J Appl Physiol
90: 1623–1629, 2001.

highlighted topics

Physiological and Genomic Consequences of Intermittent Hypoxia
Selected Contribution: Role of spleen emptying in prolonging apneas in humans

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Received 10 October 2000; accepted in final form 16 January 2001

Schagatay, Erika, Johan P. A. Andersson, Magnus Hallén, and Birger Pålsson. Selected Contribution: Role of spleen emptying in prolonging apneas in humans. *J Appl Physiol* 90: 1623–1629, 2001.—This study addressed the interaction between short-term adaptation to apneas with face immersion and erythrocyte release from the spleen. Twenty healthy volunteers, including ten splenectomized subjects, participated. After prone rest, they performed five maximal-duration apneas with face immersion in 10°C water, with 2-min intervals. Cardiorespiratory parameters and venous blood samples were collected. In subjects with spleens, hematocrit and hemoglobin concentration increased by 6.4% and 3.3%, respectively, over the serial apneas and returned to baseline 10 min after the series. A delay of the physiological breaking point of apnea, by 30.5% (17 s), was seen only in this group. These parameters did not change in the splenectomized group. Plasma protein concentration, preapneic alveolar P_{CO_2}, inspired lung volume, and diving bradycardia remained unchanged throughout the series in both groups. Serial apneas thus triggered the hematological changes that have been previously observed after long apneic diving shifts; they were rapidly reversed and did not occur in splenectomized subjects. This suggests that splenic contraction occurs in humans as a part of the diving response and may prolong repeated apneas.

diving response; breath-hold; bradycardia; vasoconstriction; blood pressure

IN SOME MAMMALS, THE SPLEEN is known to serve as a dynamic red blood cell reservoir. Erythrocyte release from the spleen causes elevated hematocrit (Hct) and increased hemoglobin (Hb) concentration, e.g., during exercise (26) or, in aquatic mammals, during diving (16, 27). Release of red blood cells into the circulation may contribute to prolonged dives in some diving mammals like the Weddell seal, which can stay submerged for more than 1 h (2). A rapid increase of Hb and Hct was observed during the first 10–12 min of diving in Weddell seals by 48 and 44%, respectively (27). The red blood cells were removed from the circulation within 12–16 min of recovery. The rapid sequestration of the red blood cells in the spleen may serve to lower blood viscosity between periods of diving activity, thereby meeting the conflicting demands of high circulatory perfusion and increased oxygen storage when needed (7). Splenic emptying has also been observed in humans (8, 10, 21). A two-thirds decrease of the spleen erythrocyte content was observed in humans at maximal exercise (21). A 20% decrease in splenic volume accompanied by a 10% increase in Hct and a 9% increase in Hb was found in working ama divers after a 3-h diving shift (17). However, hemoconcentration due to diuresis was not excluded as the cause. Because no measurements were performed during the diving activity period (17), it was not known whether the increase in Hct and Hb or the splenic contraction occurred early or late in the period of intense serial diving. The splenic size and Hct were unaffected by repetitive breath-hold diving in untrained control subjects.

Apneas repeated with <10-min intervals have been shown to prolong human breath-hold duration (13, 14, 19, 20, 34), but the responsible mechanisms have not

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been fully explained. In humans, with a limited duration of single dives but the ability to perform short-interval serial dives for extended periods, splenic contraction induced by the first dives of a series could possibly help explain the increase in apneic time observed with repeated apneas.

An apnea can be divided into two phases by the "physiological breaking point," which is reached when mainly the elevated arterial P_{CO_2} (Pa_{CO_2}) triggers involuntary breathing movements (14, 22). These phases have been termed the "easy-going phase" (EP) and the "struggle phase" (SP) because of the way they are experienced by the subject (4). During the latter, the diver feels an increasing urge to breathe until the apnea is terminated at the individual breaking point, when the need to breathe can no longer be psychologically tolerated (4, 14, 22). By recording the thoracic movements and identifying the first involuntary breathing movements, the physiological and psychological phases of apnea can be studied separately, allowing evaluation of their relative contributions to any prolongation of apneic time. The prolongation is caused by both physiological and psychological factors (14, 31). Apneic duration could be enhanced by a number of physiological factors involving the metabolic and/or gas storage levels. If hyperventilation occurs, leading to a progressively lowered alveolar P_{CO_2} ($P_{A_{CO_2}}$) before apneas, this may contribute to an increase in the EP and apneic time (13). However, a delay of the physiological breaking point has been observed despite preapneic $P_{A_{CO_2}}$ remaining at a constant level (30).

During apneic diving, humans react with a "diving response" (6), which is similar to that observed in aquatic mammals. The main features of this response are *1*) a vagally mediated bradycardia and *2*) a sympathetic α-adrenergic constriction of the peripheral arteries (11). Blood is diverted from organs that can tolerate transient asphyxia to the heart and brain (6), leading to oxygen conservation and prolonged apnea (1, 29, 33). The human diving response can be induced by simple apnea, but it is reinforced by face immersion in cold water (18). A delay of the onset of arterial oxygen desaturation by repetition of dives has been observed, leading to the suggestion that repetitive apneas may strengthen the diving response, thus enhancing the oxygen conservation and prolonging successive apneas (34). However, further study has revealed that reinforcement of the diving response is not involved in prolonging serial apneas (31).

The aim of this study was to investigate the effects of repeated apneas on Hct and Hb in intact and splenectomized subjects to reveal whether any observed changes could be linked to spleen function. Reversible increases in Hct and Hb occurring without an increase in plasma protein concentration and observed in intact subjects but not in splenectomized subjects would indicate spleen emptying as their cause. We hypothesize that, if spleen contraction occurs as a part of the human diving response, it may help explain the delay of the physiological breaking point seen with repeated apneas. This would be supported by a lack of delay of the physiological breaking point in splenectomized subjects.

METHODS

Subjects. Twenty healthy volunteers participated in these studies. Ten adults, 2 women and 8 men, were recruited for participation in the intact subject group, and 10 subjects, 2 women and 8 men, who had undergone splenectomy at least 4 yr before testing, participated in the splenectomized subject group. The splenectomized subjects were recruited by asking former patients, identified from medical records. Their indications for splenectomy were traumatic rupture of the spleen (5 subjects), Hodgkin's disease (3 subjects), and idiopathic thrombocytopenic purpura (2 subjects). All were considered cured from their respective medical condition. The ages and physiological data of the two groups did not differ significantly, and their levels of physical activity per week were in the same range (Table 1). None of the participants was a well-trained breath-hold diver, but some of the subjects had practiced breath-hold diving at a recreational level. There were four tobacco users (cigarettes or snuff) among the intact subjects and five among the splenectomized subjects. The experimental protocol was conducted in conformity with the principles of the Declaration of Helsinki and were ethically approved by the Research Ethics Committee of the Faculty of Medicine, Lund University.

Procedures. The procedures and potential risks involved were thoroughly explained and the equipment was demonstrated to the subjects, after which they gave written consent for participating in the study. The vital capacity was recorded in the standing subjects, and other physiological baseline values were collected. The subject then assumed a prone position on a mattress with the head on a rigid pillow that covered a water container. The chest bellows for recording thoracic movements were positioned, and vital capacity was measured in the prone position. The subject had a venous catheter inserted in the right arm, which was positioned at the heart level, and the probes used for cardiovascular recordings were attached to the right hand. The subjects rested in the prone position for 30 min, to ensure blood mixing and stabilization of the transcapillary fluid exchange (24). The ambient temperature was kept between 22.0 and 23.5°C, and water temperature was maintained between 9.5 and 11.0°C. The subjects were instructed to avoid hyperventilation and to

Table 1. *Subject characteristics for the two groups*

Subject Group	Age, yr	Height, cm	Weight, kg	Vital Capacity, liters	Physical Activity, h/wk
Intact	29 ± 7	180 ± 7	73 ± 7	5.6 ± 1	1.5 ± 2
Splenectomized	35 ± 7	176 ± 1	82 ± 2	5.1 ± 1	1.5 ± 3

Values are means ± SD. Physical activity refers to any sports or exercise that affects the aerobic capacity. No significant differences were observed between the groups.

keep the chest relaxed and refrain from swallowing or exhaling during apneas. They were instructed to expire to the residual volume and take a deep but not maximal breath through the spirometer mouthpiece before the apneas and to exhale completely through the mouthpiece after the apneas. Thus both inspired and expired lung volume before and after apneas were recorded, as well as the $P_{A_{CO_2}}$ at the start and end of apneas. The spirometer mouthpiece was held in the left hand by the subject and was taken out of the mouth during the apneas with face immersion. A nose clip was attached 30 s before the apnea; when 15 s remained, the cover to the water container was removed, and, after countdown during the last 10 s, apnea with face immersion was initiated. Each subject performed five apneas with face immersion in water spaced by 2 min of rest. The apneas were terminated at the individual breaking point without any information about the breath-hold duration. After each apnea, the nose clip was removed and the face was dried. During the 2-min periods of nonimmersed breathing, the head was supported on the cover of the water container. Cardiovascular and respiratory parameters were continuously recorded noninvasively. Venous blood samples of 2.5–3.5 ml were taken immediately before the first apnea and immediately after apnea *numbers 1*, *3*, and *5* and also after 3, 10, and 20 min of prone rest following the series of apneas. The venous catheter was flushed with a total volume of ~12 ml of sterile 0.9% NaCl solution. The total amount of blood taken from each subject was ~30 ml. This included waste samples taken before the collection of blood for analysis to account for the catheter dead space.

Measurements. Lung volumes were measured using a spirometer (Spirolite 201, Vise Medical, Chiba, Japan), with the mouthpiece connected to a CO_2 analyzer (Engström Eliza duo CO_2/O_2 analyzer, Gambro Engström, Bromma, Sweden) for end-tidal CO_2 sampling. Respiratory movements were recorded with the pneumatic chest bellows connected to an amplifier and an analog paper recorder. Continuous recording of cardiovascular parameters was done noninvasively. Heart rate (HR) and mean arterial pressure (MAP) were recorded with a photoplethysmometer (Finapres 2300, Ohmeda, Madison, WI) with the cuff on the middle finger. Skin blood flow was recorded with a laser-Doppler flowmeter (Advance Laser Flowmeter 21, Advance, Tokyo, Japan) with the probe connected to the thumb. The velocity measurements obtained with this technique are proportional to the flow, assuming that the capillary cross-sectional area remains unchanged (15). Analysis of Hb was done immediately in duplicate, and blood samples for Hct were drawn in triplicate. Blood samples for Hct and total protein analysis were stored for 1–2 h in a +8°C cooler. Microhematocrit and total protein blood samples were centrifuged, Hct was determined, and the plasma was stored at −20°C. Total plasma protein analysis was done within 1 wk on duplicate samples using the method of Lowry et al. (23). The method used to measure Hb has a resolution of 0.1 g/dl. The accuracy is ±0.3 g/dl. However, the duplicate analysis of one sample rarely differed, and, if they did, the difference was not larger than 0.1 g/dl. The Hct was determined manually using a standard Hct scale with a resolution of 0.5% units. It was determined as blinded samples with the triplicates separated. The triplicate analysis did not differ more than 0.5%.

Data analysis. Each subject served as his or her own control. Control values for HR, skin blood flow, and MAP were obtained from the period 90–30 s before each apneic episode. The apneic mean values of HR, skin blood flow, and MAP were obtained from the period 30–50 s into the apneas. For each apnea, the percent change from the corresponding control value was calculated. The physiological breaking point was identified from the recording of thoracic movements, and the durations of the EP and total breath-holding time were compared between the apneas. The analysis of EP only included subjects that reached a clearly detectable physiological breaking point in at least four of the apneas. Paired *t*-test with Bonferroni correction for multiple comparisons was used to compare the values of the first, third, and fifth apneas for all parameters, including lung volumes and $P_{A_{CO_2}}$ values within groups and to compare the Hb and Hct values between samples. Unpaired *t*-test was used for comparisons between groups. Significance was accepted at the 5% level.

RESULTS

Apneic time. The apneic time increased over the series of apneas in both groups, by 50.6 s (54.1%, $P < 0.01$) in intact subjects and by 23.5 s (40.9%, $P < 0.01$) in splenectomized subjects. In the intact group, the physiological breaking point could be detected in at least four of the five apneas in nine of the subjects and in the splenectomized group in six of the subjects. In the subjects with a detectable physiological breaking point, the increase in apneic time from *apnea 1* to *5* was 51.9 s (56.3%, $P < 0.01$) in intact subjects and 23.0 s (34.2%, $P < 0.05$) in splenectomized subjects (Fig. 1). When the contribution of the EP and SP to the increase in apneic time was evaluated in subjects with a detectable physiological breaking point, the EP duration was found to increase only in the intact subjects, by 16.6 s (30.5%, $P < 0.01$; Fig. 1). There was an increase in the SP duration by 35.3 s (93.2%, $P < 0.05$) in the intact subjects and by 17.7 s (147.2%, $P < 0.05$) in splenectomized subjects. The EP duration was the same in the two groups in the first apnea (intact = 54.2 s and splenectomized = 55.2 s), but the SP duration was longer in the intact subjects (intact 37.2 s and splenectomized 12.0 s, $P < 0.05$).

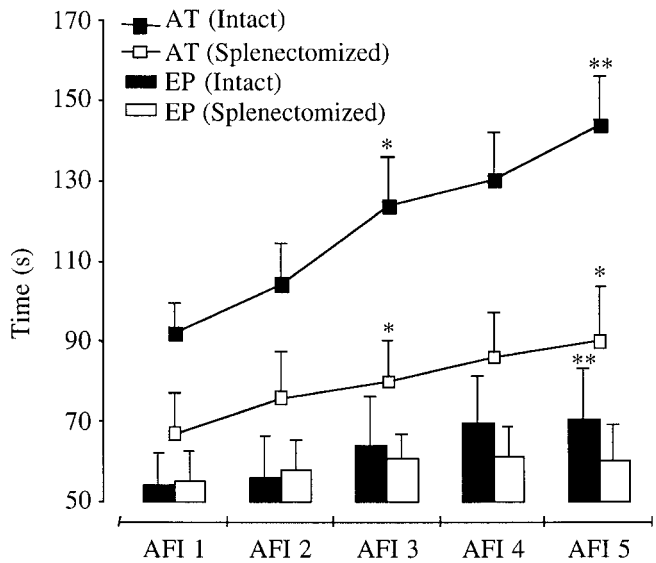

Fig. 1. Apneic time (AT) and duration of the easy-going phase (EP) over 5 apneas with face immersion (AFI) in intact ($n = 9$) and splenectomized ($n = 6$) subjects. Values are means ± SE. *Significant difference compared with AFI 1, $P < 0.05$; **significant difference compared with AFI 1, $P < 0.01$.

Table 2. *End-tidal P_{CO_2} and inspired and expired lung volume before and after the apneic face immersions in the intact and splenectomized subjects*

	Pre-AFI P_{CO_2}, Torr		Post-AFI P_{CO_2}, Torr		Insp LV, liters		Exp LV, liters	
	Intact	Splenect	Intact	Splenect	Intact	Splenect	Intact	Splenect
AFI 1	37.7 ± 1.2	36.7 ± 2.0	52.6 ± 1.4	52.0 ± 1.6	4.2 ± 0.2	3.8 ± 0.3	3.5 ± 0.4	3.9 ± 0.4
AFI 2	37.4 ± 1.4	34.5 ± 5.1	54.7 ± 1.1	51.8 ± 1.6	3.9 ± 0.3	3.9 ± 0.3	3.5 ± 0.2	3.8 ± 0.4
AFI 3	37.5 ± 1.3	33.6 ± 3.3	54.8 ± 1.0	48.4 ± 3.5	3.8 ± 0.3	3.9 ± 0.4	3.4 ± 0.3	3.7 ± 0.4
AFI 4	37.2 ± 1.3	33.3 ± 2.3	54.8 ± 1.4	55.7 ± 1.4	3.9 ± 0.2	3.8 ± 0.3	3.3 ± 0.3	3.7 ± 0.3
AFI 5	36.3 ± 1.1	33.4 ± 2.4	55.4 ± 1.3	53.7 ± 1.2	4.0 ± 0.3	3.8 ± 0.4	3.2 ± 0.2	3.3 ± 0.4

Values are means ± SE. Pre-AFI P_{CO_2}, end-tidal P_{CO_2} in the last breath before apnea; post-AFI P_{CO_2}, end-tidal P_{CO_2} in first breath after apnea; Insp LV, inspired lung volume before apnea; Exp LV, expired lung volume after apnea; AFI 1–5, apnea with face immersion *1–5;* Splenct, splenectomized. No significant differences for AFI 1–5 were found between any parameter or group.

Respiratory parameters. Preapneic and postapneic end-tidal $P_{A_{CO_2}}$ remained unchanged over *apneas 1–5* in both groups (Table 2). In addition, inspired lung volume before apneas remained unchanged at 75–80% of the prone vital capacity throughout the series in both groups (Table 2). Expired lung volume also did not change significantly throughout the series.

Hematological parameters. Hct and Hb increased significantly over the series of apneas in intact subjects, by 6.4 and 3.3%, respectively (Fig. 2*A*). Ten minutes after the final apnea, the values had returned to the levels observed before the series of apneas. In splenectomized subjects, no significant change of Hct or Hb was observed (Fig. 2*B*). No change in the total plasma protein concentration was observed in any of the groups over the apnea series (Fig. 3). There was a higher baseline Hct and a higher total plasma protein concentration in the splenectomized subjects (Hct = 40.9% and plasma protein = 80.1 mg/ml) than in the intact subjects (Hct = 37.2% and plasma protein = 65.7 mg/ml, $P < 0.05$). The Hb was the same in both groups (both = 14.2 g/dl).

Cardiovascular parameters. A diving response was observed in both groups during apnea (Fig. 4). In both groups, the reductions in HR and skin blood flow remained the same in each of the apneas of the series.

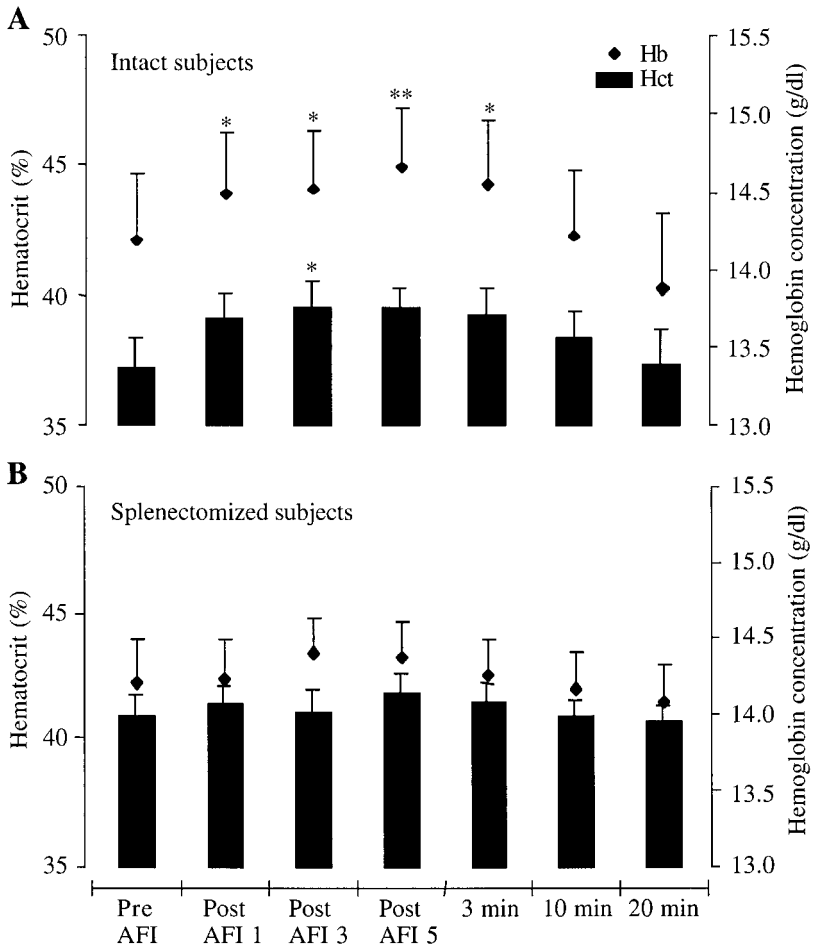

Fig. 2. Hematocrit (Hct) and hemoglobin (Hb) concentration before apneas with face immersion immediately after AFI 1, 3, and 5 and after 3, 10, and 20 min of recovery after the AFI series in intact (*A*) and splenectomized (*B*) subjects. Values are means ± SE. *Significant difference compared with pre-AFI, $P < 0.05$; **significant difference compared with pre-AFI, $P < 0.01$.

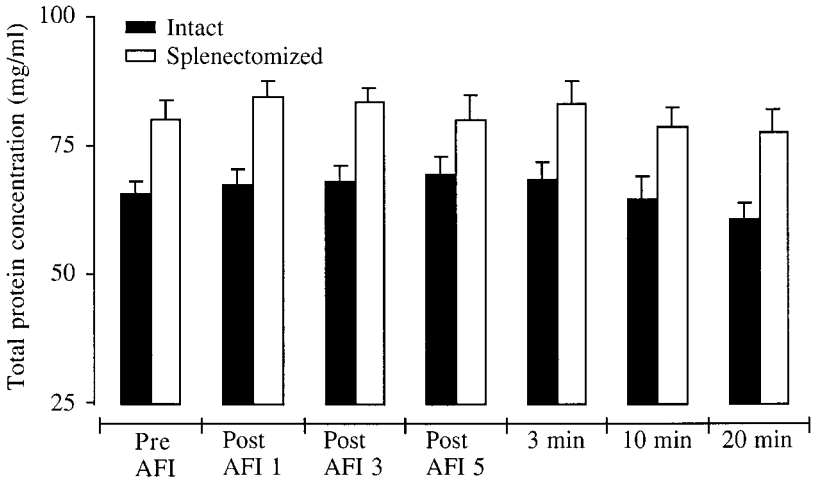

Fig. 3. Total plasma protein concentration before apneas with face immersion immediately after AFI 1, 3, and 5 and after 3, 10, and 20 min of recovery after the AFI series in intact and splenectomized subjects. Values are means ± SE.

The increases of the MAP during apneic immersion were greatest during the first apnea but remained of the same magnitude over the next four apneas in both groups (Fig. 4). Skin blood flow was more reduced during *apneas 2–5* in the intact than in the splenectomized subjects ($P < 0.05$), whereas the mean reductions in HR and rises in MAP were of the same magnitude in both groups (Fig. 4).

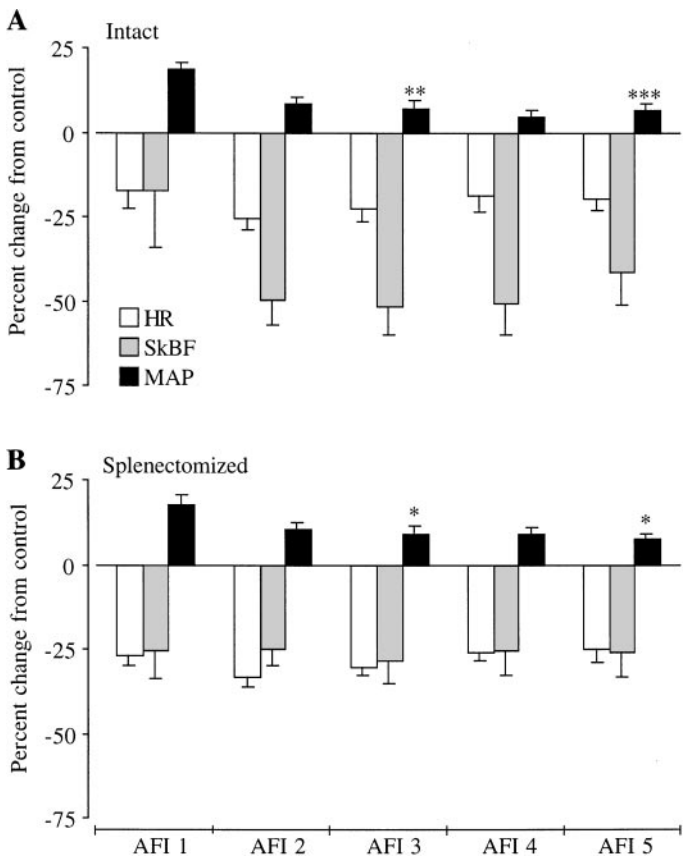

Fig. 4. Changes from control in heart rate (HR), skin capillary blood flow (SkBF), and mean arterial pressure (MAP) induced by the series of apneas with face immersion in intact (*A*) and splenectomized (*B*) subjects. Values are means ± SE. *Significant difference compared with AFI 1, $P < 0.05$; **Significant difference compared with AFI 1, $P < 0.01$; ***significant difference compared with AFI 1, $P < 0.001$.

DISCUSSION

The increases in Hct and Hb seen over the series of apneas in the intact subjects were reversed within 10 min, which shows that the increases were not the result of diuresis. The increases in Hct and Hb were not accompanied by an increase in total plasma protein content. This speaks against the cause being hemoconcentration due to fluid leaving the vascular system by increased filtration. Taken together, this indicates that the cause may be splenic contraction, which is further supported by the lack of Hct and Hb increase in the splenectomized subjects. Thus the increases in Hb and Hct previously found in the diving ama after hours of diving were probably caused, at least in part, by the observed splenic contraction (17). The magnitudes of increase in Hct and Hb seen in our nondivers (6.4 and 3.3%, respectively) were lower than those in the ama divers tested by Hurford and associates (17) (10.5 and 9.5%, respectively) but greater than those in their control nondivers (Hct = no effect, and Hb = 3%). This difference between trained and untrained subjects (17) could be due to a training effect or to the different lengths of diving shifts during experiments. However, the response is rapid in onset according to our results. An important factor might instead be the time for blood sampling after diving. In our study, the elevated Hct and Hb had already started to decline 3 min after the last apnea and returned to baseline after 10 min. It is not clear precisely at what point after the apneas the blood samples were collected by Hurford and associates (17).

The higher Hct but similar Hb found in splenectomized compared with intact subjects may be explained by a greater portion of circulating old red blood cells, with lower Hb when the cell-destroying effect of the spleen has been lost (12). The difference in plasma protein concentration between the groups could, at least in part, reflect the elevation of the immunoglobulin concentrations (IgG, IgA, and IgM) reported after

splenectomy (5). The larger relative reduction in skin blood flow during *apneas 2–5* in the intact group reflected a higher recovery blood flow after apneas, which has been reported earlier (31).

The increase of apneic duration seen in our intact subjects consisted of an increase of both the period before (EP) and the period after (SP) the physiological breaking point. This is in accordance with earlier findings and indicates that both physiological and psychological factors were affected by the repetition (14, 31). However, the increase of apneic duration in the splenectomized subjects was due to a prolongation of the SP only, thus mainly to an increased psychological tolerance or effort through the series (14).

The end of the EP is reached mainly when the elevated Pa_{CO_2} triggers involuntary breathing movements (22). Factors that influence the EP are thus linked to the level of Pa_{CO_2} at the beginning of apnea and the rate of Pa_{CO_2} increase during apnea. Hyperventilation (reducing the Pa_{CO_2} at the beginning of apnea) and a large lung volume or a pronounced diving response (leading to a slower increase of Pa_{CO_2}) would prolong the EP. However, there was a constant preapneic $P_{A}CO_2$ and inspired lung volume, and the diving response did not increase through the series of apneas in any group. Consequently, these factors cannot explain the prolonged EP with repeated apneas in the intact subjects.

There were different SP durations in the two groups, which may reveal different psychological tolerances between the individuals. The question arises regarding whether this could affect the hematological responses. We therefore compared the increases in Hct and Hb in the intact subjects of the present study with those of a group of five subjects that performed longer apneas when using the same protocol. We found no differences between their magnitudes of increases in Hct and Hb (Schagatay and Andersson, unpublished observations).

We therefore suggest that splenic contraction occurring in our intact subjects over the serial apneas caused an increased oxygen and carbon dioxide storage capacity, which prolonged the EP and apneic time. The unchanged EP duration in the splenectomized subjects supports this view. The return to baseline Hct and Hb within 10 min accords with the known duration of the effect of repeated apneas on apneic time (31). Thus spleen contraction may facilitate apneic diving in humans as in aquatic mammals. Spleen size in humans could allow such a contribution. The volume of blood in the spleen can be estimated to be 300 ml with a Hct of 80% (21). With the assumption that the same spleen emptying occurs as it does at maximal exercise (200 ml) and that the circulating blood volume is 8% of body weight in our intact subjects, splenic contraction could account for up to 60% of the increase in Hct seen in our study. Possible explanations for the remaining increase of Hct and Hb could be a net reduction in plasma volume, whereas loss to diuresis is excluded as the effect was completely reversible. A flow of small proteins across the vascular wall cannot be excluded. Increased capillary filtration of plasma into the interstitial spaces could be enhanced by the periods of increased blood pressure, but the peripheral vasoconstriction during apneas may counteract this effect, making the net effect uncertain. A high skin capillary blood flow accompanied by an elevated MAP was present during recovery after apneas. However, the main increase in Hct and Hb was found already directly after the first apnea; thus hemoconcentration by increased filtration during recovery cannot be responsible.

Splenic contraction, which is mediated through sympathetic nerves and α-adrenoceptors (25), appears to be a part of the human diving response. However, whereas the triggering of the peripheral vasoconstriction and HR reduction occurs within 30 s of each apnea, the splenic contraction requires more than one apnea to be fully initiated. Due to this difference in onset time, we suggest that humoral factors, e.g., epinephrine release, may also be important for the splenic contraction (28). These observations may be relevant to professional breath-hold divers as well as to competitive apneic divers participating in long-term breath holding (static apnea) or deep diving competitions, where work-up dives would be advantageous before record attempts. For a working ama diver, the diving shifts last for several hours with up to 30 dives/h (32), which makes an onset time of three to five apneas functionally acceptable. Hct increase is known to occur not only during diving but also during sleep apnea in elephant seals (3). Therefore, there may be important consequences of the present study to sleep apnea patients, for whom an increased blood viscosity at repeated apneas might be strenuous to the heart. Sleep apnea has been reported to occur frequently in angina patients (9). Another implication of these results may be to offer another argument for avoiding splenectomy when possible, as the spleen may contribute in previously unknown ways to the gas transportation ability of the blood in extreme situations.

In conclusion, we have shown that a series of five simulated dives is sufficient to induce increases in Hct and Hb in human nondivers and that these changes do not occur in splenectomized subjects. This coincides with a prolongation of the EP only in the subjects with spleens. We suggest that, when other factors that may have a possible role in the prolongation of apneas remained constant through the apnea series, emptying of the spleen delays the physiological breaking point of apnea by increasing blood gas storage and facilitating recovery.

We want to express our sincere gratitude to all subjects who participated in this study and to the students working with temporary research projects in our laboratory, which contributed in different ways. We also want to thank Assoc. Prof. Boris Holm for support; Inger Matsson, Hanna Wallin, and Gunilla Ljunggren for assistance with blood sampling and analysis; and Jonas Larsson for assistance during the experiments.

This study was supported by the Magnus Bergvall foundation, The Swedish National Centre for Research in Sports, and The Royal Physiographic Society in Lund.

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