# Short communication

## Preliminary observations on the effect of hypoxic and hyperbaric stress on pulmonary gas exchange in breath-hold divers

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**Key words**
Breath-hold diving, physiology, pulmonary function, pulmonary oedema, carbon monoxide, nitric oxide

**Abstract**
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**Aim:** To evaluate pulmonary alveolar-capillary membrane integrity and ventilation/perfusion mismatch after breath-hold diving.
**Methods:** Pulmonary diffusing capacity to carbon monoxide (DLCO) and nitric oxide (DLNO), haemoglobin (Hb) and haematocrit (Hct) were measured in six elite divers before and at 2, 10 and 25 minutes after a maximal breath-hold dive to a depth of 10 metres' sea water.
**Results:** Compared to pre-dive, DLCO showed a slight increase at 2 minutes in five subjects and a tendency to decrease at 25 minutes ($P < 0.001$) in all subjects. DLNO showed an increase at 10 minutes in three divers and a slight decrease at 25 minutes in five subjects. There was a small but significant ($P < 0.001$) increase in Hb and Hct at 2 minutes, possibly affecting the DLCO measurements.
**Conclusions:** An early but transient increase in DLCO in five divers may reflect the central shift in blood volume during a breath-hold dive. The late parallel decrease in DLCO and DLNO likely reflects alveolar-capillary distress (interstitial oedema). The DLNO increase in three subjects at 10 minutes may suggest ventilation/perfusion mismatch.

### Introduction

Breath-hold diving leads to significant changes in the pulmonary system, such as lung-volume reduction by depth, increased blood flow and central pooling of blood, as well as local hypoxic vasoconstriction and heterogeneous blood redistribution.[1,2] Whereas heterogeneous redistribution of pulmonary capillary blood may lead to ventilation/perfusion mismatch, thus affecting pulmonary gas exchange, the acute increase in trans-capillary pressure may stretch the alveolar wall and weaken its integrity, possibly leading to interstitial-alveolar oedema or even haemorrhage.[3–5]

Pulmonary blood shift, early subclinical interstitial odema and alveolar haemorrhage can be functionally distinguished by different modifications of diffusing lung capacity to carbon monoxide (DLCO): respectively, early and transient increase, late and consistent decrease, early and persisent increase.[5,6] DLCO is affected by both alveolar surface characteristics (area, thickness and integrity) and pulmonary blood flow and volume.[7] It has been shown that the presence of ventilation/perfusion mismatch can be inferred in the single-breath diffusing capacity to DLCO when coupled with nitric oxide (DLNO) in the same breath (since DLNO measurements are independent of pulmonary capillary blood volume and flow), thus representing the true membrane diffusing capacity.[8] Therefore, we decided to measure, in a preliminary study, the DLCO and DLNO before and after a maximum-duration apneic dive to a depth of 10 meters' sea water (msw) in a small group of volunteer divers in order to elicit the effects of both hydrostatic pressure and hypoxia on early post-dive pulmonary function.

### Methods

#### SUBJECTS

Six healthy, non-smoking, elite breath-hold divers (five male and one female), with no known medical problems and at least five years of practice and personal depths exceeding 30 msw, were studied. None reported any history of barotraumatic lung injury or decompression sickness, and physical examinations were normal. No drugs or alcohol were taken within the five days before the study, and subjects were not allowed to dive prior to the test dives. The study was conducted in accordance with the principles of the declaration of Helsinki and 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 protocols were considered not to affect health risks related to diving activity. Written informed consent was obtained from all participants in the study.

#### EXPERIMENTAL PROCEDURES AND MEASUREMENTS

The study was performed in June at Asinara Island (Italy). A dive site with the bottom at 10 msw close to a small pier was chosen. Water temperature was about 24$^{O}$C. The test dive consisted of a maximum-duration, immersed breath-hold dive to 10 msw depth. This was preceded by two warm-up dynamic breath-hold dives also to 10 msw. Baseline measurements were preceeded by 10 minutes of acclimatisation on the water surface. All measurements pre- and post-dive were made with the subjects in the sitting position, wearing their diving suits.

The following measurements were taken: venous blood samples from an antecubital fossa vein for haemoglobin (Hb) and haematocrit (Hct); trans-jugular echo Doppler for cardiac output estimation (MY LAB 30, Esaote);[9] (both via removable Velcro patches in the suit at the elbow and neck); spirometric indices and lung volumes; DLCO, DLNO, alveolar volume ($V_A$) single breath measurement, the last by helium dilution technique, using a portable computerised spirometer (Sensor Medics). The spirometer was calibrated prior to the study for barometric pressure, ambient temperature, humidity, lung flow and volume. Duplicate measurements of DLCO, DLNO and $V_A$ were repeated within 5 minutes. Following these baseline measurements, the divers prepared for diving, the time to get ready being 80 ± 20 seconds. All measurements, excluding spirometry, were repeated at 2, 10 and 25 minutes post-dive.

Spirometry and lung volumes were performed according to ERS-ATS guidelines and reference values were derived from Quanjer.[10,11] The single-breath diffusion tests were also performed according to ERS-ATS guidelines, and European reference equations for CO and NO lung transfer were used.[8,12–14] DLCO values were adjusted for the measured haemoglobin level and CO back-pressure in order to distinguish genuine changes in gas transfer over time from measurement variability.[12] Blood samples were stored in a portable fridge and analysed within 4 hours of sampling at a nearby hospital laboratory.

STATISTICAL ANALYSIS

Temporal trends of DLCO, DLNO, Hb and Hct were estimated performing a Friedman test with a 4-level within-subject factor (TIME: basal, 2, 10, 25 minutes).[15] Variations (d) of DLCO, Hb and Hct between pre- and post-dive values were estimated (dDLCO, dHb and dHct respectively) and correlations between dDLCO on the one hand and dHb or dHct on the other were performed using Spearman's rank correlation. Differences with a $P$-value < 0.05 were considered significant.

## Results

Pre-dive, all subjects had normal respiratory function and lung-diffusion indices, and these and demographics of the subjects are shown in Table 1. The test dive lasted for a mean time of 270 (SD 53) s. Friedman test on DLCO showed a significant time effect ($P < 0.01$), whereas no significant effect was found for DLNO. Compared to pre-dive, DLCO showed

**Table 1**
**Descriptive characteristics of subjects; functional values reported as single determinations as well as % predicted. $FEV_1$ – forced expiratory volume in one second; $V_A$ – alveolar volume; DLCO – diffusing lung capacity for carbon monoxide; DLNO – diffusing lung capacity for nitric oxide**

| Parameter | Mean | SD |
|---|---|---|
| Age (years) | 34 | (8) |
| Body mass index (kg $m^{-2}$) | 22 | (2) |
| Vital capacity (VC) (L) | 6.26 | (1.06) |
| $FEV_1$/VC (% pred) | 98 | (5) |
| Total lung capacity (L) | 7.13 | (1) |
| | | |
| DLCO (mL $min^{-1}$ $mmHg^{-1}$) | 12.5 | (2.25) |
| DLCO/$V_A$ (% pred) | 103 | (21) |
| DLNO (mL $min^{-1}$ $mmHg^{-1}$) | 49.8 | (9) |
| DLNO (% pred) | 141 | (25) |
| Haemoglobin (g $L^{-1}$) | 133 | (16) |
| Haematocrit (%) | 41 | (4) |
| Cardiac output (L $min^{-1}$) | 3.8 | (1.5) |

**Figure 1**
**Changes in DLCO over time following a maximal duration breath-hold dive to 10 msw ($n$ = 6); a significant decrease was seen at 25 minutes post-dive ($P$ < 0.001)**

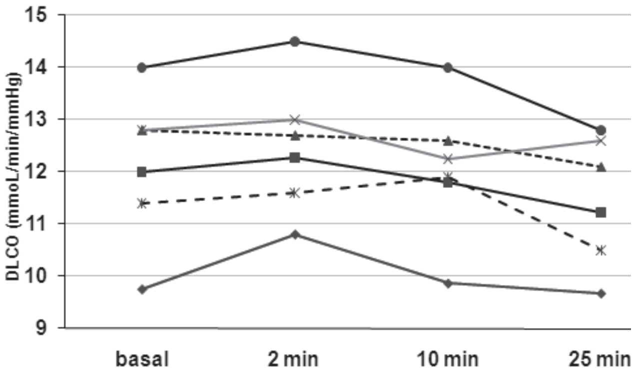
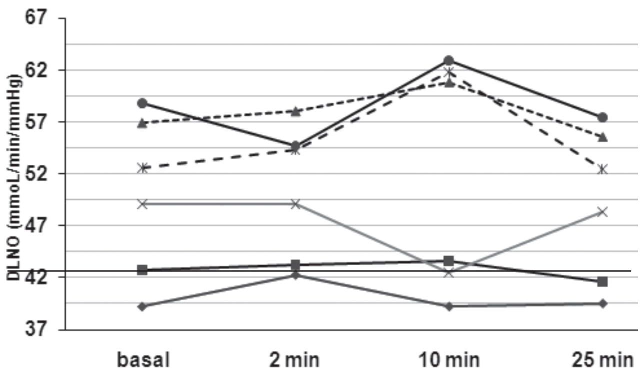



**Figure 2**
**Changes in DLNO over time following a maximal duration breath-hold dive to 10 msw ($n$ = 6); not significant**



an increase at 2 minutes in five subjects and a tendency to decrease at 25 minutes ($P < 0.001$), with a decrease in all six subjects (Figure 1). In contrast, DLNO showed no clear trend at 2 minutes, an increase in three subjects at 10 minutes and a slight decrease at 25 minutes in five subjects (Figure 2). Hb and Hct showed a significant time effect ($P < 0.03$ and $P < 0.04$ respectively), both increasing at 2 minutes ($P < 0.001$; mean (SD) % change in Hct = 1.77 (0.8)). Cardiac output showed a slight but non-significant decrease after the dive. Although correlations between dDLCO and dHb or dHct did not reach statistical significance ($r = 0.78$, $P < 0.08$ and $r = 0.82$, $P < 0.07$, respectively), probably because of the small sample size, a trend was evident.

## Discussion

These are the first observations of the mechanical and hypoxic effects of maximum-duration, immersed apnea at depth on alveolar-capillary membrane integrity, as assessed by the DLNO and DLCO tests. Our observations appear to be consistent with the initial hypothesis from a previous study, based on DLCO only, that breath-hold diving may elicit damage to the alveolar-capillary membrane.[5] An early, transient DLCO increase after a dive may reflect the central shift of blood into the pulmonary circulation, and this is further supported by the separation of the DLNO and DLCO trends at that point. On the other hand, both the slight DLNO and significant DLCO tendencies to decrease at 25 minutes likely reflect alveolar-capillary distress, such as interstitial pulmonary oedema.

In addition, the increase in DLNO at 10 minutes in three subjects may suggest ventilation/perfusion mismatching, secondary to pulmonary capillary blood flow redistribution. Finally in this study, DLCO measurement appeared likely to be affected by Hb and Hct but not by cardiac output. Furthermore, the increases in Hb and Hct early post-dive are consistent with the diving response (splenic contraction).[1]

Limitations of our study were primarily the small size of the data sample (six subjects observed at four time points) and large variability between subjects. Also, due to technical constraints (length of the challenge), only one dive was studied per subject, so that the reproducibility of observations within subjects could not be investigated. Yet, in order to support the observed effects by means of statistical evaluations and to verify the existence of time trends similar for each individual, regardless of the measured parameter weights, the use of non-parametric tests such as Friedman and Spearman's rank tests was considered a good compromise. These tests are essentially unaffected by inter-subject variability because they are calculated on the basis of variable ranks.[15] Our sample is too small to draw firm conclusions on the hypotheses we are dealing with, but the results seem to be promising and merit further detailed study to confirm these preliminary observations.

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