Spleen and cardiovascular function during short apneas in divers

Ivan Palada,1 Davor Eterović,2 Ante Obad,1 Darija Bakovic,1 Zoran Valic,1 Vladimir Ivancev,1 Mihajlo Lojpur,3 J. Kevin Shoemaker,4 and Zeljko Dujic1

Departments of 1Physiology and 2Biophysics and Scientific Methodology, University of Split School of Medicine; 3Department of Anesthesiology, Clinical Hospital Split, Split, Croatia; and 4Neurovascular Research Laboratory, School of Kinesiology, The University of Western Ontario, London, Ontario, Canada

Submitted 13 February 2007; accepted in final form 11 October 2007

Palada I, Eterović D, Obad A, Bakovic D, Valic Z, Ivancev V, Lojpur M, Shoemaker JK, Dujic Z. Spleen and cardiovascular function during short apneas in divers. J Appl Physiol 103: 1958–1963, 2007. First published October 18, 2007; doi:10.1152/japplphysiol.00182.2007.—We investigated the spleen volume changes as related to the cardiovascular responses during short-duration apneas at rest. We used dynamic ultrasound splenic imaging and noninvasive photoplethysmographic cardiovascular measurements before, during, and after 15–20 s apneas in seven trained divers. The role of baroreflex was studied by intravenous bolus of vasodilating drug trinitrosan during tidal breathing. The role of lung volume was studied by comparing the apneas at near-maximal lung volume with apneas after inhaling tidal volume, with and without cold forehead stimulation. In apneas at near maximal lung volume, a 20% reduction in splenic volume (P = 0.03) was observed as early as 3 s after the onset of breath holding. Around that time the heart rate increased, the mean arterial pressure abruptly decreased from 89.6 to 66.7 mmHg (P = 0.02), and cardiac output decreased, on account of reduction in stroke volume. Intravenous application of trinitrosan resulted in a 6-mmHg decrement in mean arterial pressure, while the splenic volume decreased for ~13%. In apneas at low lung volume, the early splenic contraction was also observed, 10% without and 12% with cold forehead stimulation, although the mean arterial pressure did not change or even increased, respectively. In conclusion, the spleen contraction is present at the beginning of apnea, when the heart rate is increased and the mean arterial pressure decreased (3, 24), contrary to subsequent hemodynamic conditions, e.g., hypertension and bradycardia. Thus the mechanisms of splenic contraction may differ from the mechanisms of the classical features of the diving response; in particular, the contribution of downloading of baroreceptors should be considered.

To evaluate this issue we undertook the intrapneic dynamic, ultrasound measurements of spleen volume in conjunction with simultaneous continuous noninvasive measurements of cardiovascular parameters. Our first aim was to provide the first continuous data on spleen volume and peripheral hemodynamics during and after short apnea at near maximal lung volume. We chose to study short, 15-s apneas because we were interested in splenic and hemodynamic changes at the very beginning of apnea, when the diving response has not yet developed. We could use longer apneas and then focus on the first 15 s or so, but in that case we could not see what happens after such short apneas, which mimic, in part, the sleep apnea syndrome. We previously showed that maximal apneas cause adverse pulmonary hemodynamic changes that last some 40 min after apnea (9). However, in this study the divers were mentally prepared for the maximal apneic attempt, so that the early effects of the central command were likely the same as in apneas of longer duration.

Next, we tested the hypothesis of involvement of baroreflex in early spleen contraction. To do so we examined whether application of vasodilating substance, which causes some 5- to 10-mmHg fall in mean arterial pressure (during tidal breathing), triggers the spleen contraction. This method of downloading the baroreflex could also suggest which part of the spleen (if any) is innervated by the vasomotor center efferents. The spleen contraction following the application of vasodilating...
substance would favor the hypothesis of contraction of the spleen capsule, rather than of splenic blood vessels.

The cause of the early intra-apneic fall in blood pressure may be a decrease in cardiac output, secondary to obstruction of venous return. The obstruction of venous return could be due to increased intrathoracic pressure, produced by the thoracic and lung recoil in conditions when the large lung volume is kept with glottis closed (12). To test this hypothesis we compared the apneas at near maximal volume with apneas after inhaling the respiratory volume during tidal breathing. Finally, we also evaluated whether the cold pressor test, which is known to increase the diving response, affects also the splenic contraction in the early phase of apnea, when the main features of the diving response are not present.

**METHODS**

All experimental procedures in this study were performed in accordance with the Declaration of Helsinki on the treatment of human subjects and were approved by the ethical committee of the University of Split School of Medicine. Each method and potential risks were explained to the participants in detail and they gave their written informed consent before the experiment. Seven apnea divers, aged 27.4 ± 4.6 yr (mean ± SD, range 22–37), participated in the study, with the average body mass index 25.9 ± 3.4 (range 22.9–32.6), height 186 ± 9 cm (range 173–200), and body fat index 17.6 ± 7.5 (% body fat/kg; range 5.4–20.7). Mean of the forced vital capacity (FVC) was 115.7 ± 19.4% of the predicted values (range 85.4–142). All participants were experienced apnea divers with engagement in breath-hold diving 7.1 ± 3.6 yr (range 5–14), personal best constant weight depth was 33.7 ± 5.6 m (range 25–40), and personal best breath holding lasted 284.0 ± 34.4 s (range 240–335). All were healthy nonsmokers.

All experiments were carried out in the acclimatized environment in the morning hours with constant temperature of 22–25°C and humidity of 25–45%. Participants were instructed not to eat anything at least 4 h before arrival at the laboratory. They arrived at the laboratory 45 min before the start of the experiment for acclimatization and detailed explanation of the procedures. The subjects rested in the supine position for 30 min to ensure blood mixing and stabilization of the transcapillary fluid exchange (22).

Continuous, noninvasive monitoring of the heart rate (HR) and blood pressure (Finometer, Finapress Medical Systems, Arnhem, Netherlands) was obtained from the middle finger of the nondominant hand. The photoplethysmographic values of diastolic and systolic blood pressures were gauged using the mercury sphygmomanometer. The photoplethysmograph was previously reported to accurately record changes in mean arterial pressure (MAP) during both exercise and apnea (5, 6). The finger bearing the photoplethysmograph cuff was positioned at the heart level and kept at the same level for the duration of the study. Arterial oxygen saturation (SaO2) was monitored rather small dose of NTG (0.2 g/kg) was started to make sure that the subject was not hypersensitive. After another 5 min the dose was increased (0.4–0.6 g/kg) until MAP did not drop by 5–10 mmHg, accompanied by an increase in HR. The ultrasound assessments of the spleen size were made before NTG application and during the highest HR values and/or lowest MAP values.

On six subjects (one diver had to drop out from this part of the study) the spleen volume and cardiovascular variables were measured during tidal volume breath-hold protocol with and without forehead cold stimulation (2–3°C). The data were first collected at baseline and then during 20 s of breath holding the tidal volume without cold stimulus; after that, the cold pack was applied on the diver’s forehead, the diver breathed spontaneously for the next 50 s, and the final measurements were done during another 20 s of breath holding the tidal volume.

Ten-second averages of HR, MAP, SV, CO, TPR, and SaO2 were calculated as control values in baseline periods, ~1 min before each apnea. All cardiovascular data collected during and after apneas were transferred from the PowerLab data-acquisition screen, for the same time periods in which the spleen size monitoring was done.

The results shown in figures and text are expressed as the mean value ± SD. Comparisons between changes of variables from the control value were first tested with nonparametric Friedman analysis of variance (because of the small sample size). In case of a significant
difference, the Wilcoxon signed rank test was applied for the particular comparison. \( P < 0.05 \) was considered statistically significant. All analyses were done with Statistica 7.0 software (Statsoft, Tulsa, OK).

**RESULTS**

All divers successfully completed the study protocol. Overall, they responded homogenously to the study protocol.

Apneas at large lung volume. The mean spleen size before apnea was 301.8 ± 82.5 ml. Compared with baseline, all seven apnea divers showed reduction in spleen volume during apneas (Fig. 1). The spleen volume was reduced to 235.6 ± 55.7 ml \( (P = 0.018) \) 3 s after the start of breath holding and this effect reached minimal values of 220.2 ± 74.3 ml \( (P = 0.018) \) 12 s following the start of breath holding. This effect lasted for 5 s after the end of apnea. Figure 1 also shows that either the splenic artery diameter or VTI after the period of apneas differed from baseline, and the same holds for systolic and diastolic arterial pressures.

**Fig. 1.** Ultrasonographically assessed spleen volume, velocity time integral (VTI), and spleen artery diameter expressed as mean ± SD. *\( P < 0.05 \) when compared with baseline value by Wilcoxon’s signed rank test after Friedman’s ANOVA in case of apnea divers.

**Fig. 2.** Response in mean arterial pressure, heart rate, stroke volume, cardiac output, and total peripheral resistance presented as mean ± SD. This graph represents values obtained before, during, and after 15-s extended apnea in all subjects. *Values are statistically significant \( (P < 0.05) \) compared with baseline values.
diastolic splenic artery blood flow velocities (data not shown). Responses in MAP, HR, SV, CO, and TPR are summarized in Fig. 2. MAP decreased during apneas approximately equally for all apnea divers due to reduced SV and CO at a time when TPR was unchanged. This was followed by a MAP increase caused by an increase in TPR. This occurred immediately after the end of apnea and it reached maximal values 5 s after the end of apnea attempt. MAP was normalized ~1 min after the cessation of apneas. HR increased during apnea and quickly restored to baseline values in the time following the end of apneas in all participants. SV decreased significantly throughout both apneas (P = 0.018) and returned to baseline in ~10 s after the apnea cessation. The start of apnea was distinguished by an increase in CO (P = 0.028). Regardless, CO decreased (P = 0.028) during apnea, predominantly due to reductions in SV (Fig. 2), while HR changed biphasically (initial rise followed by reduction). TPR increased during the later stages of the apneas and reached the baseline values 45 s after the end of apnea.

**NTG test.** Intravenous application of NTG caused reduction of splenic volume by ~11% (P = 0.028). During this protocol, MAP was reduced by 9 mmHg (P = 0.047), while HR increased for ~13 beats/min. SV slightly decreased, yet CO increased on account of increased HR (Table 1).

**Apneas at small lung volume.** During breath holding the tidal volume and the spleen volume reduced by 10% (P = 0.028) in the presence of unaltered parameters of peripheral hemodynamics (Table 1). In the cold pressor test, MAP increased ~7 mmHg and HR decreased 10 beats/min during breath holding with cold stimulation compared with breath holding without cold stimulus. Cold stimulus also caused reduction in spleen volume for 12% (P = 0.021; Table 1).

Arterial oxygen saturation, P_{O_2}, and P_{CO_2} were unaffected by this short breath holding (data not shown).

**DISCUSSION**

This study provided the first intra-apneic data on spleen volume sampled with high resolution during short apnea, while we previously reported the spleen volume changes in 30-s frames during maximal apneas (11). Now we are confident that the decrease in splenic volume occurs at the very beginning of apnea, if not even before the onset of breath holding. By measuring the simultaneous changes in peripheral hemodynamics, we provided evidence on the possible mechanisms of splenic contraction in the early phase of apnea.

First, one can safely assume that splenic response to apnea is a contraction, rather than passive collapse, due to sympathetically mediated splenic arterial constriction, as suggested previously (1). The supporting evidence is unaffected by the postapneic splenic arterial flow that we observed here and after maximal apneas (11). Unfortunately we have not found it feasible to evaluate ultrasonically the splenic artery during apnea.

The commands that are behind this rapid spleen response probably come from different origins. In case of apneas at large lung volumes, the role of increased sympathetic output from the vasomotor center, due to withdrawal of inhibitory baroreceptor activity, following a significant fall in MAP, is very likely. The other possibilities include the central sympathetic stimulation, mechanical compression, and humoral stimulation. A mechanical compression, following inhalation of a large volume of air could have added to splenic contraction. The role of humoral mediators, following hypoperfusion of kidney, is theoretically possible but not likely due to rapidity of response. However, the spleen also contracted in apneas at small lung volume and even during tidal breathing upon lowering the blood pressure, e.g., in conditions when central stimulation, mechanical compression, or humorally potentiated contraction can be ruled out. Anyhow, since a relatively small decrease in MAP, induced by trinitrosan during tidal breathing, was able to induce the splenic contraction, we can safely assume that downloading of baroreceptors, following much larger decrease in MAP, was at least one of the contributors to early splenic contraction in apneas at large lung volume. Moreover, the trinitrosan trial, showing the splenic contraction in conditions of reduced vasomotor tone, and data on unchanged postapneic splenic arterial flow favor the hypothesis that vasomotor efferents innervate the splenic capsule or pulpa rather than the splenic blood vessels. This issue warrants further investigation.

A decrease in splenic volume, seen immediately after breath holding the tidal volume in conditions of unaltered blood pressure, suggests that downloading of baroreceptors contributes to early splenic contraction in apneas at large lung volume, but not in apneas at small lung volume. The immediate increase in heart rate that occurred before a drop in blood pressure and small, but steady, increases in total peripheral resistance observed in apneas at large lung volume support the hypothesis of central sympathetic stimulation. This command may be of anticipatory origin, present even before the apnea

### Table 1. Spleen and cardiovascular function responses to vasodilating drug test (NTG), and breath-holding tidal volume for 20 s with and without application of cold pack on forehead

<table>
<thead>
<tr>
<th></th>
<th>NTG Test</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Test</td>
<td>With Cold Pressor Test</td>
<td>Without Cold Pressor Test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen volume, ml</td>
<td>308±135</td>
<td>273±130*</td>
<td>285±109</td>
<td>288±102</td>
<td>259±103*</td>
<td></td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>83.7±11.1</td>
<td>75.0±13.3*</td>
<td>92.8±3.6</td>
<td>89.6±5.9</td>
<td>91.0±6.5</td>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>77.8±8.28</td>
<td>91.1±9.3*</td>
<td>73.1±17.0</td>
<td>66.8±14.0</td>
<td>64.5±17.9</td>
<td></td>
</tr>
<tr>
<td>SV, ml</td>
<td>116.2±15.0</td>
<td>108.1±12.6*</td>
<td>114.4±11.6</td>
<td>125.3±10.6</td>
<td>125.4±9.0</td>
<td></td>
</tr>
<tr>
<td>CO, l/min</td>
<td>9.0±1.4</td>
<td>9.8±1.4*</td>
<td>8.3±1.8</td>
<td>8.4±1.8</td>
<td>8.1±2.1</td>
<td></td>
</tr>
<tr>
<td>TPR, mmHg·min⁻¹·l⁻¹</td>
<td>9.5±2.0</td>
<td>7.8±2.2*</td>
<td>11.7±2.6</td>
<td>11.2±2.8</td>
<td>11.9±3.2</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as median (interquartile range) for 6 subjects completing these studies. Test values correspond to time when the difference from baseline was maximal. MAP, mean arterial pressure; HR, heart rate; SV, stroke volume; CO, cardiac output; TPR, total peripheral resistance. *P < 0.05.
onset, or associated with cessation of normal breathing-related depression of sympathetic activity (14, 16). Unfortunately we collected the baseline data 1 min before the apnea onset, but not after that time until the apnea onset.

The rapid fall in blood pressure following large volume breath holding was caused by reduced cardiac output (due to a significant fall in stroke volume that overcame the increase in heart rate). It was previously observed that intrathoracic pressure is increased when breath holding is sustained at large lung volume (19) due to elastic recoil of the lungs and chest wall in the presence of silent inspiratory muscles and closed glottis (12). The increased intrathoracic pressure may cause the obstruction of the venous return or an increase in right atrial pressure (decrease in venous return pressure gradient), which would explain the reduction in cardiac output. Our data on unchanged cardiac output and blood pressure during short apnea at small lung volume favor this hypothesis.

It is known that stimulation of facial cold receptors by cold water augments all components of the diving response (4), in association with oxygen conserving effect (6) and prolongation of maximal breath-hold time (23). Our results show that these effects include the early facilitation of splenic contraction. Stimulation of trigeminal nerve excites the vasomotor center (in addition to cardiac vagal motoneurons; Ref. 15), which is likely the mechanism of the observed augmentation of splenic contraction with cold forehead stimulation.

If early splenic contraction is included as one of the components of the diving response, one should associate it with the early effects of sympathetic stimulation, probably coming from multiple centers, upon exciting different receptors, depending on lung volume and cold forehead stimulation or a change in mental state. Anyhow, the rapid splenic contraction was observed in apneas at both large and small lung volumes, with and without cold forehead stimulation.

The cardiovascular changes in the latter portion of short apnea, the increased blood pressure, due to continuously increasing peripheral vascular resistance, propagated to post-apneic period for ~1 min. This may be related to detrimental effects of sleep apnea episodes on peripheral hemodynamics, while we previously showed the long-lasting, substantial increases in pulmonary vascular resistance after maximal apneic attempts (9).

Our model of simulated diving (dry apneas at rest) may not apply to field diving, in particular due to absence of blood volume centralization related to orthostatic pressure gradient. Thus, in field diving, the blood volume centralization may counteract the large lung volume-related decrease in venous return, resulting in the absence of the initial hypotensive response observed in this study. It is also worthwhile mentioning that in exercise the sympathetically mediated increase in muscle nerve activity is a slower phenomenon than the onset of spleen contraction observed in this study. This may be related to the differences in the related rapidity of synaptic transmissions. One should also answer why the spleen remains contracted during maximal apneic attempts (11) when the baroreceptors are stimulated, contrary to the proposed downloading of baroreceptors at the onset of apnea. We believe this can be explained by the effects of acute hypoxemia in longer breath-hold periods, which are known to inhibit the parasympathetic system and activate sympathetic system. We are planning to investigate this hypothesis.

This study provides only indirect evidence of the mechanisms of spleen contraction initiated by breath holding. Future studies using nerve activity measurements, autonomic blockade, or pharmacoplegia could provide further insights. One could also question the method of tracking the changes in cardiac output used in this study. Clearly the cardiac output cannot be measured during apnea with dye or thermodilution rebreathing of inert gas or Fick’s principle, because relatively fast, dynamic changes in HR and CO cannot be tracked by these methods. Monitoring continuous changes in SV can be made noninvasively by ultrasound impedance cardiography and by arterial pulse-wave analysis. With pulmonary hyperinflation occurring after maximal inspiration, the use of ultrasound is somewhat limited in the location of the appropriate window, while the motion artifacts remain a problem in impedance cardiography. Therefore, we used the Modelflow method that is based on computation of an aortic flow waveform from finger by simulating a nonlinear three-element model of the aortic input impedance (18). However, even this method is somewhat limited in assuming a normal aortic valve and unaffected transmural aortic pressure, whereas it may change by maximal pulmonary hyperinflation or increased intra-abdominal pressure. During maximal apneas, pulmonary volume is slightly below the total lung capacity but remains nearly constant during the apnea duration. Thus Van Lieshout et al. (26) showed very reproducible continuous measurement of SV by noninvasive Modelflow and ultrasound in patients with instantaneous fluctuations in stroke volume during arrhythmias. Another study limitation is an indirect method of measuring the splenic arterial flow; due to problems of accurately measuring the splenic artery cross-section, we used the velocity-time integral as an index of volumetric flow. Last, due to relatively complex protocol and anticipated better compliance, our subjects were trained divers and the results cannot be lightly extrapolated to persons without diving experience.

In conclusion, the splenic contraction is present at the very beginning of apnea and is accentuated by cold forehead stimulation. At large, but not small, lung volume this initial contraction is probably facilitated by baroreflex inhibition in conditions of decreased blood pressure and cardiac output. The data suggest that the splenic capsule or pulpa, rather than the blood vessels, contract in response to sympathetic stimulation of various origins.

ACKNOWLEDGMENTS

We are grateful to the unknown reviewer who contributed much to the presentation of this article.

GRANTS

This study was supported by the Croatian Ministry of Science, Education and Sports, Grant Nos. 216-2160133-030 and 216-2160133-0130.

REFERENCES