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ORIGINAL ARTICLE

Total haemoglobin mass and spleen contraction: a study on competitive apnea divers, non-diving athletes and untrained control subjects

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Abstract In diving mammals splenic contraction increases circulating red cell volume, whereas in humans increased haemoglobin concentrations have been reported. It is unknown, however, whether repetitive apnea diving also comprises an adaptive increase in total red cell volume as reported in endurance athletes. The first aim of the study therefore was to investigate the effect of repeated apnea dives on splenic size and putative red cell release in trained apnea divers (n = 10) and control subjects (SCUBA divers performing apneas without long-term apnea training, n = 7). Long-term effects of repetitive apnea diving may elevate the oxygen transport capacity by an adaptive increase in total haemoglobin mass as reported in endurance athletes. The second goal, therefore, was to compare the trained apnea divers' and the control divers' total haemoglobin mass (tHb-mass) with that of endurance-trained (n = 9) and untrained (n = 10) non-divers. Before and immediately after a series of five dives to a depth of 4 m in a heated pool, spleen volume was assessed with ultrasound tomography. tHb-mass and plasma volume were measured using the CO-rebreathing method. In the trained apnea divers, repeated apnea dives resulted in a 25% reduction of spleen size ($P < 0.001$), whereas no significant effect was observed in the control subjects. While tHb-mass did not differ between trained apnea divers, untrained SCUBA divers performing apneas and untrained non-divers, it was 30% lower than in endurance-trained non-divers. We conclude that prolonged apnea training causes marked apnea-induced splenic contraction. In contrast to athletes in endurance sports, the trained apnea divers did not present with increased total haemoglobin mass and, hence, no increase in blood oxygen stores.

Keywords Spleen · Red cell volume · Haemoglobin mass · Blood volume · Apnea diving · CO-rebreathing method

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Introduction

It is well-established that splenic contraction increases total circulating red cell volume in diving mammals (Hurford et al. 1996; Thornton et al. 2001), because in these species the spleen blood volume represents approximately 50% of the total red cell volume (Hurford et al. 1996; Stewart and McKenzie 2002). Several studies also reported the spleen contraction coincided with increased haemoglobin concentrations in human volunteers after repetitive apneas, both without (Schagatay et al. 2001, 2005) and with face immersion in water (Bakovic et al. 2003, 2005; Espersen et al. 2002). Since this increased haemoglobin concentration was not present in splenectomised volunteers (Bakovic et al. 2003; Schagatay et al. 2001), the spleen is referred to as a blood cell reservoir that contributes to prolongation of apnea duration (Bakovic et al. 2003, 2005; Schagatay et al.

2001). It should be noted, however, that the increase in haemoglobin concentration was only transient, i.e. it disappeared within 2–10 min after the apneas (Espersen et al. 2002; Schagatay et al. 2005). Since the human spleen contains about 200 ml of blood (Stewart and McKenzie 2002), spleen contraction can theoretically increase total circulating red cell volume by maximally 3–5%. The proof of such a small variation is complicated because the kinetics of erythrocyte release and uptake from the spleen interferes with the duration of the currently available methods to quantify total haemoglobin mass (tHb-mass) (Gore et al. 2005). A more reliable way to detect red cell release is the determination of haemoglobin concentration ([Hb]), which may however be affected by water immersion causing haemoconcentration (Stewart et al. 2003) as a result of immersion-induced diuresis (Epstein 1992). The first aim of the present study, therefore, was to examine the influence of repetitive "wet" apneas on spleen size and [Hb].

Apnea periods of up to 9 min require a high volume of stored oxygen, and the lung most likely represents the largest oxygen store: in fact, lung volume before diving is closely related to diving performance (Overgaard et al. 2006), and elite apnea divers present with large lung volumes (Radermacher and Muth 2002; Ferretti and Costa 2003; Muth et al. 2005). However, adaptation of tHb-mass may also contribute to an increased O_2-availability. This kind of adaptation is well known in endurance trained athletes possessing about 40–50% more haemoglobin than untrained subjects (Heinicke et al. 2001). Therefore, the second aim of this study was to quantify the tHb-mass of trained apnea divers and compare these values with that of SCUBA diving controls without long-term apnea training as well as with that of endurance-trained and untrained non-divers.

Spleen size was measured with ultrasound tomography (Hurford et al. 1990; Koga 1979), tHb-mass was assessed using a recently described CO-rebreathing method (Schmidt and Prommer 2005), the latter technique being independent of measurements of haematocrit and/or plasma protein, i.e. of changes in body water distribution between the intravascular and the extravascular space.

Materials and methods

The study protocol was approved by the Ethical Committee of the Ulm University, and prior to the study, written informed consent was obtained from each participant. Ten trained apnea divers with regular apnea training for 2–3 h 3 times per week or more over at least the past 3 years were investigated; seven experienced SCUBA divers performing apnea dives without long-term apnea training served as controls. To compare total haemoglobin mass and blood volume two groups of differently trained male non-divers were included consisting of untrained subjects (n = 10) and highly trained triathletes (n = 9). The demographic and anthropometric data of all subjects are summarised in Table 1. In addition, the individual total lung capacity and residual volume of the 17 divers are summarised in Table 2, which was measured with body plethysmography prior to the study, as well as their individual diving times. Prior to the investigation, smoking, alcohol and caffeine intake were prohibited. All subjects were asked to drink as much as needed to guarantee an adequate hydration status.

Measurements and calculations

On the day before the apnea dives as well as immediately after these dives, i.e. on the second day, the total haemoglobin mass was measured in each diver using the optimised CO-rebreathing method as recently described in detail (Schmidt and Prommer 2005). This method allows accurate determination of tHb-mass with a typical error of 1.7%, i.e. changes of tHb-mass of more than 3.4% are considered as real changes with a probability of 95%. The time lag of approximately 16–24 h between the two measurements guaranteed complete CO elimination after

Table 1 Anthropometric data of all subjects investigated

	Trained apnea divers (males)	Trained apnea divers (females)	SCUBA divers	Untrained non-divers	Endurance trained non-divers
Age (years)	35 ± 9	32 ± 6	38 ± 11	31 ± 10	31 ± 8
Body weight (kg)	76 ± 4	67 ± 11	85 ± 10	80 ± 7	79 ± 10
Size (cm)	184 ± 6	174 ± 9	180 ± 6	179 ± 4	186 ± 7
BMI (kg m^{-2})	23 ± 3	22 ± 1	25 ± 3	25 ± 2	23 ± 2
VO_2max (ml kg^{-1} min^{-1})	ND	ND	ND	44 ± 4	65 ± 6

All data are mean ± standard deviation (male trained apnea divers n = 7, female trained apnea divers n = 3, SCUBA diver controls (n = 7), untrained non-divers (n = 10), endurance trained non-divers (n = 9)

BMI body mass index, *VO_2max* maximal O_2 uptake, *ND* not determined

Table 2 Individual demographic and anthropometric data, lung volumes and diving characteristics of the eight control subjects (subject no. 1–7) and the ten trained apnea divers (subject-no. 8–17)

Subject no.	Initials	Gender/age (years)	Size (cm)/body weight (kg)	Total lung capacity (l)/residual volume (l)	Maximum single dive time (min:s)/total diving time (min:s)
1	GZ	M/47	173/87	6.4/1.8	2:57/10:25
2	BI	M/49	191/97	9.4/2.7	1:49/7:03
3	HS	M/44	182/96	11.1/4.6	1:50/8:33
4	MG	M/29	173/86	5.9/1.4	2:38/10:42
5	UE	M/30	181/72	11.0/1.8	3:19/15:02
6	OH	M/46	180/85	7.9/2.4	2:33/11:37
7	DD	M/23	182/73	9.9/3.2	2:33/10:22
8	FR	M/30	179/73	8.1/1.8	2:53/11:31
9	SP	M/34	182/70	9.8/1.8	4:08/19:18
10	TS	M/28	193/80	10.8/2.5	4:11/20:35
11	KS	M/36	185/76	9.1/2.3	4:01/19:56
12	SK	F/29	165/56	6.4/2.0	3:06/15:08
13	DV	M/26	187/78	14.0/1.7	4:11/19:56
14	KK	F/28	183/77	10.0/2.9	3:05/15:13
15	AF	M/41	186/83	12.8/2.3	4:00/16:28
16	DAB	F/39	174/67	7.6/2.4	3:32/15:51
17	WH	M/51	173/74	9.1/2.5	3:46/16:20

the first measurements and thus avoided CO accumulation in the blood, which might have compromised the subjects' capacity to hold their breath. In the non-diving groups tHb-mass was determined once. Briefly, while sitting in a semiupright position the subjects were connected to a specially designed closed spirometric system allowing a CO-bolus application followed by 2 min rebreathing of a small amount of oxygen (3.5 l). The administered amount of CO in ml was individually calculated by body mass (kg): 0.8 for male and body mass (kg): 0.7 for female subjects. The amount of CO which had not been taken up by the body was calculated from the remaining CO in the spirometer and the residual volume of the lung as well as from the CO exhaled after disconnecting the subject from the spirometer (see Schmidt and Prommer 2005). Arterialised blood samples were taken from an earlobe before and 4, 6 and 8 min after the start of the rebreathing period. The total haemoglobin mass was subsequently calculated as

$$\text{tHb} - \text{mass} = K_{\text{baro}/T} \cdot M_{\text{CO}} \cdot 100 \cdot (1.39 \cdot \Delta - \text{HbCO}\%)^{-1} \quad (1)$$

- Δ-HbCO being the difference of CO-haemoglobin fractions before and after the CO administration,
- $K_{\text{baro}/T}$ = actual barometric pressure: 760^{-1} (1 + 0.0036661 actual temperature)
- $M_{\text{CO}} = V\text{CO}_{\text{adm}} - (V\text{CO}_{\text{rem}} + V\text{CO}_{\text{exh}})$ with $V\text{CO}_{\text{adm}}$, $V\text{CO}_{\text{rem}}$, $V\text{CO}_{\text{exh}}$ being the total volumes of CO administered, the volume of CO not taken up, and the volume of CO exhaled after the subject had been disconnected from the spirometer, respectively.

HbCO and haemoglobin concentration [Hb] were analysed using the ABL 520 gas blood system (Radiometer, Copenhagen, Denmark). Haematocrit (Hct) was measured in duplicate by microhaematocrit centrifugation (EBA 21, Hettich, Tuttlingen, Germany) at 21,400*g*, 15,000 rpm for 10 min. Mean red cell haemoglobin concentration (MCHC), total blood volume (BV), red cell (RCV) and plasma volumes (PV) were then calculated using the following formulas (2)–(5):

$$\text{MCHC} = ([\text{Hb}] \cdot 100) \cdot \text{Hct})^{-1} \quad (2)$$

$$\text{BV(ml)} = (\text{tHb} \cdot 100) \cdot ([\text{Hb}] \cdot 0.91)^{-1} \quad (3)$$

$$\text{RCV(ml)} = \text{tHb} \cdot \text{MCHC}^{-1} \cdot 100 \quad (4)$$

$$\text{PV(ml)} = \text{BV} - \text{RCV} \quad (5)$$

The spleen size was measured in each diver within 2 min after finishing the last apnea dive using an ultrasound scanner equipped with a linear 3-MHz sector transducer (Siemens Sonoline SI-250). Transversal and longitudinal (oblique) images were acquired with the

subject lying in the right lateral decubitus position before and immediately after the repetitive apnea dives. Maximal longitudinal (length) and transversal (width) diameters were obtained from these images off-line, and the splenic volume was derived from the calculated cross-sectional area A_{spleen} as described previously (Hurford et al. 1990) using the method by Koga (1979) according to the formula

$$\text{Spleen volume} = 7.53\ A_{spleen} - 77.56,$$

where $A_{spleen} = 0.8$ (length – width),

Immediately before starting the series of apnea dives as well as after the post-apnea measurements had been recorded, the subjects emptied their bladder, and the urine volume was recorded.

Study protocol

After the initial measurement of the spleen volume the trained apnea divers and the control subjects performed a series of five apnea dives of incremental duration to a depth of 4.0 m in a heated pool (28°C): control subjects were instructed to dive as long as possible, which resulted in a maximum duration of a single apnea dive of 2:31 ± 0:33 min. For safety reasons the trained apnea divers performed dives up to a maximum of 4 min each (maximum duration of a single apnea dive 3:41 ± 0:30 min, $P < 0.001$ vs. controls). The duration of surface recovery period was left to the individual diver's decision. Hyperventilation prior to the dives was prohibited. During the dives, the subjects wore neoprene suits for thermal protection and face masks. At the bottom of the pool they did not perform any physical effort, and negative buoyancy was assured with weight belts. About 30 s after finishing the last apnea a capillary blood sample was drawn out of the earlobe, which was immediately followed by determination of the spleen size. After approximately 3 min the CO-rebreathing was started; i.e. the COHb concentration used for tHb-mass calculation after CO-inhalation was obtained 9 min after the last dive.

Statistic analysis

Since normal data distribution was confirmed using the Kolmogorov–Smirnov test, all data are presented as mean ± standard deviation. Data before and after the apnea dives within the same subject was compared using a paired *t*-test, while intergroup differences for the haematological variables were tested using a one-way ANOVA and a subsequent post-hoc Scheffe test. A $P < 0.05$ was considered significant.

Results

While the five consecutive dives resulted in a cumulated diving time of 10.5 ± 2.5 min in the control subjects, the trained divers achieved a significantly higher cumulated diving time of 17.0 ± 2.8 min ($P < 0.001$). The total surface recovery times between all individual dives taken together were 15.7 ± 6.8 and 19.9 ± 3.0 min ($P = 0.100$), i.e. about 4 and 5 min between each dive, in the control subjects and the trained divers, respectively. Urine production was similar in the two groups (122 ± 116 in the controls vs. 160 ± 200 ml in the trained divers, $P = 0.658$).

Figure 1 summarises the results of the spleen size estimations. Before the repeated apnea dives, the estimated spleen volume was not significantly different between the control subjects and the trained divers (229 ± 55 vs. 191 ± 47 ml, $P = 0.142$), while it was significantly higher in the control subjects after the dives (206 ± 55 ml vs. 144 ± 50 ml, $P = 0.029$), as a result of the significant spleen size reduction in the trained divers ($P < 0.001$ vs. $P = 0.121$ in the controls).

Table 3 summarises the results of the haemoglobin measurements and blood volume calculations. As expected neither tHB-mass nor [Hb], plasma or total blood volume significantly differed between before and after the dives. Absolute and relative tHb-mass values were almost identical in the trained apnea divers, SCUBA diver control

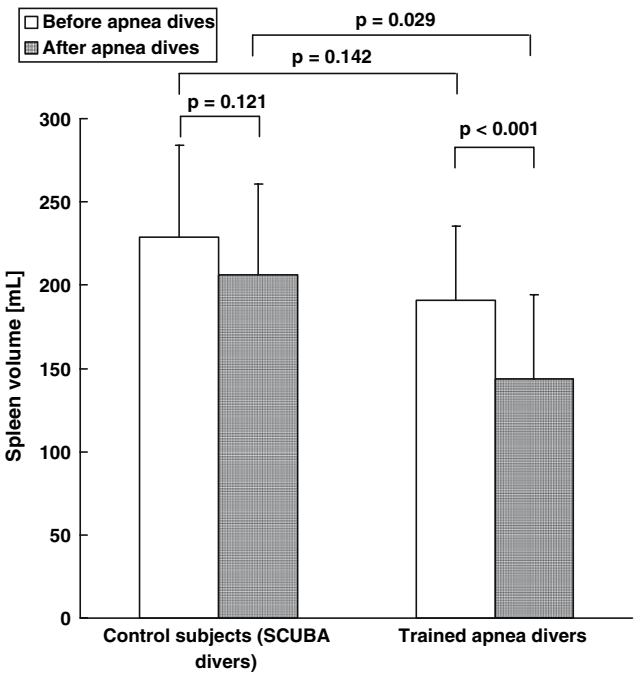

Fig. 1 Spleen volume in the control subjects (*left panel*, $n = 7$) and the trained apnea divers (*right panel*, $n = 10$) before (*open columns*) and immediately after (*hatched columns*) the repetetive apnea dives. Data are mean ± standard deviation

Table 3 Haematological findings in the control subjects (n = 7), the trained apnea divers (n = 10) and the trained (n = 9) and untrained (n = 10) non-divers

			Trained apnea divers (males, n = 7)	Trained apnea divers (females, n = 3)	Control subjects (n = 7)	Untrained non-divers (n = 10)	Trained non-divers (n = 9)
Haemoglobin concentration (g l^{-1})	Day 1		152 ± 5	141 ± 9	154 ± 9	151 ± 8	152 ± 10
	Day 2	Before	150 ± 7	141 ± 9	161 ± 9		
		After	149 ± 6	138 ± 10	156 ± 9		
P-value			0.41		0.33		
Total haemoglobin mass (ml)	Day 1		850 ± 83***	613 ± 87	863 ± 125**	871 ± 64***	1105 ± 148
	Day 2						
		After	840 ± 76	613 ± 86	857 ± 123		
P-value			1.0		1.0		
Total haemoglobin mass (g kg^{-1})	Day 1		10.8 ± 1.1***	9.2 ± 0.6	10.7 ± 1.6***	10.9 ± 0.7***	14.0 ± 1.0
	Day 2						
		After	10.7 ± 1.1	9.2 ± 0.9	10.7 ± 1.6		
P-value			1.0		1.0		
Total red cell volume (ml)	Day 1		2442 ± 198***	1812 ± 304	2464 ± 358***	2541 ± 188 ± **	3159 ± 402
	Day 2	Before	2392 ± 216	1811 ± 277	2445 ± 352		
		After	2404 ± 279	1776 ± 275	2432 ± 374		
P-value			0.95		0.67		
Plasma volume (ml)	Day 1		3702 ± 350**	2978 ± 410	3685 ± 587***	3800 ± 456***	4827 ± 569
	Day 2	Before	3770 ± 225	2982 ± 505	3416 ± 388		
		After	3780 ± 204	3126 ± 482	3600 ± 521		
P-value			0.35		0.20		
Blood volume (ml)	Day 1		6144 ± 506***		6149 ± 915***	6340 ± 606***	7986 ± 893
	Day 2	Before	6162 ± 411		5861 ± 711		
		After	6183 ± 452		6032 ± 852		

The non-diving groups were only examined once; the diving groups were examined on day 1 and on day 2 after the series of five apnea dives. Since tHb-mass was not different between day 1 and after diving on day 2, total red cell volume and plasma volume could be calculated for day 2 both before and after apnea. All data are mean ± SD. The statistical comparison between "before and after apnea" was done for the whole elite diver group (males and females)

Statistical differences between the endurance non-diving group and the other groups are indicated by ** $P < 0.01$, *** $P < 0.001$. No differences were found between the male diving groups and the untrained non-diving group

subjects and in the untrained non-diving group as well, while the endurance-trained non-divers presented with approximately 30% higher values. Similar results were obtained for red cell volume, plasma volume and blood volume. Hemoglobin concentration, in contrast, was similar in all groups.

Discussion

The aim of the present study was to investigate the short-term effects of repetitive "wet" apnea dives on spleen size and haemoglobin concentration as well as to evaluate the magnitude of tHb-mass in elite apnea divers. The key findings were (1) that the marked spleen contraction accounting for approximately the organ size was only present in the trained apnea divers, but (2) did not translate into any significant rise of the haemoglobin concentration, and (3) tHb-mass does not serve as an additional O_2-store in trained apnea divers.

Spleen size

In our investigation, only the trained apnea divers presented with a significant decrease in the calculated spleen size, whereas the control subjects did not show any difference between the values obtained before and after the dive series, respectively. These findings are virtually identical to results reported previously by Hurford et al. (1990): in Korean ama divers, these authors observed a 20% decrease in spleen volume after the divers had left the

water, whereas in scuba divers, who did not practice apnea diving and served as controls, no difference was found. Our results, however, are in contrast to more recent reports in the literature showing more pronounced spleen contraction after repeated apneas, even in subjects without particular apnea training (20–46% reduction of spleen volume vs. 26% in our study) (Bakovic et al. 2005; Espersen et al. 2002; Schagatay et al. 2005). It should be noted, however, that during these studies the repeated apneas were performed with face immersion in cold water (10°C), whereas in our investigation the subjects performed "real" apnea dives in a heated pool wearing the routine thermoinsulating neoprene suits. Hence, our study was performed under similar conditions as the one by Hurford et al. (1990) who investigated professional apnea divers wearing the regular wet suits during dive shifts in seawater of 25°C. It is well known that the so-called "diving reflex" is augmented by face immersion into cold water (Foster and Sheel 2005; Gooden 1994), and the spleen contraction is referred to be part of the diving response on "equal terms with bradycardia and peripheral vasoconstriction" (Espersen et al. 2002). Finally, in the more recently published papers (Bakovic et al. 2003; Espersen et al. 2002; Schagatay et al. 2005) the investigation of repetitive apneas without body water immersion and in subjects that did not wear neoprene suits allowed to measure the spleen size virtually immediately after the end of the apnea series, while our subjects had to leave the water and take off their wet suits before the measurement could be started. This time lag of approximately 2–3 min may in part also explain the discrepancy between our results and the literature data: in these studies, spleen size was reported to normalise within 2–10 min after the repeated apneas in the subjects without apnea training (Bakovic et al. 2005; Espersen et al. 2002; Schagatay et al. 2005).

In contrast to the previous reports (Bakovic et al. 2003, 2005; Espersen et al. 2002; Hurford et al. 1990; Schagatay et al. 2001, 2005), we did not find any change in haemoglobin concentration, neither in the trained apnea divers nor in the scuba diver control group. Except for one of the subjects, the trained apnea divers' urine production was below 3–4% of the prevailing plasma volume. Hence, we can exclude any artefactual increase in haemoglobin concentration, which might have resulted from haemoconcentration due to immersion-induced diuresis, such as discussed by Hurford et al. (1990). Assuming a haematocrit of 95% for the amount of blood released from the spleen (Stewart et al. 2003), the mean reduction in spleen size of about 25% (see Fig. 1) would have yielded an increase in the circulating tHB-mass in the trained divers of only about 15 g, which would correspond to 2 g l^{-1} in [Hb]. Such a subtle difference is within the measurement error of determining [Hb] and therefore most likely was undetectable in the prevailing study. The results of the tHB-mass measurements also support our presumption that the optimised CO-rebreathing method cannot be applied to detect short-term changes in tHb-mass: Since the average red cell splenic transit time ranges from 0.5 to 2 min (Ferrant et al. 1987; Matsuda and Uchida 1989) and the blood sampling was performed 5 min after starting the rebreathing period, we assume that the red cells stored in the spleen were at least in part labelled with CO. Consequently, the optimised CO-rebreathing method most likely does not allow to detect subtle and only transitory changes in the circulating red cell volume.

Total haemoglobin mass

One might assume that trained apnea divers have increased tHb-mass, which would enable them to store more oxygen and therefore to tolerate prolonged apnea periods. In fact, elite endurance athletes were reported to posses a 40–50% higher tHb-mass than untrained subjects (Heinicke et al. 2001), which would result in an additional O_2-availability of 500 ml when tHb-mass is 1,050 g instead of 700 g. Our results, however, clearly show that tHb-mass was identical in the trained apnea divers and in the untrained SCUBA diver controls. The endurance-trained non-diving group, however, presented with a 250 g higher tHb-mass, which is equivalent to an increased O_2-transport volume of 350 ml. The lacking elevation in tHb-mass in the trained apnea divers suggests that trained apnea divers do not have any genetic predisposition as it is probably the case in elite endurance athletes (Martino et al. 2002). Furthermore, it agrees well with data from patients suffering from moderate sleep apnea, who also show normal red cell volumes (Schmidt 2002) despite repetitive overnight falls of the arterial haemoglobin O_2 saturation. Finally, the duration of the hypoxic periods in the divers probably does not exceed the time necessary for effective erythropoiesis, which is supposed to be at least 80 min of continuous hypoxia (Eckardt et al. 1989) or cumulative 240 min of intermittent hypoxia (2.5 min of hypoxia at 10.5% O_2 in N_2 followed by 1.5 min of normoxia, Knaupp et al. 1992). Our findings, therefore, reject the hypothesis of elevated tHb-mass providing an additional O_2-store in trained apnea divers (Heinicke et al. 2003). Nevertheless, a lacking long-term effect of apnea training on tHb-mass still needs to be demonstrated in longitudinal studies.

In conclusion, while we confirmed previous reports on marked spleen contraction after repeated apnea dives, we did not find any change in haemoglobin concentrations or total red cell volume after the dives. Since diuresis-related haemoconcentration could be ruled out, our data demonstrate the modest and only transitory character of apnea-

induced rise in haemoglobin levels. Finally, the comparable values for tHb-mass in trained apnea divers and the control subjects make a special adaptation of the haemoglobin system as an oxygen store unlikely.

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