# Increase of Hemoglobin Concentration After Maximal Apneas in Divers, Skiers, and Untrained Humans

Matt Richardson<sup>1</sup>, Robert de Bruijn<sup>1</sup>, H.-C. Holmberg<sup>2,3</sup>, Glenn Björklund<sup>1,4</sup>, Helena Haughey<sup>1</sup>, and Erika Schagatay<sup>1</sup>

## **Catalogue Data**

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# Abstract/Résumé

Human splenic contraction occurs both during apnea and maximal exercise, increasing the circulating erythrocyte volume. We investigated the hematological responses to 3 maximal apneas performed by elite apneic divers, elite cross-country skiers, and untrained subjects. Post-apnea hemoglobin concentration had increased in all groups, but especially in divers. The increases disappeared within 10 min of recovery. Apneic duration across apneas also increased the most in divers. Responses in divers could be more pronounced as a result of apnea training.

La contraction de la rate chez l'être humain qui se produit pendant l'apnée et au cours d'un exercice maximal augmente le nombre d'érythrocytes en circulation. Nous avons analysé les ajustements hématologiques de plongeurs en apnée de haut niveau, de skieurs de fond de haut niveau, et de sujets non entraînés au cours de trois apnées maximales. On remarque après une période d'apnée une augmentation de la concentration d'hémoglobine dans les trois groupes, mais de façon plus marquée chez les plongeurs. L'augmentation s'estompa

<sup>&</sup>lt;sup>1</sup>Dept. of Natural and Environmental Physiology, Mid Sweden University, 85170 Sundsvall, Sweden; <sup>2</sup>Dept. of Physiology & Pharmacology, Karolinska Institut, Stockholm, Sweden; <sup>3</sup>Swedish Olympic Committee, Sofiatornet, Olympiastadion, Stockholm, Sweden; <sup>4</sup>National Winter Sport Center, Östersund, Sweden.

pendant les 10 min de récupération. La période apnéique fut plus marquée chez les plongeurs. Les adaptations plus importantes chez les plongeurs sont peut-être dues à l'entraînement à l'apnée.

# Introduction

Oxygen storage capacity is a vital component both for endurance performance in athletes and for apneic duration in apneic divers. One way to increase oxygen storage capacity is to increase the circulating hemoglobin volume by various means such as altitude training or even blood doping (Ekblom, 2000). Several studies have shown that spleen contraction is associated with near-maximal or maximal exercise (Froelich et al., 1988; Laub et al., 1993; Wolski, 1998). This spleen contraction during exercise elicits a transient increase in hemoglobin concentration (Hb) and hematocrit (Laub et al., 1993; Wolski, 1998) which may, without changes in other blood components, increase oxygen carrying capacity. An increase in circulating Hb can also be achieved by apnea-induced splenic contraction (Schagatay et al., 2001), leading to an increase in oxygen carrying capacity. Repeated apneas in series with short rest intervals increase in duration (Schagatay et al., 1999), which has been attributed to the increase in Hb due to spleen contraction (Schagatay et al., 2001). This prolongation may reflect both increased oxygen storage and improvements in CO<sub>2</sub> buffering capacity on repeated apneic attempts. Hb returns to normal values within 10 minutes of normal breathing, which is also the time known to reverse the increase in apneic duration at repetition.

The return of Hb to baseline shows that the response is not due to increased diuresis or sweating, and the constancy of protein levels shows that it is not due to extravasation of plasma by increased filtration (Schagatay et al., 2001). Apneainduced splenic contraction has been shown to be present in trained apneists (Bakovic et al., 2003; Hurford et al., 1990), and a spleen-attributable increase in Hb after apneas has been documented in untrained subjects (Schagatay et al., 2001), but it is unknown whether this response can be augmented through apneic training. It is also unknown whether spleen contraction resulting in increased Hb can be triggered in endurance athletes by apnea alone, and how this compares to the response in apneic divers and untrained subjects. The present study was aimed at describing the hematological response pattern associated with repeated maximal apneas in healthy non-divers (untrained), nondiving elite cross-country skiers (skiers), and elite apneic divers).

# Methods

#### SUBJECTS

A total of 78 volunteers from specific populations, ages 18 years and older, were recruited into three groups: elite divers, elite skiers, and untrained subjects. Of the 33 elite divers, 29 men and 4 women  $(30.3 \pm 10.6 \text{ yrs}, 75.9 \pm 12.1 \text{ kg}, 181.5 \pm 8.5 \text{ cm})$ , 17 were national team members in apnea sports from six countries, 9 were Swedish underwater rugby players, and 7 were apnea or free diving instructors from Sweden. They trained an average of  $5.5 \pm 4.0 \text{ hrs}$  a week in apneic diving and apnea related activities, and also an average of  $6.5 \pm 6.1 \text{ hrs}$  a week of physical training. No divers had been residing or training at altitude in the 6 months prior to

the study, but some of the national team members had been competing in breathholding in the month leading up to the testing period.

The 13 skiers, all male  $(20.2 \pm 3 \text{ yrs}, 72.1 \pm 5.5 \text{ kg}, 180.6 \pm 6.2 \text{ cm})$ , were all members of the Swedish national senior (n = 4), under-23 (n = 4), and junior (n = 5) cross-country ski teams. They were tested in their off-season during which they trained  $13.5 \pm 3.2$  hrs per week of ski training or skiing related physical activities. They had little or no experience in breath-holding. There had been no competitions in the previous month before testing, and none of the skiers had been living or training at altitude in the 6 months prior to the study.

The 32 untrained subjects  $(30.0 \pm 6.3 \text{ yrs}, 71.6 \pm 8.4 \text{ kg}, 177.6 \pm 8.0 \text{ cm})$  were 9 women and 23 men who had little or no experience in breath-holding. On average this group completed  $2.5 \pm 3.1$  hrs per week of physical training or athletic activities, but none were competitive athletes in any sport, and none were living at altitude.

#### EXPERIMENTAL PROCEDURE

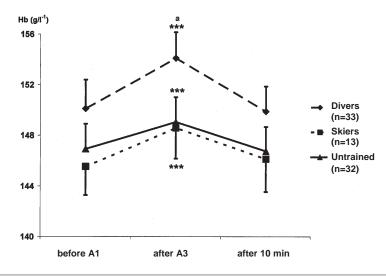
The subjects reported to the lab after 2 hrs without heavy meals and 1 hr without any meals or liquid intake. They signed informed consent documents for the test protocol, which was approved by the regional human ethics board. Following 20 min of horizontal rest, each subject performed 3 apneas of maximal, voluntary duration without time cues, spaced by 2 min of rest and normal breathing. Subjects were instructed not to hyperventilate and to immediately precede apneas by a total expiration and a deep but not maximal inspiration.

#### MEASUREMENTS

Blood samples for hemoglobin values were taken by trained personnel via intravenous catheter (Venflon Pro, Beckton-Dickson AB, Helsingborg, Sweden) and analyzed via automated blood analysis unit (Micros 60 Analyzer, ABX Diagnostics, Montpellier, France), or via capillary blood samples and analyzed by hemoglobin analyzer (B-Hemoglobin Photometer, Hemocue AB, Ängelholm, Sweden), all analyzed in triplicate. These two methods have been shown to produce similar values in previous studies (Neufeld et al., 2002; Paiva Ad Ade et al., 2004) and in our own pilot study data (unpublished). Methods were consistent within individuals and each person served as his or her own control. Blood samples were drawn immediately before the onset of the 2-min countdown for the first apnea, immediately following the final apnea, and 10 min after the final apnea. Maximum volume drawn for catheterized subjects was below 15 ml, and fingertip samples consisted of several drops from a new fingertip each time collected in the Hemocue cuvettes.

#### ANALYSIS

The values of Hb and apneic duration were compared within each group via a Student paired *t*-test, and the effects of apneas between groups were compared via unpaired *t*-test. *T*-tests were corrected for multiple comparisons using Bonferroni correction. Statistical significance was accepted at p < 0.05. Values are expressed as absolute values and, when appropriate, also as percent changes from reference.



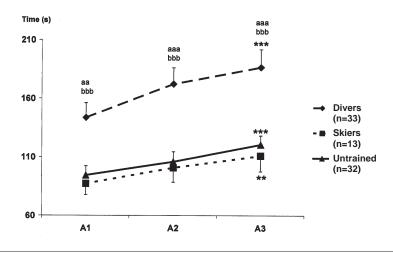
**Figure 1.** Hb values (mean  $\pm SE$ ) before the first apnea (A1), after Apnea 3 (A3), and 10 min after apneas in divers (n = 33), skiers (n = 13), and untrained subjects (n = 32). \*\*\*Significant changes of Hb from pre-apnea value within groups, p < 0.001; <sup>a</sup> Significant difference between divers and pooled non-divers, p < 0.05.

A Pearson correlation was used to analyse the relationship between apneic duration and Hb increase across the apnea series.

## Results

Baseline Hb was similar and normally distributed in all groups, with divers tending to have a higher value (divers 150.1 g/L; skiers 145.5 g/L; untrained 146.9 g/ L) (Figure 1). After the apneas, all groups responded with an increase of Hb (p < 0.001; Figure 1). The largest increase was seen in divers (4.0 g/L<sup>-1</sup>; 2.7%) compared to skiers (3.0 g/L<sup>-1</sup>; 2.1%), and untrained (2.1 g/L<sup>-1</sup>; 1.4%, p < 0.05), while skiers did not differ from either divers or untrained (Figure 1). All groups returned to baseline Hb values within 10 minutes. When the data from skiers and untrained subjects was pooled into a single non-diver group, Hb was higher in divers than in the non-diver group after 3 apneas (p < 0.05). Duration of the initial apnea was higher for divers (144 s) than for both skiers (87 s; p < 0.001) and untrained (94 s; p < 0.01; Figure 2), but the durations were the same for skiers and untrained.

The apneic duration increased in all groups across the 3-apnea series (p < 0.01 in skiers, and p < 0.001 in divers and untrained; Figure 2). The increase was more pronounced in divers (43 sec, 20%) compared to skiers (26 sec, 12.2%, p < 0.05) and untrained (24 s, 15.5%, p < 0.01; Figure 2). The apneic duration of the last apnea of the series was 187 s in divers, 111 s in skiers, and 121 s in untrained. Four subjects in the divers' group performed near-maximal apneas instead of maximal apneas and were excluded from duration analysis. However, no correlation was found between apneic duration and Hb increase across the apnea series in any group.



**Figure 2.** Duration of apneas performed by divers (n = 29), skiers (n = 13), and untrained subjects (n = 32). Significant changes of duration across 3 apneas within groups: \*\*\*p < 0.001; \*\*p < 0.01. Difference between: <sup>a</sup> divers and untrained; <sup>b</sup> divers and skiers.

### Discussion

This study shows that elite divers, elite skiers, and untrained subjects all respond with elevated Hb to 3 maximal effort apneas, but with divers showing a more powerful response. Since Hb increase after apneas has previously been shown to be absent in splenectomized subjects (Schagatay et al., 2001), we suggest that splenic contraction is likely to be the major factor behind the increase in Hb seen in the current study. This is the first observation of an apnea-induced elevation of Hb in nondiving endurance athletes, although the response is no greater than that of untrained subjects. The higher increase in Hb after apneas in the divers suggests that regular apnea practice could impart a specific training effect, affecting haematological responses to apnea in a manner that differs from that of exercise training. It may be that repeated attempts to a level of transient hypoxia, which is not achieved during submaximal or even maximal exercise, are needed to evoke a greater splenic response.

The apneic duration was higher in the trained divers than in other groups, and it could be argued that longer apneas caused the further increase in Hb. However, it has previously been observed in untrained subjects that a longer duration of individual maximal apneas did not affect the magnitude of Hb and Hct increases (pilot study reported in Schatagay et al., 2001). The lack of correlation between duration of apneas and magnitude of Hb increase within groups is in accordance with these earlier findings. The pronounced hematological response in divers could conceivably be a function of increased splenic contractility or a greater amount of stored red blood cells prior to splenic contraction after apnea training, or both. However, differences could also be due to specific genetic traits of persons who choose diving as a sport. Known effects of apnea training include increased cardiovascular diving response and apneic duration (Schagatay and Andersson 1998), but it is not known whether the spleen response can similarly be trained. Longitudinal studies of apnea training in previously untrained subjects are needed in order to explore this possibility further.

In conclusion, all groups responded to apnea with an increased Hb, likely due to spleen contraction, but this was most prevalent in divers, suggesting that these responses may be enhanced by apnea training but not by endurance ski training.

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