Time course of carbon monoxide transfer factor after breath-hold diving.

R. PREDILETTO¹, E. FORNAI¹, G. CATAPANO¹, C. CARLI¹, E. GARBELLA², M. PASSERA¹, D. CIALONI¹, R. BEDINI¹, A. L’ABBATE²

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Prediletto R, Fornai E, Catapano G, Carli C, Garbella E, Passera M, Cialoni D, Bedini R, L’Abbate A. Time course of carbon monoxide transfer factor after breath-hold diving. Undersea Hyperb Med 2009; 36(2):93-101. Breath-hold divers may experience haemoptysis during diving. Central pooling of blood as well as compression of pulmonary gas content can damage the integrity of the blood-gas barrier, resulting in alveolar hemorrhage. The single-breath carbon monoxide test (DL,CO) was used to investigate the blood-gas barrier following diving. The study population consisted of 30 divers recruited from a training course. DL,CO levels were measured before diving and at 2, 10 and 25 min after the last of a series of four dives to depths of 10, 15, 20 and 30 m. When compared to pre-diving values, DL,CO values increased significantly at 2 min following diving in all subjects except one. Thereafter values progressively decreased toward baseline at 10 and 25 min in all subjects but one, while in four divers DL,CO values decreased below baseline. The early but transient increase in DL,CO levels shortly after diving supports the persistence of capillary pooling of red blood cells following emersion. Persistence at 25 min of high DL,CO values in one subject could be attributed by lung CT to extravasation of blood into the alveoli. Early or late DL,CO values >10% below baseline values suggest the presence of pulmonary edema. The relatively high prevalence of DL,CO alterations found suggests caution on the safety of breath-hold diving activities.

Abbreviations: DL,CO = Diffusing capacity for the lungs measured using single-breath carbon monoxide test, also known as transfer factor; DL,CO/VA or KCO = Diffusing capacity for carbon monoxide per unit of alveolar volume; VA = alveolar volume; FEV1 = forced expiratory volume in one second; FEV1/VC = ratio of forced expiratory volume in 1 second to vital capacity; FVC = forced vital capacity; VC = slow vital capacity; TLC = total lung capacity; RV = residual volume; FRC = functional residual capacity; RV/TLC = ratio of residual volume to total lung capacity; BMI = body mass index expressed in Kg/m²; ATS = American Thoracic Society; ERS = European Respiratory Society; SD = standard deviation

INTRODUCTION

Breath-hold diving during underwater immersion leads to profound changes in cardiovascular parameters, including pulmonary capillary blood volume and cardiac output (1-3). In particular, hydrostatic pressure increases with depth, and as a consequence of Boyle’s law is responsible for the decrease in lung inhaled gas volume (4). Once the pulmonary gas volume has decreased approximately to the level of residual volume (usually at around 30 m depth), a significant amount of blood shifts toward the lungs, allowing the diver to descend further (4,5). This increase in pulmonary capillary blood volume, coupled with the decrease in inhaled gas volume, may contribute to increased intravascular pressure and in particular trans-capillary pressure. In turn, the increased capillary pressure may stretch the wall, weaken its integrity, and reduce natural vessel ability to sustain high mechanical stress (6). A further increase in capillary pressure may cause the rupture of the alveolar-endothelial membrane and initiate alveolar hemorrhage [(7-9). Increased intravascular pressure in the lungs has been evoked to explain cases of pulmonary
edema associated with heavy exercise (10), scuba diving, or strenuous swimming (11-13). A similar mechanism has also been hypothesized in the course of voluntary diaphragmatic contractions in breath-hold divers (14) and during thoracic squeezing when breath-holding at residual volume (15,16). Thus, in line with this view, an increased pulmonary capillary blood volume combined with decreased lung gas volume may be responsible for the diving-related risk of pulmonary edema and/or alveolar hemorrhage. However, other conditions such as poor physical training, underlying cardiovascular dysfunction, hypertension, anxiety, asthma or strenuous exertion before diving have also been proposed to explain diving lung distress (12).

The carbon monoxide transfer factor can provide information regarding the functional integrity of the blood-gas barrier and pulmonary microcirculation (17). Therefore, to obtain useful information on the dynamic changes of capillary blood volume and alveolar-endothelial membrane integrity, we decided to measure the diffusing capacity of CO immediately after, and at different times following repeated stress on the respiratory system, consisting in four consecutive breath-hold dives to progressive depths.

METHODS

2.1 Subjects
The study population consisted of 50 potential subjects, healthy volunteers, breath-hold divers participating in an international diving competition and solicited to enter the study. Twenty of them were excluded due to haemoptysis in the previous week. The remaining thirty volunteers represented the study sample. All subjects enrolled were experienced breath-hold divers with a history of more than 5 years of breath-hold diving. Mean age was 34 ± 7 years, none were current smokers, and none reported a history of cardiopulmonary disease or other diseases. At the time of the study all breath-hold divers were free of symptoms and physical signs of cardiopulmonary disease and denied having taken any drugs or alcohol during the last 5 days. Eight of the 30 breath-hold divers studied reported having had bloody sputum occasionally, after previous dives.

The study was performed during a 5-day international competition in Sharm el Sheik (Egypt). The day before the experimental procedures, most breath-hold divers dove to depths of over 50 m, as part of a personal training schedule or during instructor activity.

The experimental procedure was conducted in accordance with the principles of the declaration of Helsinki. The protocol was approved by the Institutional Review Board of the Institute of Clinical Physiology (Pisa), National Research Council of Italy. The designed pre- and post-diving protocol was considered to not affect in any way the health risks related to competitive diving activity. Written informed consent was obtained from all participants in the study.

2.2 Experimental procedures and Measurements
Prior to diving, all subjects underwent standard spirometry and measurement of lung volume, performed following the recent ERS-ATS guidelines (18,19) and using a fully equipped computerized spirometric system from Zan-Morgan Ferraris (Usa-Europe). Then the single-breath CO diffusing capacity (DL,CO), as well as the alveolar volume by methane gas dilution technique (VA), were measured according to the recent ERS-ATS guidelines (20). Reference values for spirometry were from ERS (18), and for diffusion parameters were derived from Cotes (21). Baseline DL,CO and VA were measured twice within a time interval of 5 min and the values averaged. Baseline DL,CO value was then adjusted for an
estimated hemoglobin level of 14.6 gr/dl (20). All parameters were expressed as percentage of reference predicted values. The measurements were done with divers in a comfortable sitting position, wearing their suits. Once the lung function tests were accomplished, the subject entered the water.

The experimental design consisted of a series of four dives, each at progressively greater sea-depths of 10, 15, 20, and finally 30 m (Fig. 1). The subject was required to dive to the scheduled depth following a guide line which, however, was not to be actively used during diving. Descent and ascent were performed wearing fins and swimming at constant weight (“poids constant”)—that is, not being allowed to drop the weights during the dive. The depth of 30 m was chosen because large lung volume changes were expected to occur at this level, and this depth was easily attained by all divers (maximum personal depth around 50 m).

After completion of the baseline measurements, all breath-hold divers started to dive. For safety, each breath-hold diver was kept in sight by two expert divers during the phases of descent, ascent and at the surface, following each dive. Two other SCUBA divers with breathing apparatus observed the entire procedure at 15 and 30 m. After the first two dives, the breath-hold diver rested at the sea-surface for 3-4 min; finally, following a 7-min rest after the third dive, the subject performed a breathing maneuver from resting tidal volume to his maximum inspiratory volume and lastly dove to 30 m. Breath-hold divers wore a comfortable wetsuit during diving; the procedure was performed in October, and water temperature was about 26°C. The instrumental equipment was checked and calibrated for barometric pressure, ambient temperature, humidity, flow and volume according the ERS/ATS guidelines.

At the end of the last dive to 30 m, the breath-hold divers were helped to exit the water and dry off, without any further effort; within 2 min (2 ± 0.86 min) they repeated the DL,CO and VA tests. Then the breath-hold divers rested, sitting comfortably dressed in bathrobes in a shaded area outdoors; there was no wind and air temperature was 28°C.

Measurements were repeated after 10 (11.3 ± 3.5) and 25 (23.6 ± 6.3) min. Thus, in summary, the transfer factor of CO and VA were measured three times (a single determination each time) at different time intervals after the end of the last dive at 30-m depth, while spirometry was performed only at baseline. Spirometry was not repeated post-dive in order to spare the breath-hold diver extra work and to guarantee the stability of his condition, as well as due to limitations of the time schedule.

2.3 Statistical analyses

Group data are expressed as the means and standard deviation. Variability of DL,CO
and VA values between the two baseline values were calculated using the Bland-Altman method (22) in order to distinguish genuine changes in gas transfer over time from measure variability. Analysis of variance for repeated measurements was used to evaluate the differences between pre- and post-diving values at 2, 10 and 25 min. Differences with a p-value < 0.05 were considered significant.

RESULTS

The characteristics of our study population are shown in Table 1, along with baseline spirometry and diffusing capacity values. No breath-hold diver presented signs of airflow limitation or of hyperinflation at baseline.

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<th>Table 1. Baseline characteristics of the study population (subjects n=30) Data are presented as mean and SD</th>
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Table 1. Data are presented as mean and standard deviation. For abbreviations: BMI: Body mass index; FVC: forced vital capacity; FEV1: forced expiratory volume in one second; FEV1/VC: ratio of forced expiratory volume in one second to vital capacity; TLC: total lung capacity; VC: slow vital capacity; RV/TLC: ratio of residual volume to total lung capacity; DL,CO: Diffusing capacity for the lungs measured using carbon monoxide, also known as transfer factor; DL,CO/VA or KCO: Diffusing capacity for carbon monoxide per unit of alveolar volume; VA: alveolar volume.

Variability of DL,CO and VA measured twice at baseline was within 5%. According to ERS/ATS reference values, the pre-diving values of DL,CO were within normal range in 26 breath-hold divers while in the remaining 4, DL,CO values were higher (150 ±13% of predicted).

The average durations of the four dives were 77 s at 10 m, 63 s at 15 m, 67 s at 20 m and 82 s at 30 m (Fig. 1). There were no serious episodes of loss of consciousness during the succession of dives. Two breath-hold divers showed signs of dizziness at the surface, and a third showed excess of mucus, cough, and bloody sputum—all events which followed the last dive to 30 m.

As compared to baseline values, the transfer factor measured immediately after the final immersion to 30 m was significantly increased in all breath-hold divers (p < 0.0001). On the average, values were over 30% higher than the pre-dive values (116 ± 20 vs 147 ± 30 % predicted) (Fig. 2). Thereafter, values progressively decreased toward baseline at 10 min (123 ± 30 % predicted) and generally returned to baseline on the average at 25 min (109 ± 15% vs 116 ± 20 baseline).

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At variance with the general trend, the behavior of the DL,CO levels after the early post-diving measurement was different in 5 of the 30 subjects (Fig. 3, above). In particular, in one diver (black closed squares) high values of DL,CO were recorded throughout the entire observational period were associated to haemoptysis and evidence of intrapulmonary hemorrhage at CT scanning. In the remaining four divers late DL,CO values decreased >10% below the baseline (lower horizontal dashed line) compatible with fluid effusion into the lungs. Two of the above breath-hold divers (open circle and large open squares) complained of symptoms of dizziness after emersion.

Blood gas analysis showed the presence of hypoxemia, and CT scan revealed the presence of patchy opacities in both lungs at the level of the superior as well as parahilar zones (Fig. 4, upper panel). The subject was treated with systemic corticosteroids, antibiotics, supplemental oxygen and unfractionated heparin, with a progressive improvement of symptoms and objective findings in the next few days. The single breath carbon monoxide diffusing capacity, repeated after 5 days, had returned to baseline values. The breath-hold diver was released 1 week later after a complete remission of clinical, blood gas and lung function alterations. Lung morphology, controlled by CT scan 40 days later, resulted normal (Fig. 4, lower panel).

Regarding the additional four subjects whose late DL,CO values decreased below baseline, two presented symptoms of dizziness
immediately upon returning to the surface. Their symptoms disappeared rapidly, and they were able to perform the complete series of respiratory tests.

As shown in Fig. 5, alveolar volume did not change from baseline at any time following diving (105 ± 9.5 at baseline vs 106 ± 10.7; 107 ± 9.9 and 105 ± 9.7 % predicted at 2, 10 and 25 min post-dive, respectively) Thus, the DL,CO/VA pattern reproduced that of DL,CO documenting that changes in alveolar volume did not play any role in the observed changes in CO diffusion.

DISCUSSION

This is the first study using measurement of carbon monoxide transfer factor to assess possible structural and/or functional alterations of the alveolar-endothelial membrane following breath-hold diving during apnea at total lung capacity. The biomarker was measured at different times following a series of consecutive dives to progressively increasing depths (10, 15, 20 and 30 m).

The study clearly showed that compared to the pre-diving value, the diffusing capacity for carbon monoxide was significantly increased soon after (2 ± 0.86 min) emersion, while alveolar volume was unchanged.

This finding can be interpreted as the result, relative to baseline, of enhanced CO uptake by a larger amount of hemoglobin abutting the membrane of ventilated alveoli. Theoretically, this can be attributed to various factors, which are expanded pulmonary capillary blood volume, increased hematocrit or increased cardiac output secondary to diving muscular exercise. Unfortunately these factors could not be distinguished in the present study. However, apart from the early increase in DL,CO values, the main finding of our study is its decreased below baseline value in four outliers in the successive measurements. This finding leads us to conclude that distress of the alveolar-capillary barrier actually occurred in some divers and that expansion of the pulmonary capillary blood volume is most likely responsible for this phenomenon. Thus, according to this interpretation the increase in pulmonary blood volume occurring during diving was not entirely reversed to normal at the time of the first measurement, a few minutes following emersion. Reversion of capillary blood volume to normal occurred only later as documented by the return of DL,CO values towards baseline in successive measurements, similarly to what was reported by Manier et al. after maximal exercise (23). This finding is also in accordance with the study of Lindholm et al. (16) who investigated the effect of apnea initiated at residual, rather than maximal, pulmonary volume on CO diffusing capacity. The authors found increased values of DL,CO immediately following diving and their reversal to baseline 25 min following emersion.
The common finding of early enhanced CO diffusion in our study, where apnea was started at total lung capacity, and in Lindhom’s study (16), where apnea was performed at residual volume, points out the negligible role of lung air volume in modulating the changes in CO diffusion observed in our study. Moreover, the results of our study are in line with those observed by Greening and Hughes (24).

It should be considered that increased CO diffusion caused by increased pulmonary capillary blood volume may easily mask possible alterations in CO diffusion occurring in the opposite direction, such as the decrease in CO transfer factor caused by interstitial or alveolar edema (17, 20, 25). In this instance, decreased diffusion of CO possibly becomes apparent only after normalization of capillary blood volume and in the condition that alteration in membrane conductance lasts for a sufficiently long time.

In our study five breath-hold divers deviated from the general pattern described above, i.e. early increase in CO transfer factor followed by normalization.

In the first breath-hold diver, DL,CO level increased at 2 min, as in the others, but remained elevated at 10 and at 25 min post-emersion. This subject reported symptoms at emersion and had extensive intra-alveolar hemorrhage documented by lung CT scan a few hours later. It seems reasonable to conclude that in this case the presence of a large quantity of red blood cells in the alveoli was responsible for the persisting high uptake and retention of CO.

In the remaining four breath-hold divers, deviation from the general pattern was in the opposite direction, i.e. DL,CO values in late measurements were clearly below baseline values. In these instances, we interpreted this deviation as suggestive of pulmonary edema, unmasked at 10 or 25 min only after the reduction of pulmonary blood volume had taken place. Two of the aforementioned four breath-hold divers presented symptoms of dizziness immediately upon return to the surface, although there were no objective signs of pulmonary edema, and one of them declined to complete the testing.

### 4.1 Study limitations

One hypothetical factor affecting DL,CO results in our study could be the interference of five consecutive single-breath CO tests. It is well known that the inhalation of carbon monoxide repeated four times can increase the level of HBCO by 3% and this may be responsible for a decrease in DL,CO by 3.5% (20). In our study the late DL,CO values considered abnormal in four subjects were far below 3.5% of baseline, thus suggesting an actual decrease.

A further factor potentially influencing DL,CO results is the finding of high pre-diving values of DL,CO in four divers. Excluding methodological bias on the basis of the low variability observed in double measures, one possible explanation might be related to the fact that some subjects in the days before the study had made dives to 50 m, thus stressing their respiratory systems more than the others.

As previously mentioned, a further limitation is that in our study we did not measure either hematocrit or hemoglobin, nor cardiac output—all factors potentially affecting CO diffusing capacity through increased muscular exercise or splenic contraction and hemoconcentration (26,27). However, it should be noted that all these factors act in the opposite direction (amplification of CO diffusion) relative to the reduction in CO diffusion observed in the four outlier divers of our study.

Finally, we limited spirometric measurements to the pre-diving session alone. This choice was made in order to avoid interfering with the repeated measurements of DL,CO and VA following diving. Possibly,
post-diving spirometric tests would have provided supplementary data to the diagnosis of pulmonary edema, as shown by Lindholm et al. (16).

CONCLUSIONS

To date, there are no reports in the literature on controlled studies aimed at evaluating the incidence of functional and/or structural lung injury among divers. This is the first field observation study on pulmonary diffusion capacity following sea diving up to 30-m depths. Results are compatible with the occurrence of pulmonary injury in 16% of our breath-hold diver population. This proportion could be even higher if one considers that the increase in pulmonary capillary blood volume, consistently observed early in the post-diving period, can mask short-lived alterations at the level of the alveolar-endothelial membrane.

Considering the increasing number of people performing competitive or recreational breath-hold diving, a potential risk for pulmonary injury in such a proportion deserves further attention.

The continuous insults to the alveolar-capillary membrane, i.e. rupture and subsequent repair, could elicit the subsequent development of chronic lung disease. Unfortunately, from the data available no conclusions can be drawn on the outcome of diving-activities in terms of development of interstitial fibrosis or pulmonary hypertension.

Our study outlines the need for better knowledge of the acute as well as chronic effects of diving activity on the lungs, especially breath-hold diving. Epidemiological studies clarifying the prevalence and incidence of lung diseases in breath-hold divers are also required.

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