Engan et al., 2013; Sperlich et al., 2015). After eight weeks of training, relative spleen contraction was attenuated (58% before versus 46% after training), while absolute splenic contraction remained the same (142 ± 52 mL before training versus 139 ± 34 mL post training). This difference between absolute and relative contraction can be explained by the fact that baseline splenic volumes were enhanced.

In accordance with previous research (Bakovic et al., 2005;

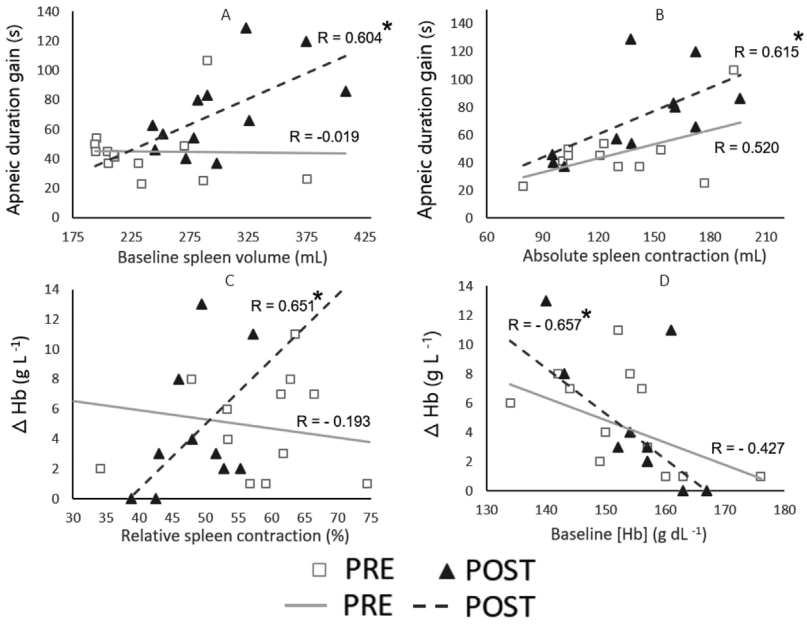

Fig. 4. Correlation for acute increase in apnea duration and baseline spleen volume (panel A) and absolute spleen contraction (panel B), and acute increases in [Hb] and relative spleen contraction (panel C) and baseline [Hb] (panel D) before (PRE) and after (POST) eight weeks of training (n = 12). The regression line is represented by the solid (PRE) and dashed (POST) lines.
*Significantly correlated at $p < 0.05$.

Richardson et al., 2005; Schagatay, 2009), an acute increase in [Hb] of approximately 2% immediately following apnea was seen across all test days. Given the role of the spleen contraction in the acute increase in [Hb] (Schagatay, 2009) and considering that no enhanced spleen contraction could be observed, it is logical that acute increases in [Hb] did not improve either. This is in contrast to our second hypothesis in which training was expected to enforce both acute responses. This hypothesis was based on the more pronounced spleen contraction (Bakovic et al., 2003) and increase in [Hb] (Richardson et al., 2005) observed in cross-sectional studies in trained subjects as compared to non-apnea trained individuals. Yet, Engan et al. (2013) also could not observe differences in relative spleen contraction and [Hb] increase, after only two weeks of training. Thus both Engan et al. (2013) and our data seem to indicate that differences between breath-hold divers and untrained subjects are not the result of short term training. However, the observed increases in baseline spleen volumes following training, combined with the smaller relative, yet similar absolute spleen contraction, might lead us to speculate that structural adaptations facilitating a more pronounced response, have already occurred within the eight weeks of training, but are not (yet) functional. For example, the degree of hypoxia and hyperoxia that our subjects could tolerate after training might not yet be severe enough to elicit maximal spleen contraction and therefore, they might not achieve the full potential of the increased spleen volume yet. Therefore, a longer training period might be needed.

Chronic adaptations following the apnea training protocol were observed for both baseline spleen volume and [Hb]. First, spleen volume was significantly higher after eight weeks of training, changing from 241 ± 55 mL at the pre test to 299 ± 51 mL at the post test. To our knowledge, this is the first demonstration of elevated spleen size following a training intervention. Yet, it is in accordance to the observations of Schagatay et al. (2005, 2012) that spleen volumes were larger in trained breath-hold divers as compared to non-divers. Schagatay et al. (2012) could not distinguish whether this difference is training induced or caused by individual predisposition, while our data suggest that this is, at least partially, the effect of training. On the contrary, a recent study (Ilardo et al., 2018) demonstrated higher spleen volumes in the Bajau people, a Sea Nomad population known for a lifestyle based on breath-hold diving, more specifically spear and shell fishing. Differences between Bajau and Saluan people (non-diving population which is genetically close to the Bajau) were observed and could be linked to genetic variations. This study did not find differences between Bajau divers and non-divers, contradicting our results.

Secondly, baseline [Hb] increased by 3.3% following apnea training. Although an apnea-specific training effect on hematological values has been hypothesized in previous research (de Bruijn et al., 2008; Lemaître et al., 2010), it is not clear whether the increase in [Hb] in our study is directly caused by the training program. As we measured concentrations, results are prone to misinterpretation due to potential plasma volume changes. Although we implemented several precautions to avoid the influence of plasma and blood volume changes on [Hb], we cannot exclude potential influences on the data. Research measuring HbMass and/or plasma volume, as advocated by Otto et al. (2017) is thus highly needed to confirm whether the observed increase in [Hb] following apnea training reflects a true improvement in Hb.

5. Conclusion

This study was the first to provide longitudinal data on the impact of training on the acute spleen contraction and increase in [Hb] following apnea. In accordance with our first hypothesis, acute responses were observed on each test day. However, contradicting our second hypothesis, the magnitude of the responses was unaltered. Yet, spleen volume had increased giving room for larger contractions, and correlations between apneic time, spleen volume and [Hb] (baseline [Hb], ΔHb) emerged post training, indicating some training adaptations. Based on our data, the debate on the inherent whether training induced origin for differences in acute responses to apnea between trained and untrained individuals remains open. We can however conclude that eight weeks of training is not sufficient to elicit significant training adaptations on the acute response, but shows promise for more long term training protocols. Additionally, this study is the first to deliver evidence that the larger spleen volumes in trained divers can be, at least partially, training induced.

Author contributions

Conception and design: JB, KC, JS, WD, PVE, JGB, JB.

Acquisition, analysis and interpretation: JB, KC, JS, FL, LL, PVE, JGB, JB.

Article drafting and revisions: JB, KC, JS, FL, WD, LL, PVE, JGB, JB.

All authors read, revised and approved of the final version and agree to be accountable for all aspects of the work. Questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

All authors who are listed qualify for authorship and all those who qualify are listed.

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Conflict of interest

The authors have no conflict of interest to disclose.

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