

The human spleen as an erythrocyte reservoir in diving-related interventions

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Espersen, Kurt, Hans Frandsen, Torben Lorentzen, Inge-Lis Kanstrup, and Niels J. Christensen. The human spleen as an erythrocyte reservoir in diving-related interventions. *J Appl Physiol* 92: 2071–2079, 2002. First published October 12, 2001; 10.1152/jappphysiol.00055.2001.—Twelve subjects without and ten subjects with diving experience performed short diving-related interventions. After labeling of erythrocytes, scintigraphic measurements were continuously performed during these interventions. All interventions elicited a graduated and reproducible splenic contraction, depending on the type, severity, and duration of the interventions. The splenic contraction varied between ~10% for “apnea” (breath holding for 30 s) and “cold clothes” (cold and wet clothes applied on the face with no breath holding for 30 s) and ~30–40% for “simulated diving” (simulated breath-hold diving for 30 s), “maximal apnea” (breath holding for maximal duration), and “maximal simulated diving” (simulated breath-hold diving for maximal duration). The strongest interventions (simulated diving, maximal apnea, and maximal simulated diving) elicited modest but significant increases in hemoglobin concentration (0.1–0.3 mmol/l) and hematocrit (0.3–1%). By an indirect method, the splenic venous hematocrit was calculated to 79%. No major differences were observed between the two groups. The splenic contraction should, therefore, be included in the diving response on equal terms with bradycardia, decreased peripheral blood flow, and increased blood pressure.

splenic contraction; splenic venous hematocrit; simulated diving; diving response

IN MANY ANIMAL SPECIES, THE spleen serves as a reservoir for red blood cells (2, 18, 19, 22, 28, 31, 37). Contraction of the spleen is induced by food ingestion (8), exercise (19, 31, 37), arousal (19), hypoxia (21, 26), bleeding (2, 18), decrease in blood pressure (19), diving (22), and injection of catecholamines with a subsequent increase in hematocrit and hemoglobin concentration in the peripheral blood (19, 37). The variations in hematocrit and hemoglobin concentration induced by exercise are eliminated after splenectomy (21, 31).

This splenic reservoir function for the red blood cells has been questioned in humans as older studies showed no reservoir function for the red blood cells (1,

9), whereas newer studies using scintigraphic techniques have demonstrated considerable contraction of the spleen during exercise (11, 13, 29, 34).

Diving induces a series of hemodynamic changes described by the diving response or reflex (4, 16). The diving reflex consists of bradycardia, a decrease in cardiac output with preserved stroke volume, and peripheral vasoconstriction with a decrease in the peripheral blood flow in both animals and humans (7, 39). Several animal species perform diving under normotension (6, 40), but, in humans, simulated breath-hold diving (in the following, shortened to “simulated diving”) elicits an increase in blood pressure (39).

The diving reflex can be elicited by simulated breath-hold diving in both animals and humans (6, 7, 16, 39, 40), i.e., immersion of the face in water without other parts of the body getting wet. In humans, the diving reflex is reinforced when cold water (5–10°C) is used (16).

It has previously been shown that, in Korean Ama divers, a splenic contraction and a subsequent increase in hematocrit take place during breath-hold diving (23). The performance of this study was done with repetitive dives during a long time interval with a delay of 10–15 min before ultrasonic investigation of the splenic size.

In the present study, we wanted to test the hypothesis that the human spleen acts as a reservoir for red blood cells with splenic contraction and synchronously increases in hemoglobin content after diving-related interventions and to investigate the immediate time course of the splenic contraction under laboratory settings with continuously monitoring. Second, we wanted to test whether diving experience will reinforce the splenic contraction during diving-related interventions.

METHODS

Subjects. Twelve men without diving experience (*group I*) and ten men with diving experience (*group II*) were included in the study. All were volunteers and healthy. In *group I*, several subjects were going to a sports college. In *group II*, six of the subjects were performing underwater rugby on a medium-to-high level, and four were performing scuba diving on

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a regular basis. We distinguished between the two groups to investigate whether specific training (breath-hold techniques, habituation to water in the face) could influence the responses in splenic contraction and hematocrit. The demographic data of the volunteers were, in *group I*, 26 yr (23–29 yr), 180 cm (179–182 cm), and 72 kg (70–76 kg), and, in *group II*, 25 yr (24–28 yr), 183 cm (180–186 cm), and 80 kg (74–85 kg) (median with interquartile ranges in parentheses) for age, height, and weight, respectively. All were fasting, abstained from tobacco, and had not performed considerable exercise at least 12 h before the start of the study. The study was approved by the local ethics committee for the county of Copenhagen.

On arrival, an intravenous catheter (Venflon 2.0) was inserted in an antecubital vein, and a blood sample was drawn for labeling of the erythrocytes.

The *in vitro* labeling of the erythrocytes with ^{99m}Tc -per-technetate was performed with a commercially available preparation kit (CAD CA-6100, Cadema, Middletown, NY) with the addition of tin and hypochlorite (NaOCl , 0.1%) to obtain a high-binding efficiency and binding stability of the radioactive isotope without centrifugation of the erythrocytes (25). The binding efficiency of ^{99m}Tc to the erythrocytes was 96% (95–97%) in *group I* and 95% (94–97%) in *group II*.

Ten milliliters of blood were drawn 4–5 min after injection of ^{99m}Tc -labeled erythrocytes in 10 subjects in *group I*, and the count rate was measured on the same gamma camera after all of the interventions were performed and corrected for background emission and the ^{99m}Tc decay. The counting efficiency of the gamma camera was calculated as the ratio of measured radioactivity using the LEAP collimator and a known amount of radioactivity of ^{99m}Tc .

The blood volume was estimated by the injected amount of radioactivity divided by the radioactive counts per milliliter of blood times the counting efficiency of the gamma camera.

A dynamic scintigraphy was started 1 min before each stimulus with 2 s per frame and a duration of 254 s. Regions of interest (ROIs) were drawn manually over the spleen before, at the end of the intervention, and 1 and 2 min after the stimulus in *group I*. In *group II*, only three ROIs were drawn: before, at the end of the intervention, and 2 min after the intervention. In these ROIs, the spleen area and the count rate in the spleen were calculated. Three to four separate areas were used to minimize movement artifacts and to register the size of the spleen over time. Movement of the target organ with regard to the gamma camera during the interventions was minimized partly by a slight fixation of the subjects to the gamma camera and partly by definition of the ROI for each analysis period during the data collection.

The count rate in the spleen is an estimate of the content of erythrocytes in the spleen. Splenic contraction in the different interventions was determined as the reduction in content of erythrocytes and in the splenic area.

A measurement of the depth of the spleen was performed in two subjects in *group I*. The subjects had, in the prone position, a 5-cm radioactive indicator placed on the back, the gamma camera was placed vertically along the left side of the thorax, and the distance to the center of the spleen was measured. The measurement was the same for the two subjects and was, consequently, used for all of the subjects in *group I*. An attenuation factor, obtained using this distance in an attenuation curve for the gamma camera, was used to estimate the scintigraphic volume of the spleen. The scintigraphic volume of the spleen was estimated as the radioactivity count in the spleen times the attenuation factor divided by the radioactivity count in the blood. The scintigraphic volume of the spleen was calculated in the same interven-

tions, in which the measurement of the splenic volume by ultrasound was performed.

Ultrasonic estimations of splenic volume were performed before and 1–2 min after the intervention at two simulated diving interventions in eight subjects from *group I* in the prone position. A 3.0-MHz mechanic sector scanner (model 1846, Brüel and Kjær, Copenhagen, Denmark) was used to visualize the spleen. An image of the spleen in a longitudinal section was frozen on the ultrasound monitor. The splenic circumference on the monitor was marked with a light pen, and the splenic sectional area could then be calculated by the ultrasound scanner. Before and after each simulated dive, five estimates of the splenic sectional area were performed, and the median value was used in the calculation of the splenic volume according to Koga (27).

The heart rate was continuously monitored with an electrocardiogram, and the blood pressure was measured continuously (beat to beat) with a noninvasive method with a cuff on the third finger and the hand placed at heart level (Finapres system). The heart rate and blood pressure were analyzed over a 2-min interval before and 1 min after the interventions. The data analyses during the interventions were performed at the start and at the end of all interventions over approximately five heartbeats. The heart rate was measured as the mean R-spoke frequency at the electrocardiogram or from the systolic blood pressure peaks. In the same time interval, the mean systolic and diastolic blood pressure was estimated, and mean arterial pressure was calculated from the area under the blood pressure curve. Blood flow was measured in the right calf with venous occlusion plethysmography using the strain-gauge technique. The blood flow in the calf was estimated as relative changes and expressed in arbitrary units. The blood flow values were calculated as a mean of at least three measurements before and after the interventions and as single values during the interventions.

Hemoglobin concentration was measured on a CO-oximeter (OSM-3, Radiometer, Copenhagen, Denmark) as the mean of double measurements and hematocrit with ultracentrifugation (12,000 rpm, 5 min; Haemofuge A, Sepatech, Heraeus, Osterode, Germany) as the mean of three measurements. The hematocrit tubes had a length of 100 mm. The length of the column of erythrocytes was measured with a 0.5-mm accuracy by the same person throughout the whole study. The coefficients of variation for the measurements of hemoglobin concentration and hematocrit were estimated by 10 repeated measurements of the same blood sample twice during the study. The coefficients of variation for hemoglobin concentration were 0.99 and 1.12% and for hematocrit measurements were 0.7 and 0.5%.

The hemoglobin concentration in the spleen ($\text{Hb}_{\text{spleen}}$) was calculated by use of blood volume (BV), hemoglobin concentration before ($\text{Hb}_{\text{before}}$) and after diving (Hb_{after}), and the reduction in scintigraphic volume of the spleen ($\Delta\text{volume}_{\text{spleen}}$) by the following formula

$$\text{Hb}_{\text{spleen}} = [(\text{BV} + \Delta\text{volume}_{\text{spleen}}) \times \text{Hb}_{\text{after}} - (\text{BV} \times \text{Hb}_{\text{before}})] / \Delta\text{volume}_{\text{spleen}}$$

Plasma epinephrine (E) and norepinephrine (NE) were measured with a radioenzymatic technique (5). Intra-assay coefficients of variation for NE and E in samples containing normal basal values were 6 and 8%, respectively ($n = 10$). Corresponding values of interassay coefficients of variation for NE and E were 7 and 11%, respectively. The sensitivity of the assay, calculated as three times the standard deviation of the analytic blank, was, for intra-assays, 0.3 and 0.5 pg/assay for E and NE, respectively. Corresponding values for interas-

Table 1. *Relative content of erythrocytes in the spleen and relative splenic area in subjects without diving experience (group I)*

Intervention	Before	Relative Content of Erythrocytes			Relative Splenic Area		
		End	1 min After	2 min After	End	1 min After	2 min After
Apnea	100	84.6† (82.6–87.4)	94.9 (92.4–102.8)	100.0 (100.0–103.0)	88.3† (85.6–90.0)	96.5 (92.2–98.6)	100.0 (100.0–103.0)
Cold clothes	100	87.2* (85.4–92.9)	96.6 (93.3–98.9)	99.3 (96.5–101.4)	88.3* (81.9–96.2)	97.4 (93.7–100.0)	100 (100.0–100.0)
Simulated diving	100	71.3†‡ (67.0–73.7)	89.3 (85.6–93.7)	99.0 (97.0–103.0)	73.3†‡ (67.9–75.4)	89.3 (85.3–96.2)	100.0 (97.0–103.0)

Values are median values before, at the end of, 1 min after, and 2 min after different interventions (in %). Interquartile ranges are in parentheses. * $P < 0.05$ and † $P < 0.01$ compared with the initial values. ‡ $P < 0.01$ compared among the different values at the end of the interventions.

says were 0.5 pg/assay for both E and NE. The method has been described by Knudsen et al. (26).

Procedure. All experiments were performed in the morning. After a labeling period of 1 h, the subjects were placed in the prone position, and the ^{99m}Tc -labeled erythrocytes were injected. A gamma camera (Siemens, ZLC) with a “low-energy, all-purpose” collimator was horizontally placed immediately over the spleen region.

After 15 min of rest, all subjects performed “apnea” once or twice, independent of respiration phases and without any instructions, and simulated diving (breath hold during immersion of the face in 10°C cold water) five times in *group I* and three times in *group II*, and ice-cold, wet clothes were placed on the face and on the front of the neck once with the subjects still breathing normally (“cold clothes”). Each intervention lasted 30 s. The subjects in *group II*, in addition, performed apnea and simulated diving as long as possible (“maximal apnea” and “maximal simulated diving”) twice each. Ten to fifteen minutes of rest were interposed between each stimulus. The interventions were performed in the same sequence, unknown to the subjects.

Heart rate, blood pressure, and blood flow in the calf were continuously registered during the whole study. Blood samples were drawn 1 min before and 1 min after each stimulus for measurements of hemoglobin concentration, hematocrit, and plasma concentration of E and NE.

Statistics. All data are presented as median values and interquartile ranges.

The scintigraphic data and the values from the plethysmographic blood flow measurements are shown in relative values.

Nonparametric analysis of variance by repeated measurements (Friedman test) was performed to estimate 1) whether the changes in repeated performance of the same intervention were constant; 2) whether initial values of hemoglobin concentration, hematocrit, plasma catecholamines, heart rate, and blood pressure were unchanged compared with each intervention; and 3) whether there were differences in the response to each intervention. A Wilcoxon rank-sum test for paired data was performed if there was a significant difference in the Friedman test. The changes in hemoglobin concentration, hematocrit, and plasma catecholamines in each intervention were also tested with Wilcoxon rank-sum test for paired data. In comparison between groups, the Mann-Whitney rank-sum test for unpaired data was used.

A P value < 0.05 was regarded as significant.

RESULTS

Group I. The relative content of erythrocytes in the spleen and the relative splenic area in the different interventions from *group I* are presented in Table 1.

Apnea, simulated diving, and cold clothes contracted the spleen significantly between 10 and 29%. The splenic contractions in the different interventions were significantly different, with regard to both the content of erythrocytes and the splenic area, and the simulated diving caused a larger splenic contraction than did the other interventions.

The size of the spleen was quickly normalized, being 88–97% 1 min after and 99–100% 2 min after each intervention.

The spleen diminished its area and erythrocyte content between 27 and 37% in the repeated simulated diving. There was no significant difference in the decrease in the content of erythrocytes or in the decrease in splenic area among the different simulated dives (data not shown). The mean coefficient of variation for the five repeated simulated dives for the subjects was 0.09 with a standard deviation of 0.03.

Ultrasonic measurements of the volume of the spleen, performed in two simulated dives in eight subjects, were estimated to be 214 ml (196–298 ml) before and 200 ml (182–285 ml) 1–2 min after the simulated dives ($P < 0.05$). The concurrent scintigraphic estimate of the splenic volume was 249 ml (193–273 ml) before the stimulus.

The hemoglobin concentration and hematocrit before and after the different interventions are shown in Table 2. There were no changes in the initial values of hemoglobin, hematocrit, or plasma E and NE among the interventions.

Table 2. *Hemoglobin concentration and hematocrit in subjects without diving experience (group I)*

Stimulus	Hb, mmol/l		Hct, %	
	Before	After	Before	After
Apnea	8.9 (8.8–9.9)	8.9 (8.7–9.9)	41.3 (37.8–42.8)	40.8 (38.1–42.5)
Cold clothes	9.0 (8.9–9.8)	9.0 (8.9–9.8)	40.4 (37.7–41.9)	40.7 (37.8–43.4)
Simulated diving	8.9 (8.8–9.9)	9.0*† (8.9–9.9)	40.7 (38.0–41.6)	41.0*† (39.4–42.9)

Values are median values before and after each intervention. Interquartile ranges are in parentheses. Hct, hematocrit. * $P < 0.05$ compared with start value; † $P < 0.05$ compared among the interventions.

Table 3. Catecholamine values from subjects without diving experience (group I) and subjects with diving experience (group II) during the different interventions

Intervention	Group I				Group II			
	Epinephrine		Norepinephrine		Epinephrine		Norepinephrine	
	Before	After	Before	After	Before	After	Before	After
Apnea	0.035 (0.03–0.04)	0.04* (0.035–0.05)	0.155 (0.135–0.20)	0.195† (0.155–0.24)	0.035 (0.023–0.045)	0.04* (0.03–0.06)	0.14 (0.09–0.19)	0.15 (0.09–0.19)
Maximum apnea					0.035 (0.03–0.045)	0.055* (0.375–0.083)	0.153 (0.085–0.198)	0.18 (0.155–0.27)
Simulated diving	0.04 (0.03–0.05)	0.055* (0.04–0.08)	0.17 (0.12–0.195)	0.235‡ (0.17–0.315)	0.033 (0.022–0.047)	0.048† (0.027–0.06)	0.139 (0.097–0.2)	0.175† (0.112–0.313)
Maximum simulated diving					0.04 (0.0275–0.0575)	0.0675† (0.055–0.108)	0.1975 (0.1075–0.3025)	0.2575† (0.225–0.4025)
Cold clothes	0.03 (0.02–0.05)	0.04 (0.025–0.04)	0.165 (0.155–0.215)	0.205† (0.175–0.225)	0.03 (0.02–0.04)	0.03 (0.025–0.05)	0.16 (0.115–0.26)	0.185 (0.12–0.255)

Values are median values before and after (>2 min) interventions. Interquartile ranges are in parentheses. * $P < 0.05$ and † $P < 0.01$ compared with initial value; ‡ $P < 0.05$ compared among the interventions.

A significant increase in hemoglobin concentration of 0.1 mmol/l and hematocrit of ~0.5% was seen after simulated diving, but there were no changes due to the other interventions.

The catecholamine values are represented in Table 3. Apnea and simulated diving led to significant increases in plasma E and NE concentrations, whereas cold clothes only increased the NE concentration. The increase in plasma NE after simulated diving was greater compared with the other interventions (Mann-Whitney, $P < 0.05$).

The hemodynamic data for all the interventions are shown in Table 4. The initial values for heart rate and blood pressure were not different in the different interventions.

A primary rise in heart rate immediately after the start of the intervention, a secondary fall in heart rate at the end of the intervention (~25%), an increase in blood pressure (~21%), and a reduction in blood flow in the calf (~70%) at the end of the intervention were seen after the simulated diving.

During the application of the cold clothes, a reduction in heart rate ($P < 0.05$) was seen without any changes in mean arterial pressure or blood flow in the calf.

At the end of apnea, a rise in mean arterial pressure (~14%) and a reduction in blood flow in the calf (~25%) were seen without any changes in heart rate.

The initial values for heart rate and blood pressure and the values obtained 1–2 min after the end of the interventions were similar.

The reduction in heart rate and blood flow and the rise in blood pressure were greater in the simulated diving compared with the other interventions ($P < 0.0001$, $P < 0.001$, and $P < 0.0001$, respectively). The diving response (bradycardia, reduction in peripheral blood flow, and rise in blood pressure) was unchanged in the five repeated simulated dives.

The blood volume was calculated to be 4.97 liters (4.33–5.75 liters), equivalent to 71 ml/kg (57–77 ml/kg).

The hemoglobin concentration in the splenic vein was calculated to be 17.5 mmol/l (17.2–17.9 mmol/l) after the simulated diving. This corresponds to a hematocrit of 79% in the splenic vein with a mean cell hemoglobin concentration of 22 mmol/l.

Because of the shortage of gamma camera capacity, radioactive labeling of the erythrocytes and scintigraphy was not performed in two of the subjects in group I, but all of the other data from these subjects were used in the analysis.

Five of the subjects in group I did not have blood samples taken 1 min after each intervention.

Group II. The relative scintigraphic content of erythrocytes in the spleen and the relative scintigraphic splenic area during the different interventions are presented in Table 5.

Table 4. Hemodynamic data in subjects without diving experience (group I)

Intervention	Heart Rate, beats/min				Mean Arterial Blood Pressure, mmHg				Calf Blood Flow, relative values		
	Before	Start	End	After	Before	Start	End	After	Before	End	After
Apnea	63 (58–81)	62 (60–79)	58 (53–78)	62 (59–81)	89 (88–103)	94* (93–106)	103* (96–113)	92 (91–102)	100	75* (64–90)	112 (102–116)
Cold clothes	67 (59–71)	66 (56–73)	59* (52–69)	66 (59–72)	92 (84–105)	90 (80–108)	90 (86–110)	90 (86–108)	100	70 (55–97)	100 (94–102)
Simulated diving	68 (63–88)	87* (65–95)	51*† (41–59)	66 (57–78)	95 (93–110)	101 (97–113)	118*† (117–144)	97 (94–106)	100	31*† (14–47)	103 (89–122)

Values are median values before, at the start of, at the end of, and after (>2 min) the interventions, with the interquartile ranges in parentheses. * $P < 0.05$ compared with initial value; † $P < 0.01$ compared among the interventions.

Table 5. Relative content of erythrocytes in the spleen and relative splenic area in subjects with diving experience (group II)

Intervention	Before	Relative Content of Erythrocytes		Relative Splenic Area	
		End	2 min After	End	2 min After
Apnea	100	89.9* (87.3–94.9)	99.0 (98.4–100.6)	89.9* (87.3–94.9)	99.4 (96.9–100.0)
Cold clothes	100	93.2* (88.7–96.8)	97.4 (94.4–101.3)	91.9* (80.9–96.9)	100.0 (97.9–100.0)
Simulated diving	100	77.0* (69.4–79.6)	96.0 (92.9–98.7)	79.8* (74.6–84.0)	98.4 (94.6–110.2)
Maximum apnea	100	73.4* (71.0–83.8)	97.1 (96.3–105.1)	75.7* (73.5–79.5)	100.0 (96.6–105.7)
Maximum simulated diving	100	66.0* (55.9–70.9)	96.4 (87.9–99.6)	69.0* (62.4–74.2)	94.0 (89.8–112.0)

Values are median values (in %). Interquartile ranges are in parentheses. * $P < 0.01$ compared with initial value.

Significant reductions in the content of erythrocytes in the spleen and the splenic area were shown at the end of all interventions, similar to and of the same differentiated magnitude as that in *group I*. The splenic contraction under maximal simulated diving was significantly greater compared with simulated diving and maximal apnea, which again were significantly greater compared with apnea and cold clothes ($P < 0.001$). Two minutes after each intervention, the content of erythrocytes and the splenic area were restored.

Maximal simulated diving resulted in a greater splenic contraction than simulated diving in *group I* (Mann-Whitney, $P < 0.05$).

The scintigraphic measured volume of the spleen was calculated to be 302 ml (241–371 ml), not significantly different from that in *group I*.

The duration of maximal apnea and maximal simulated diving was 82.5 s (65–107 s) and 93.5 s (76–130.5 s), respectively ($P < 0.05$). There was no difference between first and second performance of both of these interventions: 83 s (67–100 s) and 84 s (64–114 s) for maximal apnea and 89 s (65–116 s) and 99 s (83–143 s) for maximal simulated diving, respectively.

The hemoglobin concentration and hematocrit before and after the different interventions are shown in Table 6. There were no changes in initial values of hemoglobin concentration, hematocrit, and plasma E and NE among the interventions. Increases in hemo-

globin concentration in the range of 0.1–0.3 mmol/l and in hematocrit in the range of 0.4–1.0% were found in maximal simulated diving, simulated diving, and maximal apnea. No changes were seen in hemoglobin concentration and hematocrit in apnea and cold clothes. Catecholamine values are presented in Table 3. Increases in E and NE concentration from before to after the interventions were found in maximal simulated diving and simulated diving, but in maximal apnea only a rise in E concentration was seen. Increases in hemoglobin concentration, hematocrit, and catecholamines in the interventions were of similar magnitude as those in *group I*.

The hemodynamic data for all of the interventions are shown in Table 7. The initial values for heart rate and blood pressure were not different for the different interventions.

A rise in heart rate was seen immediately after the start of the interventions in apnea, simulated diving, and maximal simulated diving. In all interventions except apnea and cold clothes, a reduction in heart rate, a rise in blood pressure, and reduction in peripheral blood flow at the end of the intervention were seen. Only a rise in blood pressure and reduction in peripheral blood flow at the end of the intervention were seen in apnea. The reduction in heart rate and the rise in blood pressure at the end of maximal simulated diving, simulated diving, and maximal apnea were equal. The

Table 6. Hemoglobin concentration and hematocrit in subjects with diving experience (group II)

Intervention	Hb, mmol/l		Hct, %	
	Before	After	Before	After
Apnea	9.9 (9.5–10.0)	9.7 (9.6–9.9)	40.2 (39.1–41.9)	40.3 (39.9–42.6)
Cold clothes	9.9 (9.5–10.1)	10.0 (9.6–10.1)	41.0 (40.0–43.5)	41.1 (40.0–42.5)
Simulated diving	9.8 (9.5–10.0)	9.9† (9.6–10.1)	40.5 (39.7–43.3)	41.1† (40.1–42.8)
Maximum apnea	9.9 (9.5–10.1)	10.0* (9.6–10.1)	41.1 (39.8–42.5)	42.0† (40.6–43.0)
Maximum simulated diving	9.8 (9.5–10.1)	10.1† (9.6–10.2)	41.3 (39.5–42.6)	42.3† (40.4–43.2)

Values are median values before and after each intervention. Interquartile ranges are in parentheses. * $P < 0.05$ and † $P < 0.01$ compared with initial value.

Table 7. Hemodynamic data in subjects with diving experience (group II) during the different interventions

Intervention	Heart Rate, beats/min				Mean Arterial Pressure, mmHg				Calf Blood Flow, relative values		
	Before	Start	End	After	Before	Start	End	After	Before	End	After
Apnea	57 (50–75)	62* (52–85)	59 (49–72)	58 (51–72)	90 (86–97)	94 (92–104)	108* (96–120)	91 (87–98)	100	69* (49–86)	96 (75–102)
Cold clothes	62 (57–74)	62 (56–77)	66 (56–73)	66 (57–75)	95 (89–107)	98 (88–111)	102 (90–108)	102 (94–107)	100	93 (69–123)	106 (90–112)
Simulated diving	70 (55–79)	80* (63–100)	49* (46–64)	66 (54–75)	98 (90–104)	101 (95–115)	122* (94–130)	98 (91–103)	100	43*† (23–62)	94 (87–102)
Maximum apnea	73 (55–81)	69 (56–96)	56* (49–73)	70 (54–79)	91 (79–99)	93 (80–105)	111* (91–150)	95 (82–99)	100	39*† (36–48)	80 (65–109)
Maximum simulated diving	69 (58–84)	80* (77–106)	46* (44–62)	66 (58–77)	98 (92–108)	97 (96–112)	120* (116–150)	101 (95–106)	100	25*† (16–36)	90 (68–104)

Values are median values before, at the start of, at the end of, and after (>2 min) interventions. Interquartile ranges are in parentheses. * $P < 0.05$ compared with initial value; † $P < 0.05$ compared with apnea of the reduction in blood flow.

reductions in peripheral blood flow were equal in maximal simulated diving, simulated diving, and maximal apnea but greater than in apnea ($P < 0.05$). In cold clothes, no changes in the measured hemodynamic variables were seen. The reduction in heart rate and the increase in blood pressure in simulated diving were greater in *group I* than in *group II* (both $P < 0.05$). In the other comparative interventions, there was the same response in the two groups.

The blood volume was calculated to be 4.98 liters (4.7–5.7 liters). The hemoglobin in the splenic vein was calculated to be 14.7 mmol/l (12.2–19.4 mmol/l) in simulated diving, 14.3 mmol/l (12.5–21.9 mmol/l) in maximal simulated diving, and 13.7 mmol/l (13.4–23.8 mmol/l) in maximal apnea.

DISCUSSION

Contraction of the spleen could be demonstrated in all interventions in both groups. The reduction in both the splenic area and the content of erythrocytes in the spleen ranged between 12 and 30% in *group I* and between 7 and 32% in *group II* by the end of the interventions. This is in agreement with many animal studies and the latest human studies, where a splenic contraction of 40–70% is shown at maximal exercise (11, 13, 29, 34).

The splenic contraction in maximal simulated diving was significantly greater than the splenic contraction in simulated diving and maximal apnea, which again was greater than the splenic contraction in apnea and in application of cold clothes in *group II*. The reduction in size was 25% or more in the first three mentioned interventions. The same differences in splenic contraction in the different interventions were seen in *group I*, where the splenic contraction in simulated diving was greatest.

According to the above, an increase in the hemoglobin content in the blood was seen in *group I* in simulated diving and in *group II* in maximal simulated diving, simulated diving, and maximal apnea. This is in concordance with Qvist et al. (33), who found hematocrit increases in Korean diving women, when diving was performed without breathing equipment, but not

during apnea in the laboratory. In both groups, changes in the hematological variables were found only in situations with a high degree of splenic contraction, whereas less pronounced contraction (<25%) gave no detectable changes in hemoglobin concentration and hematocrit. The most reasonable explanation for this is that the sensitivity of the used equipment for measuring the hemoglobin concentration and hematocrit was too small to detect these relatively small changes in hematological variables in apnea and cold clothes.

The splenic contraction was repeatable and reproducible, as shown in the repeated simulated diving in *group I*. No fatigue or adaptation of this phenomena was shown, indicating that the time span between each intervention was sufficient. This was also supported by the fact that the scintigraphic splenic area had returned to control dimensions after ~2 min.

The reduction in the size of the spleen and in the content of erythrocytes in simulated diving is in accordance with the findings of Hurford et al. (23) in Korean diving women, but the time for the measurements was not the same. Hurford et al. found a prolonged reduction in the size of spleen (~20%) 10–20 min after 3 h of intermittent breath-hold diving without diving equipment. An explanation for this extended reduction in splenic size could be dehydration, hypovolemia, or extravascular volume displacement in connection with prolonged exercise and insufficient hydration, because we show an immediate normalizing of the splenic size after the termination of diving interventions. All of these explanations could be included in the assumption of Hurford et al. about decreased plasma volume. This can also explain the considerable increase in hemoglobin concentration and hematocrit values found in this study (23). Whole body immersion in thermoneutral water is followed by hemodilution (20), but immersion in cold water (<25°C) reduces plasma volume because of increased diuresis (17). There was no reduction in splenic size in subjects without experience in performing breath-hold diving form in the same study (23). This is also contradictory to our results, in which subjects both with and without diving experience developed a 30% reduction in splenic area during 30 s of simulated diving.

The splenic vein hematocrit is estimated to be almost the double of the value in the peripheral blood using the indirect method, as described in METHODS. This is in agreement with earlier animal studies (10, 12, 14, 15) and in patients with hematological disease with and without splenomegaly (41). In healthy subjects, the splenic hematocrit has not been determined before.

All interventions induced splenic contraction. The obvious explanation must be a direct stimulation via the sympathetic nerves to the spleen because of the short duration of the interventions and the fast restoration of the spleen. A differentiated nervous response due to the different interventions was obtained. The apnea elicited a sympathetic response mainly via α -receptors (vasoconstriction) with an increase in mean arterial blood pressure and a reduction in blood flow in the calf. The cold clothes elicited, contrary to this, a weak vagal stimulation (bradycardia), which must be elicited from thermoreceptors in the face. The simulated diving, which is a combination of apnea and a cold and wet stimulus of the face, had a mixed sympathetic and vagal stimulation (vasoconstriction and bradycardia) as mentioned earlier (16). The simulated diving elicited, in our study, an increase in both E and NE concentration, where the increase in NE concentration was significantly greater compared with that in apnea and cold clothes. Both the increase in NE concentration and the changes in the hemodynamics indicate an additive and/or synergistic effect of apnea and cold clothes in simulated diving, as shown earlier (30).

In *group II*, the duration of maximal simulated diving was 13% longer than the duration of maximal apnea ($P = 0.01$). This is in accordance with Schagatay and Andersson (35), who showed that the apneic time was prolonged during simulated breath-hold diving in relation to apnea in air. These results are in contrast to those of others (3). The greater splenic contraction with subsequently greater emptying of oxygenated blood and thereby an increase in oxygen transport capacity in the blood can explain the prolonged endurance seen in maximal simulated diving seen in our study. This suggests an oxygen-conserving mechanism in subjects with diving experience.

The greatest cardiovascular changes in diving-related interventions have been reported with 5–10°C cold water (30) lasting ~30 s (24). The interventions in our study are in accordance with this.

No correction for background emission was done. The correct placement of the ROI for measurement of background radioactivity emission was in the area from where the spleen had retracted during the splenic contraction. The placement of this area was not possible in the interventions, where the splenic contraction was modest. If the correction for background radioactivity emission were done only in selected interventions, the comparison between the interventions would have been on a wrong premise. An area of interest for background correction was placed in the area from where the spleen had retracted during one simulated dive in one subject. With this correction procedure, the splenic contraction was ~46% compared with 30%

without background correction. This means that the shown values for splenic contraction are minimum values and the background-corrected splenic contraction in our study was in the same range as splenic contraction during maximal exercise (11, 13, 29, 38). In other studies (11, 13, 29, 34), a correction for background emission was performed in different ways or not at all in one study.

Earlier it was postulated that the human spleen could not contract because of the lack of smooth muscle tissue in the spleen. Pinkus et al. (32) have shown that the human spleen contains smooth muscle tissue with the use of highly specific rabbit antibodies to human uterine smooth muscle myosin and an indirect immunoperoxidase technique. These smooth muscle cells are localized in short, regular, orderly, and repetitive bands parallel to the longest axis of the sinus in red pulpa. In the white pulpa, the reticular cells contain myosin (32). These contractile elements containing myosin are constructed so that the documented splenic volume changes can presumably be due to a direct activation of these repetitive structures (32). Several histological studies performed in animals have shown a high density of sympathetic innervation of splenic capsule, trabeculae, and the red pulpa, which further explains the capability of the spleen to function as a reservoir.

The simulated diving in the two groups induced the diving response with bradycardia, decreased peripheral blood flow, and increasing blood pressure, as shown earlier (16). In our study, there was a significant short increase in heart rate immediately after the start of simulated diving, as shown before (39). This rise in heart rate could be due to an early, rapid sympathetic activation of the heart and/or an inhibition of the vagal (parasympathetic) tone by the touch of the cold water. The latest statement is the most probable explanation because of this short-lasting rise in heart rate during normotension. Other explanations could be a psychological arousal, hyperventilation before the start of the simulated diving (36), or an active movement of the upper body, but, in these circumstances, one would expect a concomitant increase in blood pressure. An attempt was made to control these factors in the subjects, who were unaware of the start of the intervention until a few seconds before start, and by a light fixation to the gamma camera.

The spleen with its contractile properties and the splenic reservoir are or can be involved in several compensatory mechanisms. All findings in humans and animals would indicate more consideration to the hemodynamics in splenectomized patients during medical procedures.

Conclusion. The human spleen acts as a reservoir for red blood cells, and contraction of the spleen leads to synchronous increases in hemoglobin content after exposure for diving-related interventions.

All interventions, even very short (~30 s), elicited a graduated splenic contraction, depending on the type, severity, and duration of the interventions. The splenic contraction varied between ~10% for apnea and cold

clothes and ~30–40% for simulated diving, maximal apnea, and maximal simulated diving. The response time for the splenic contraction was short, with a rapid time-dependent contraction and with a rapid restoration of the spleen to initial values.

The strongest interventions (simulated diving, maximal apnea, and maximal simulated diving) elicited an increase in hemoglobin concentration and hematocrit. These modest, but significant, increases have an uncertain clinical importance. The subsequent increase in the oxygen transport capacity of the blood can, however, contribute to the longer duration of maximal simulated diving compared with maximal apnea.

The splenic contraction was reproducible in repeated exposure of simulated diving. Therefore, the splenic contraction should be included in the diving response on equal terms with bradycardia, decreased peripheral blood flow, and increased blood pressure.

With an indirect method using the increase in hemoglobin concentration, the reduction in splenic volume, and the total blood volume, the splenic hematocrit was estimated to be considerably higher (79%) than the hematocrit in the peripheral blood.

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