Spleen volume and blood flow response to repeated breath-hold apneas

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The purpose of this study was 1) to answer whether the reduction in spleen size in breath-hold apnea is an active contraction or a passive collapse secondary to reduced splenic arterial blood flow and 2) to monitor the spleen response to repeated breath-hold apneas. Ten trained apnea divers and 10 intact and 7 splenectomized untrained persons repeated five maximal apneas (A1–A5) with face immersion in cold water, with 2 min interposed between successive attempts. Ultrasonic monitoring of the spleen and noninvasive cardio-pulmonary measurements were performed before, between apneas, and at times 0, 10, 20, 40, and 60 min after the last apnea. Blood flows in splenic artery and splenic vein were not significantly affected by breath-hold apnea. The duration of apneas peaked after A3 (143, 127, and 74 s in apnea divers, intact persons) was mainly completed throughout the first apnea. The spleen did not recover in size between apneas and only partly recovered 60 min after A5. The well-known physiological responses to apnea diving, i.e., bradycardia and increased blood pressure, were observed in A1 and remained unchanged throughout the following apneas. These results show rapid, probably active contraction of the spleen in response to breath-hold apnea in humans. Rapid spleen contraction and its slow recovery may contribute to prolongation of successive, briefly repeated apnea attempts.

IN MANY TERRESTRIAL AND AQUATIC mammals, during periods of inactivity, the spleen contains a significant volume of a thick blood, which is partly released to active circulation during increased physical activity or diving (18). The redistribution of red blood cells (RBC) between splenic and active circulation switches between a low-hematocrit, low-viscosity state, when oxygen transport capacity exceeds the body needs, and a high-hematocrit state, when oxygen and carbon dioxide transport is stressed. In the seal, the activation of the sympathetic nervous system produces a reduction in the splenic size, probably by the activation of α1-adrenergic receptors on the smooth muscle within the splenic capsule (4, 9).

In humans, the spleen contains ~200–250 ml of densely packed blood cells. The splenic venous hematocrit is estimated to 80%, thus human spleen probably contains ~8% of total body RBC (12). Compared with a 50% splenic content of RBC in horse (19) and seal (4, 6, 9), it appears that splenic RBC reservoir function is not well developed in humans. Up to 50% of this thick blood is transferred to active circulation during maximal exercise or apneic diving (7, 18). Although the direct proof that splenic contraction either augments the aerobic performance or prolongs apnea diving in humans is still lacking, it is beyond doubt that the human spleen may decrease in size substantially and deliver much of its blood content in response to increased adrenergic activity (19).

However, the human spleen contains relatively few adrenergic fibers (18). It was suggested that spleen volume changes after an increase in sympathetic activity represent a passive collapse, rather than active contraction. The assumption is that only splenic arterial vessels are sufficiently sympathetically innervated. In that case, an increased sympathetic tone would decrease the splenic inflow and intravascular pressures, leading to passive collapse of the spleen. The supporting data was provided by Allsop et al. (1), who tracked intrasplenic kinetics of RBC, granulocytes, and platelets. Labeled RBC equilibrated rapidly between the spleen and circulating blood after injection, whereas labeled platelets and granulocytes equilibrated more slowly. After a brief period of maximal exercise, RBC exited the spleen within 60 s, whereas platelets and granulocytes required 10 min to leave it. These differences in kinetics of different blood cells both at rest and after exercise were taken as an indication that, after exercise, spleen passively collapses secondary to reduced blood flow.

Our first aim was to test this hypothesis in apnea breath hold simulated diving by measuring the apnea-induced changes in spleen volume, arterial inflow, and venous outflow. We reasoned that decreased arterial

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inflow and unchanged or increased venous outflow would be found in a case of passive collapse, whereas unchanged flow in the splenic artery and increased flow in the splenic vein would be found in a case of active spleen contraction.

Next, in the field apnea diving, a series of dives are usually performed. It is also known from experience that several introductory dives are useful before attempting the longest one. Therefore, our second aim was to provide data on a series of repeated apneas that mimic field apnea diving. We wanted to see whether splenic contribution to apnea breath hold varies between successive apneas. We hypothesized that the spleen empties continuously throughout the first apnea and does not recover in the short period before the next attempt. If so, the second and later apneas would be at an advantage over the first one in having a larger circulating RBC pool at the onset of apnea. This could explain the prolongation of maximal apneic time in repeated apneas, an important issue in competitive diving. This issue was addressed by Schagatay and Andersson (17) in terms of hematological sequelae of repeated apneas, but without monitoring the spleen itself. In the second related research, Espersen et al. (5) included radionuclide measurements of the spleen volume, but without having interest in the cumulative effect of repeated apneas. Lastly, neither of these studies included trained apnea divers. Trained divers are the population that performs most apnea dives. In them, because of the effect of training, the role of the spleen in diving might be the most prominent.

We attempted to isolate the contribution of spleen emptying in apnea breath hold from vagally mediated bradycardia and sympathetic α-adrenergic vasoconstriction of peripheral vasculature, i.e., from the so-called diving reflex (2, 16). To do so, along with ultrasonic measurements of spleen size and blood flows, we included noninvasive measurements of cardiopulmonary parameters.

**METHODS**

**Participants.** All experimental procedures were conducted in accordance with the American Physiological Society’s Guiding Principles for Research Involving Animals and Human Beings and were approved by Research Ethics Committee of the University of Split School of Medicine. Each method and potential risks were explained to the participants in detail, and they gave written, informed consent before the experiment. Twenty-seven men participated in the study. There were 10 trained breath-hold divers (4 of them were members of the Croatian national apnea diving team) and 10 intact and 7 splenectomized persons without diving experience. Splenectomized persons were recruited from the Department of Surgery, Clinical Hospital Split database to match the other study participants in sex and age. In all of them, the spleen had been removed after a traffic accident at least 2 yr before the study, and they were otherwise healthy. The only two smokers were among the intact untrained persons. The groups were anthropometrically matched (Table 1).

**Protocol.** All experiments were carried out in the acclimatized environment in the afternoon hours. One participant was tested each day. Participants were instructed not to eat anything at least 4 h before the arrival to laboratory and to abstain from smoking in the previous 12 h. They were directed to arrive to the laboratory 30 min before the start of the experiment for acclimatization and detailed explanation of the procedures. The participant was instructed to rest for 15 min in a prone position on a bed with the head placed on the cover of a container filled with cold water (12°C). After collection of baseline parameters, each participant performed five maximal breath holds with face immersed into cold water. The participants were instructed to expire to residual volume and take a deep but not maximal breath before the apneas and to expire completely after the apneas. Successive apneas were separated by 2-min intervals, allowing for data collection, which is the same diving protocol used by Schagatay et al. (18). First, immediately after apnea, the size of a spleen was measured. This lasted ~30 s and was followed by blood flow measurements, which also lasted ~30 s. Simultaneously, the blood pressure was measured. Other parameters were measured continuously. The participants were instructed not to hyperventilate before diving. They wore nose clips and were not allowed to exhale into the water during simulated diving. Times of apneas were recorded by use of a stopwatch. After the last breath hold, the participants were followed up for an hour, with measurements performed at 0, 10, 20, 40, and 60 min.

A more detailed protocol was applied in three apnea divers. We wanted to observe the intra-apneic and postapneic dynamics of the spleen volume changes, in conjunction with other parameters of the diving response. To accomplish this, starting with the onset of a single maximal apnea, in these three divers, the spleen volume was measured every 30 s, and heart rate and oxygen saturation were monitored continuously, up to the full recovery of spleen volume.

**Ultrasonographic spleen measurement.** All ultrasonographic measurements were taken by the same physician (experienced in abdominal ultrasonography) with a 1.5–3.3 MHz phase array probe (Vivid 3, GE, Milwaukee, WI). The participants rested supine for 15 min before baseline measurements. In times between apneas, the participant switched from prone to supine posture. In this body position it is not possible to obtain a cross section in which both maximal length and maximal width of the spleen are clearly visible. Therefore, on each subject, two images of the spleen were obtained, one for length and one for width measurements. Both images were acquired in the 10th intercostal space, during deep inspiration. The images were stored on a personal computer. At a later date, by using scanner software, length and width of the spleen were determined three times, by the same author (A. Obad). Repetitive estimates were consistent within 1 mm. Cross-sectional area and the

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Values are means ± SE. FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 s.
estimated volume of the spleen were calculated as described by Koga (12). The mean of three estimates was reported. In addition, diameters of the splenic artery and vein, as well as blood velocities within them, were recorded in the near proximity (~2 cm) to the spleen hilus and saved for later analysis. All recordings were done at baseline, in periods between apneas A1–A5 and at 0, 10, 20, 40, and 60 min after cessation of the apnea series.

**Cardiopulmonary measurements.** Arterial blood pressure was measured noninvasively with the ECG synchronized, combined oscillometric-auscultatory method (Tango, Sun-Tech Medical Instruments, Raleigh, NC) with the cuff of the instrument positioned on the dominant upper arm. Mean arterial pressure (MAP) was calculated as diastolic pressure + 1/3 pulse pressure. Oxygen saturation was monitored continuously by pulse oximetry (Dash 2000, GE, Marquette, WI) with the probe placed on the middle finger of the dominant arm. Heart rate was continuously recorded on a personal computer by the use of Polar belts positioned around the subject’s chest. The transcutaneous partial pressures of oxygen (PtcO2) and carbon dioxide (Ptc CO2) were measured on the subject’s skin. The transcutaneous measurement of oxygen and carbon dioxide was performed (Quark PFT, Cosmed, Rome, Italy) in accordance with the American Thoracic Society recommendations both in upright and prone positions. During those measurements, all participants were thoroughly acquainted with the vital capacity maneuver.

**Data analysis.** All results were expressed as means ± SE. Comparisons between variables before and after apneas were first tested with nonparametric Friedman’s ANOVA (because of the small sample sizes: n = 10, 10, and 7). In case of a significant difference, Wilcoxon’s signed-rank test was applied for the particular comparison. Differences in response to apneas between study groups were tested by two-way analysis of variance (larger sample sizes, n = 9 × 9, 9 × 10, and 9 × 7, allowed us to benefit from this parametric statistics versatility). This was done by comparing the relative values of variables, defined as percent of baseline value after apneas A1–A5 and at four times after the last apnea, between three study groups, using the phase of measurement (categorical variable having 9 values in total) as the other way in ANOVA. In this case, Tukey’s honestly significant difference test was used for post hoc comparisons. In case of duration of apnea, absolute values of variables were used. P < 0.05 was considered significant.

**RESULTS**

All participants successfully completed the study protocol.

**Baseline comparisons.** The groups of participants were anthropometrically similar, except for trained apnea divers being heavier than the other participants. Spirometry in two body postures yielded almost identical values (data not shown); the reported values correspond to upright posture (Table 1).

**Splenic blood flows and vessel diameters.** The splenic arterial blood flows were not significantly changed compared with baseline values after apneas A1–A5 and at four times after the last apnea (Fig. 1). However, the splenic arterial blood flow decreased after cessation of breath-hold apneas, reaching 88% of baseline value at 60 min in both groups. The splenic venous blood flows were also unaffected by apnea breath holds, except for an increase after A1 in untrained persons (P = 0.03). The diameter of the splenic artery was not significantly changed after breath-hold apneas, whereas the diameter of the splenic vein increased after A1, followed by gradual return to baseline value at 20 min after the last attempt both in trained and untrained persons (Fig. 2).

**Spleen volume.** The spleen volume decreased 18% in divers and 14% in untrained persons after A1 and did not change significantly in subsequent apneas. After the cessation of apneas, the spleen volume recovered slowly, reaching 95 and 92% of baseline values at 60 min in apnea divers and untrained persons, respectively. The reduction in the spleen volume was greater in apnea divers than in untrained persons (P < 0.001, Fig. 3). In three apnea divers who were tested sepa-
rately, the spleen started to decrease in size immediately after the onset of apnea, reaching ~75% of the baseline volume at 150 s at the end of apnea. This response was simultaneous with a transient increase in heart rate (followed by more pronounced decrease, a nadir before the apnea and subsequent increase toward baseline), whereas oxygen saturation started to decline in the third quarter of apnea duration. The recovery phase was slower, and it took ~8 min for the complete spleen volume recovery, unlike much faster restoration of the heart rate and oxygen saturation (Fig. 4). Thus, in serial apneas, the spleen volume recovery in 2-min periods between apneas was probably negligible, in contrast to other manifestations of the diving reflex.

**Duration of apnea.** Apneas A2–A5 were longer than A1 both in apnea divers and untrained intact persons, but not in splenectomized persons. Overall, apnea divers performed longer apneas than untrained intact persons, who in turn performed longer apneas than splenectomized persons ($P < 0.001$) (Fig. 5).

**Diving response.** The apnea-related changes in MAP and heart rate were in concord with previous studies: MAP increased mostly after A1 and subsequently decreased, but it was still at elevated values, even in the 1-h period after cessation of diving in all groups. Heart rate increased anticipatory before the onset of A1 and subsequently decreased under the baseline values approximately equally after all apneas. The heart rate response to serial apneas differed significantly between the three groups (Figs. 6 and 7). The heart rate was quickly restored to baseline values in the 2-min periods between apneas in all participants (data not shown).

**Transcutaneous estimates of blood gases.** The preapneic PtcO$_2$ and PtcCO$_2$ in the tissue were constant over all apneas (data not shown), ensuring the constant baseline conditions in blood gases for each apnea attempt. In apnea divers, the oxygen saturation decreased steadily over the subsequent apneas, reaching ~88% after A5. Oxygen desaturation was less emphasized in intact and in splenectomized persons (Fig. 8). Oxygen saturation restored in the period between apneas in all groups and in all apnea attempts (data not shown).

**DISCUSSION**

We have shown that, in simulated apnea diving, the reduction of the spleen volume is fast with unchanged flow in the splenic artery. This rules out the possibility...
of passive collapse and shows that in apnea diving the spleen is not part of the periphery with reduced blood flow secondary to elevated sympathetic tone. The spleen contracts immediately on the onset of apnea, in parallel with simultaneous increase in the heart rate, when arterial blood gases are yet unaffected. This rapidity of the splenic response to apnea diving argues against peripheral triggers and favors the existence of a centrally mediated feed-forward mechanism.

The splenic contraction was only moderately greater in trained than in untrained persons, which suggests that apnea training does not much influence the ability of spleen to participate in the diving response.

In our study, once the spleen has contracted, it took ~8 min for full recovery after a single apnea and

![Fig. 4. Intra-apneic and postapneic sampling of the spleen volume (assessed ultrasonographically), transcutaneous arterial oxygen saturation and heart rate in 3 trained apnea divers who performed maximal simulated apnea dives with face immersion in cold water. The values are expressed as percent of baseline value and averaged over 3 divers. The duration of apnea was 150 s in all divers. Note that the spleen starts to contract immediately on the apnea onset, decreases in size steadily throughout apnea duration, and slowly recovers after the end of apnea diving. In contrast, arterial oxygen saturation and heart rate have different intra-apneic dynamics and recover quickly after cessation of apnea.](./image1)

![Fig. 5. Duration of 5 successive maximal simulated apnea dives with face immersion in cold water, separated by 2-min recovery periods, in 10 trained apnea divers and 10 intact and 7 splenectomized untrained persons (means ± SE). Note that apneas A2–A5 were longer than A1 both in apnea divers and untrained intact persons, but not in splenectomized persons; *P < 0.05 when compared with baseline value, by Wilcoxon’s signed-rank test, following Friedman’s ANOVA in case of apnea divers (*) and untrained persons (†). Overall, the duration of apnea was greater in apnea divers than in untrained intact persons, who dived longer than splenectomized persons (P < 0.001, by Tukey’s honestly significant difference test, following 2-way ANOVA).](./image2)

![Fig. 6. Mean arterial pressure, expressed as percent of baseline value (means ± SE), after each of 5 successive maximal simulated apnea dives with face immersion in cold water, separated by 2-min recovery periods, in 10 trained apnea divers and 10 intact and 7 splenectomized untrained persons. Mean arterial pressure increased mostly after A1 and subsequently decreased in untrained and splenectomized subjects, but not in trained divers; *P < 0.05 when compared with baseline value, by Wilcoxon’s signed-rank test, following Friedman’s ANOVA in case of apnea divers (*) and untrained persons (†).](./image3)

![Fig. 7. Heart rate, expressed as percent of baseline value (means ± SE), after each of 5 successive maximal simulated apnea dives with face immersion in cold water, separated by 2-min recovery periods, in 10 trained apnea divers and 10 intact and 7 splenectomized untrained persons. Heart rate increased before the onset of apnea diving and subsequently decreased below baseline value approximately equally after all apneas, in all groups; *P < 0.05 when compared with baseline value, by Wilcoxon’s signed-rank test, following Friedman’s ANOVA in case of apnea divers (*), untrained persons (†), and splenectomized persons (‡).](./image4)
more than 1 h after five successive apneas. This slow spleen relaxation agrees with the ultrasonically observed reduction of splenic size in Korean ama (professional divers) measured 10–20 min after their 3-h lasting open-sea apnea diving shift (10). On the contrary, Espersen et al. (5) observed fast postapneic recovery of the spleen size, lasting 2 min only. This gross difference between the studies may be related to different measuring techniques. Espersen et al. used scintigraphic, gamma camera imaging to track the changes in the spleen size. This method, using labeled RBC, generates the images of the intrasplenic pool (volume of distribution) of RBC, rather than the spleen itself. Thus these scintigrams estimate the intrasplenic volume of distribution of RBC, not the volume of spleen itself. Second, the scintigrams are quantitative, count-based images and may track the intrasplenic changes of RBC mass directly, not relying on the geometrical model. Some of these features may be considered advantageous over morphological imaging. However, gamma camera images have poor resolution and the outlining of the organ border may be difficult. This, in conjunction with substantial and time-changing background radiation (radiation from the labeled RBC in blood vessels of the tissue above and behind the spleen in the imaging projection), may hinder tracking of relatively small volume changes, as is the case in spleen response to apnea diving. Several variants of the method used by Espersen et al. were also used by others in measuring the changes in spleen RBC content and volume after exercise (19), but neither of these methods has ever been validated objectively. On the other hand, several articles reported very good correlation between ultrasonic estimates of splenic maximal length, cross-sectional area or volume, and actual splenic morphometric parameters obtained after the removal of the organ at surgery or autopsy (11, 12, 14). The reproducibility of ultrasonic spleen measurement in this study was also good, judging from the fact that the spleen diameters were obtained as the mean of three measurements differing 1 mm or less. Thus we believe that ultrasound B-scanning is better suited for tracking spleen response to apnea diving than radio-nuclide scintigraphy with labeled RBC.

We observed that reduction in splenic size occurred immediately at the apnea onset, thereafter decreasing in size steadily throughout apnea duration. The spleen did not relax in the 2-min recovery period and exhibited little change in the volume in the next apneas. Thus the increase in circulating RBC mass was only gradual in the first apnea, whereas the next attempts were at an advantage in having larger circulating RBC mass already at the apnea onset. This could contribute to successive prolongation of apnea duration in trained and untrained intact persons, but not in splenectomized persons. Schagatay et al. (18) arrived at the same conclusion by analyzing the hematological changes induced by apnea diving.

However, many other factors may have influenced the apnea duration. These include physiological factors, such as preapneic level of blood gases, inspired volume of air, degree of the diving response (potentially having oxygen-conserving effects), as well as psychological tolerance to apnea diving. The apnea consists of two phases: the “easy-going phase” and the “struggle phase.” These phases are separated by the “physiological breaking point,” which is reached when elevated arterial CO₂ tension triggers involuntary breathing movements (17). The two phases can be separated by recording the thoracic movements and identifying the first involuntary breathing movement. We were not equipped to perform this measurement. We also did not record the volume of inspired gas before the apnea onset in the study participants. However, all of our participants performed several vital capacity measurements before the apnea series, and there is little doubt that highly trained divers inspired reproducible volumes of air in the serial apneas. Also, the constant preapneic transcutaneous estimates of blood gases and the fact that the diving response did not increase over the successive apneas in our study argues for the contribution of splenic contraction to successive prolongation of maximal apnea duration.

Besides exercise and diving, the spleen emptying may be involved in periods of breath-halt in obstructive sleep apnea. Also, granulocytes and platelets pool in the human spleen to a much greater extent than RBC, accounting for ~40% of both body populations (3, 8, 15). The physiological implications of the spleen as the reservoir of various blood cells are suggested in the case of RBC in exercise and diving but are otherwise unknown.

In conclusion, we showed that the spleen decreased in volume in simulated apnea diving in the presence of conserved arterial splenic flow, which rules out the...
possibility of its passive collapse, as suggested for splenic response to brief maximal exercise. Second, in serial apneas the spleen emptied steadily throughout the first attempt and did not recover in the short period between successive attempts. If the blood ejected from the spleen materially increased the circulating RBC pool, this would put the second and subsequent apneas at an advantage over the first one in having larger blood gas storage at the apnea onset. This could contribute to the well-known prolongation of duration of successive, briefly repeated apnea attempts. To further elucidate this topic, comparison of the intra-apneic changes in blood gases over serial apneas remains to be elucidated.

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DISCLOSURES

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