



Gas exchange and cardiovascular responses during breath-holding in divers

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ABSTRACT

To check whether the evolution of alveolar pressures of O₂ (P_AO₂) and CO₂ (P_ACO₂) explains the cardiovascular responses to apnoea, eight divers performed resting apnoeas of increasing duration in air and in O₂. We measured heart rate (f_H), arterial pressure (AP), and peripheral resistances (TPR) beat-by-beat, P_AO₂ and P_ACO₂ at the end of each apnoea. The three phases of the cardiovascular response to apnoea were observed. In O₂, TPR increase (9 ± 4 mmHg min⁻¹) and f_H decrease (-11 ± 8 bpm) were lower than in air (15 ± 5 mmHg min⁻¹ and -28 ± 13 bpm, respectively). At end of maximal apnoeas in air, P_AO₂ and P_ACO₂ were 50 ± 9 and 48 ± 5 mmHg, respectively; corresponding values in O₂ were 653 ± 8 mmHg and 55 ± 5 mmHg. At end of phase II, P_AO₂ and P_ACO₂ in air were 90 ± 13 mmHg and 42 ± 4 mmHg respectively; corresponding values in O₂ were 669 ± 7 mmHg and 47 ± 6 mmHg. The P_ACO₂ increase may trigger the AP rise in phase III.

1. Introduction

The evolution of alveolar O₂ and CO₂ partial pressures (P_AO₂ and P_ACO₂, respectively) during breath-holding was investigated by several authors (Agostoni, 1963; Ferretti et al., 1991; Hong et al., 1971; Lanphier and Rahn, 1963; Lin et al., 1974; Lindholm and Lundgren, 2006; Otis et al., 1948), whether at rest or at exercise, whether by breathing air or oxygen before breath-holding. These studies lead to the development of two concepts: the volitional or conventional breaking point of apnoea, which represents the time at which it becomes impossible to keep the glottis voluntarily closed, and the physiological breaking point (PBP) of apnoea, which was defined as the time of appearance of the first involuntary diaphragmatic contraction (Ferretti, 2001; Fitz-Clarke, 2018; Lin et al., 1974). The O₂ – CO₂ diagram of Rahn and Fenn (1955) was used to represent these concepts graphically (Agostoni, 1963; Ferretti, 2001; Lin et al., 1974; Rahn and Fenn, 1955). Hong et al. (1971) investigated also the time courses of cardiovascular changes and alveolar gas composition during breath-holding: they obtained alveolar gas composition by means of a sampler bag, and measured arterial pressure by an invasive method, and cardiac output (\dot{Q}) by means of a dilution technique. They could obtain only heart rate (f_H) on a beat-by-beat basis, so they could not infer on possible relations between cardiovascular responses and alveolar gas composition.

Further technical developments allowed also continuous

measurements of arterial blood pressure, stroke volume (Q_S) and \dot{Q} on beat-by-beat basis. So, beat-by-beat recordings of cardiovascular variables during maximal dry apnoeas were carried out (Costalat et al., 2017, 2015, 2013; Fagoni et al., 2017, 2015; Lemaître et al., 2008; Perini et al., 2010, 2008; Sivieri et al., 2015; Tocco et al., 2012, 2013). The patterns of cardiovascular changes, especially in the final phase of maximal apnoeas, suggest that they may have some link with the patterns of alveolar gases. More specifically, some authors hypothesized that the transition between the second steady phase (phase II) and the third unsteady state phase (phase III) of the cardiovascular parameters' time course (Perini et al., 2010, 2008) may be linked to a precise alveolar gas composition (Fagoni et al., 2017, 2015; Perini et al., 2008; Sivieri et al., 2015; Taboni et al., 2018). Fagoni et al. (2017) coupled the study of beat-by-beat cardiovascular responses to apnoea with determinations of alveolar gas composition, but the latter was obtained only at the end of maximal apnoeas and at the end of the phase II of the cardiovascular response.

The aim of this study was to perform a simultaneous investigation of the time courses of alveolar gas composition and the beat-by-beat cardiovascular parameters, in order to shed light on the mechanisms involved in the respiratory responses to breath-holding. The main question was to check whether the patterns of alveolar gases may explain some features of the cardiovascular response to breath-holding. It was a deductive study, without a firm hypothesis. It was carried out at rest, while breathing either air or oxygen before the apnoeas in order to

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assess the different role of hypoxia and hypercapnia. Coupling of cardiovascular patterns with alveolar gas patterns at end of phase II was expected to inform on possible correspondence with PBP. Eventually, a model for PBP and the conventional breaking point of apnoea is proposed.

2. Materials and methods

2.1. Subjects

Eight male well trained divers were enlisted. They were 36 ± 6 years old, 174 ± 4 cm tall and 77 ± 6 kg heavy. All divers were attending an advanced level diving course, they were healthy and non-smokers and none of them had previous history of cardiovascular, pulmonary, or neurological diseases, or was taking any medication at the time of the study. All gave their informed consent after having received a detailed description of the methods and experimental procedures of the study. The study was approved by the local ethical committee and conformed to the Declaration of Helsinki.

2.2. Experimental procedure

Experiments were carried out at sea level, in an air-conditioned room at 23–24 °C, with stable relative humidity of 60–65%, and with a barometric pressure of 99–103 kPa. Subjects were asked to come to the laboratory on two occasions: the first for the tests in air and the second for the tests in oxygen. The same procedures were applied on both occasions.

Upon arrival in the laboratory, the subjects took the supine position. After instrumentation, they familiarized with the procedures. Recording started after 5 min of quiet rest. The experimental protocol consisted of 5 min of recording during quiet rest to obtain pre-apnoea control values (PRE). Then the subjects performed a series of breath-holds of varying duration, administered in random order. Selected apnoea times were 30, 60, 90, 120, 150, 180 s, plus one maximal apnoea for the protocol in air, and 30, 60, 120, 180, 240 s, plus one maximal apnoea, for the protocol in oxygen. Each breath-hold was followed by 3 min of recovery to re-equilibrate alveolar gases. The subject did not know apnoea duration in advance; he was always asked to start a maximal breath-hold, which was interrupted upon request of the experimenter, except for the maximal apnoea, which was prolonged to the volitional breaking point.

The day of the test in oxygen, experiments were carried out during oxygen breathing (inspired partial pressure of $O_2 = 715 \pm 5$ mmHg). The gas was delivered by means of a two-way non-rebreathing T-shape valve (Hans Rudolph, Inc., Shawnee, KS, USA). The inhalation port was connected to a 200 l Douglas bag, which was used as a pressure buffer and was filled with 100% oxygen coming from a high-pressure tank. Ten minutes of quiet breathing was allowed after the connection to the O_2 delivery system, until attainment of alveolar gas equilibration (Darling et al., 1940; Fagoni et al., 2015; Taboni et al., 2018), then parameters' recording started.

In both conditions, apnoeas were preceded by the subject's pre-dive routine, usually consisting of some deep respiratory acts, ending with a deep inspiration, so that the lung volume at which the apnoeas started was close to the subject's total lung capacity. At the end of each breath-hold, the subject performed a maximal expiration, to allow for expiratory gas analysis.

2.3. Measurements

The parameters recorded during the entire procedure were: (1) beat-by-beat non-invasive arterial blood pressure profile (Portapres[®], Finapres[®] Medical Systems, Enschede, The Netherlands); (2) beat-by-beat peripheral haemoglobin oxygen saturation (SpO_2) at an earlobe by

red and infrared light absorption analysis (ear clip transducer TSD124B with the oximetry amplifier OXY100E module, BIOPAC[®] Systems Inc., Goleta, CA, USA); (3) beat-by-beat f_H by electrocardiography (ECG100C module, BIOPAC[®] Systems Inc., Goleta, CA, USA); (4) breath-by-breath inspiratory and expiratory flows by ultrasonic flowmeter (Spiroson[®], ECO MEDICS AG, Duernten, Switzerland) calibrated with a three-litre syringe; (5) continuous air fraction of oxygen (FO_2) and carbon dioxide (FCO_2) at the mouth by means of a paramagnetic oxygen analyser (O2100C module, BIOPAC[®] Systems Inc., Goleta, CA, USA) and an infrared CO_2 analyser (CO2100C module, BIOPAC[®] Systems Inc., Goleta, CA, USA) respectively, calibrated with two gas mixtures of known composition.

All signals were collected and sampled at 200 Hz (MP150 system with AcqKnowledge acquisition and analysis software, BIOPAC[®] Systems Inc., Goleta, CA, USA) and stored on a personal computer for subsequent analysis. FO_2 , FCO_2 and respiratory flows traces were aligned for the time delay between the flowmeter and the gas analysers. Flat flowmeter signals provided beginning, ending and duration of breath-holds.

2.4. Data treatment

Partial pressure of O_2 and CO_2 were computed as FO_2 and FCO_2 times the dry barometric pressure, respectively. P_{AO_2} and P_{ACO_2} were obtained from the alveolar plateau recorded during the maximal expiration at the end of each breath-hold. Arterial blood pressure profiles were analysed, to obtain beat-by-beat values of systolic (SAP), diastolic (DAP), and mean (MAP) blood arterial pressure using the Beatscope[®] software (Finapres[®] Medical Systems, Enschede, The Netherlands). The same software provided also Q_S by means of the Modelflow method (Wesseling et al., 1993). Beat-by-beat \dot{Q} was computed as the product of single-beat Q_S times the corresponding single-beat f_H . Total peripheral resistances (TPR) were calculated as the ratio between MAP and \dot{Q} . An automated procedure implemented under MATLAB (version 7.6.0.324, MathWorks[®], Natick, MA, USA) was used to identify the three phases of apnoea in maximal breath-holds both in air and in oxygen (Fagoni et al., 2017, 2015; Sivieri et al., 2015; Taboni et al., 2018) by means of linear regression analysis, allowing the detection of changes in slope between successive phases. The determination of the alveolar gas composition at the end of phase II was obtained interpolating linearly the alveolar gas composition of the interrupted apnoeas preceding and following the time at the end of phase II.

2.5. Statistical analysis

Data are presented as mean \pm standard deviation. Differences between air and oxygen apnoeas were investigated by means of Student's *t*-test. Differences among the final values of the interrupted apnoeas were examined with a one-way ANOVA and isolated by means of Tukey test. Comparison of our data with those from the literature (see Discussion) was done with the Welch's *t*-test (Welch, 1947). Parameters of the conventional breaking point and of PBP models have been determined with non-linear least squares fitting of experimental data. Differences were considered significant when $p < 0.05$, otherwise they were considered non-significant (NS). The statistical software Prism (version 7, GraphPad[®], La Jolla, CA, USA) was used for this aim.

3. Results

Maximal apnoea duration was 200 ± 11 s in air and 354 ± 35 s in oxygen ($p < 0.05$). In both conditions, the time course of cardiovascular parameters during maximal apnoeas showed the three phases originally described by Perini et al. (2008) (see Fig. 1). The first unsteady phase (phase I) lasted 16 ± 4 s, both in air and in oxygen. Phase II lasted 82 ± 27 s and 162 ± 61 s ($p < 0.05$) and phase III lasted 101 ± 34 s and 175 ± 43 s respectively ($p < 0.05$), in air and in

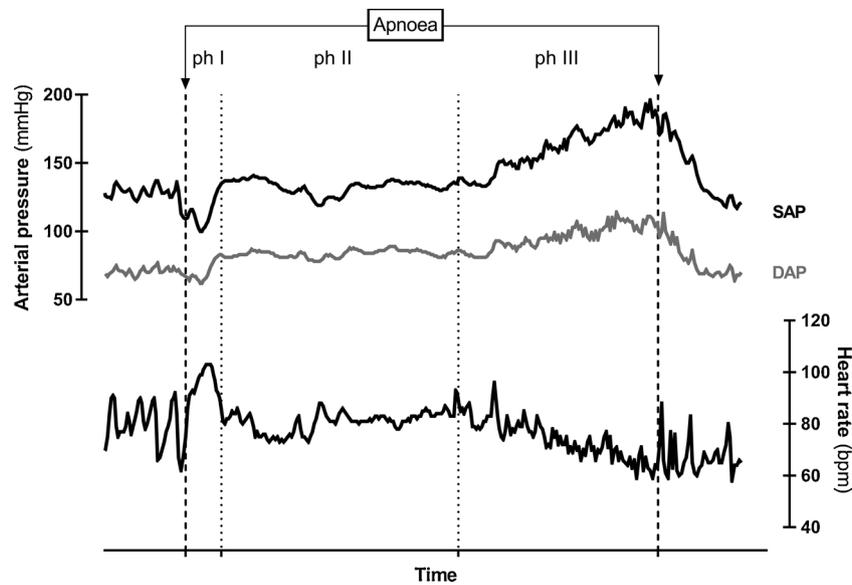


Fig. 1. Time course of heart rate and arterial blood pressure during a maximal apnoea in air from a given subject. The three phases of cardiovascular responses are shown. SAP: systolic arterial pressure; DAP: diastolic arterial pressure; ph I, ph II, and ph III: phases I, II, and III of cardiovascular responses to apnoea, respectively.

oxygen respectively.

When apnoeas were performed in air, PRE values of main cardiovascular parameters were: SAP: 154 ± 17 mmHg; DAP: 75 ± 7 mmHg; f_H : 90 ± 10 bpm; Q_S : 111 ± 10 ml; \dot{Q} : 10.0 ± 1.5 l min^{-1} ; TPR: 10.0 ± 1.4 mmHg min^{-1} . In pure oxygen, PRE values were: SAP: 143 ± 18 mmHg; DAP: 74 ± 8 mmHg; f_H : 79 ± 10 bpm ($p < 0.05$ vs. PRE f_H in air); Q_S : 107 ± 11 ml; \dot{Q} : 8.3 ± 1.1 l min^{-1} ($p < 0.05$ vs. PRE \dot{Q} in air) TPR: 11.7 ± 1.7 mmHg min^{-1} . The mean values of SAP, DAP, f_H , Q_S , \dot{Q} , and TPR measured on the last 10 beats of each apnoea, both performed in air and in oxygen, are shown in Fig. 2.

In PRE, SpO_2 was $100 \pm 0\%$ both in air and in pure oxygen. When apnoeas were performed in oxygen SpO_2 remained always equal to 100%. When apnoeas were performed in air, the minimum SpO_2 was attained at 19 ± 2 s after the end of each breath-holds. The time course of minimum SpO_2 is plotted in Fig. 3.

In air, PRE $\text{P}_{\text{A}}\text{O}_2$ was 125 ± 7 mmHg and PRE $\text{P}_{\text{A}}\text{CO}_2$ was 31 ± 3 mmHg. In oxygen, PRE $\text{P}_{\text{A}}\text{O}_2$ was 683 ± 5 mmHg and PRE $\text{P}_{\text{A}}\text{CO}_2$ was 30 ± 5 mmHg. End apnoea $\text{P}_{\text{A}}\text{O}_2$ and $\text{P}_{\text{A}}\text{CO}_2$ are plotted as a function of time of apnoea in Fig. 4.

At the end of maximal apnoeas in air, $\text{P}_{\text{A}}\text{O}_2$ was 50 ± 9 mmHg and $\text{P}_{\text{A}}\text{CO}_2$ was 48 ± 5 mmHg; while at the end of maximal apnoeas in oxygen, $\text{P}_{\text{A}}\text{O}_2$ was 653 ± 8 mmHg and $\text{P}_{\text{A}}\text{CO}_2$ was 55 ± 5 mmHg. In

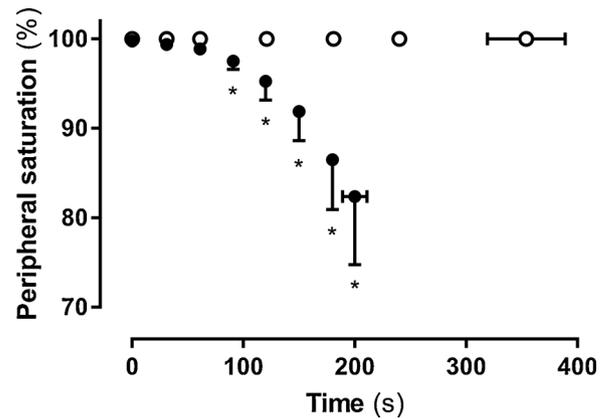


Fig. 3. Minimum peripheral oxygen saturation attained after the end of each apnoea in air (full dots), and in pure oxygen (open dots). *: significantly different vs. time 0 value in air.

air, interpolated $\text{P}_{\text{A}}\text{O}_2$ and $\text{P}_{\text{A}}\text{CO}_2$ at the end of phase II were 90 ± 13 mmHg and 42 ± 4 mmHg respectively; in oxygen, at the end of phase II, $\text{P}_{\text{A}}\text{O}_2$ was 669 ± 7 mmHg and $\text{P}_{\text{A}}\text{CO}_2$ was 47 ± 6 mmHg.

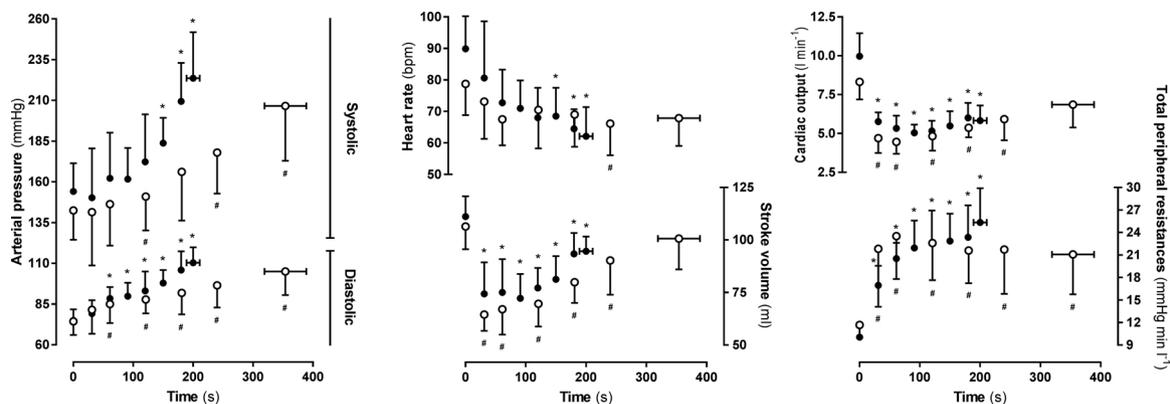


Fig. 2. Mean values and standard deviation of systolic and diastolic arterial pressure (left panel), heart rate and stroke volume (central panel), and of cardiac output and total peripheral resistances (right panel) obtained on the last 10 beats of each apnoea are plotted against time of apnoea. Time 0 of each panel represents the pre-apnoea mean value. Results from both apnoeas performed in air (full dots) and in pure oxygen (open dots) are shown. *: significantly different vs. time 0 value in air; #: significantly different vs. time 0 value in oxygen.

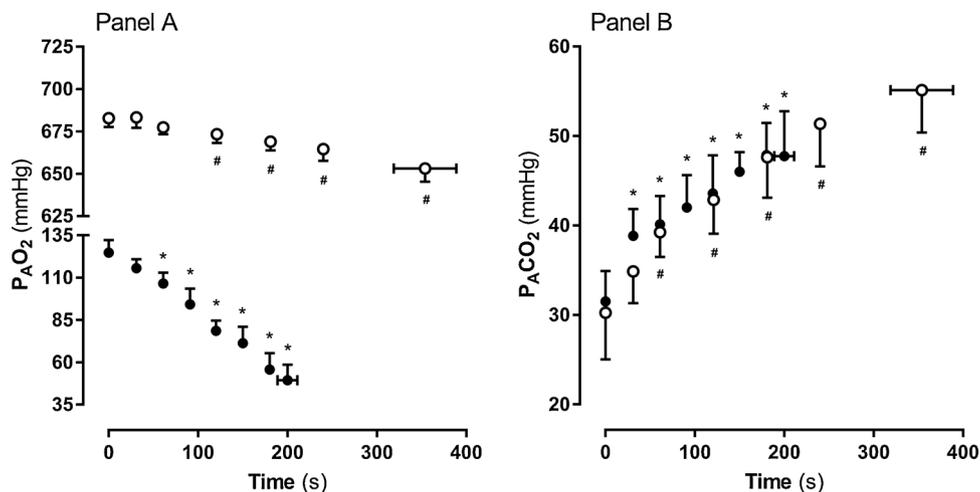


Fig. 4. Mean alveolar partial pressures of O₂ (P_AO₂, panel A) and of CO₂ (P_ACO₂, panel B) plotted as a function of time of apnoea in air (full dots) and in oxygen (open dots). *: significantly different vs. time 0 value in air; #: significantly different vs. time 0 value in oxygen.

All values were significantly different from those at the end of the corresponding maximal apnoea.

Mean alveolar gas compositions at the end of all apnoeas are reported on a P_AO₂ – P_ACO₂ diagram in Fig. 5 for both apnoeas performed in air and in oxygen. The alveolar gas composition moves upward and leftward with the apnoea duration.

Main cardiovascular parameters were plotted as a function of P_AO₂ and of P_ACO₂ in Fig. 6 for apnoeas performed in air and in Fig. 7 for apnoeas performed in pure oxygen. The alveolar gas composition interpolated at the end of phase II is reported on the same Figures.

4. Discussion

The time courses of arterial pressure and f_H in air and in oxygen are consistent with previous findings, in fact, in both conditions the three phases of the cardiovascular response to apnoea, originally defined by Perini et al. (2008), could be clearly recognized, as well as an increase in the duration of phases II and III in oxygen with respect to air (Fagoni et al., 2015). The mean values of Q̇ and Q_S at the end of interrupted apnoeas in air agree with those found by Costalat et al. (2013) during dry apnoeas. Nevertheless, a final drop in f_H (i.e. phase III) was reported also in pure oxygen, but with an unchanged SpO₂ equal to 100%; thus, it is not the change in blood oxygenation to prompt the f_H drop in phase III.

The patterns of alveolar gases were consistent with those observed in previous studies implying apnoeas of progressively increasing duration (Ferretti et al., 1991; Hong et al., 1971; Lanphier and Rahn, 1963). Notwithstanding, the time necessary to attain the conventional breaking point differed among studies depending on metabolic rate, it

was shorter than in the present study when apnoeas were performed in sitting posture (Lin et al., 1974), and on preceding hyperventilation manoeuvres, it was longer than in the present study when 1 min of hyperventilation preceded breath-holding in supine posture (Ferretti et al., 1991). To the best of our knowledge, this was the first time that the beat-by-beat recordings of several cardiovascular variables of interest were coupled with an analysis of the time course of alveolar gas composition.

4.1. On the cardiovascular responses to apnoea

The relationship between SAP and P_ACO₂ was positive (Figs. 6 and 7), with a sudden increase in slope after attainment of the interpolated P_ACO₂ value at the end of phase II, which appears to act as a kind of threshold triggering the dramatic SAP increase in phase III.

In contrast with SAP, the relationships between f_H and P_AO₂ or P_ACO₂ in air followed different patterns from those in pure oxygen. In fact, after the initial decrease in f_H at the beginning of apnoea, which was interpreted as a baroreflex response in previous studies (Fagoni et al., 2017, 2015; Sivieri et al., 2015), the decrease over time continued in air, but was softened in pure oxygen. As a consequence, the f_H drop turned out bigger in air than in oxygen (–28 ± 13 bpm and –11 ± 8 bpm respectively, p < 0.05). This finding may support the hypothesis that apnoea-induced bradycardia is not only the result a vagal reflex as commonly stated, but it is also sustained by incoming hypoxia. As such, it could be assimilated to a kind of initial diving response (Andersson and Evaggelidis, 2009; Andersson and Schagatay, 1998a; Ferretti, 2001; Ferrigno et al., 1997; Fitz-Clarke, 2018).

Both in air and in pure oxygen, the Q_S fell at the beginning of

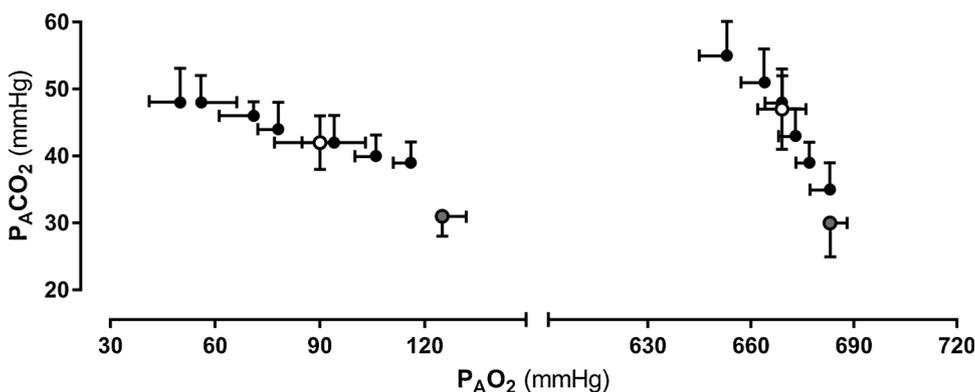


Fig. 5. Mean alveolar partial pressures of oxygen and carbon dioxide (P_AO₂ and P_ACO₂, respectively) in pre-apnoea (grey dots) and at the end of apnoea of incremental duration (black dots). On this graph are plotted data from apnoea performed in air (on the left side) and in oxygen (on the right side). The interpolated alveolar gas composition at the end of phase II (white dot) is also reported with its standard deviation.

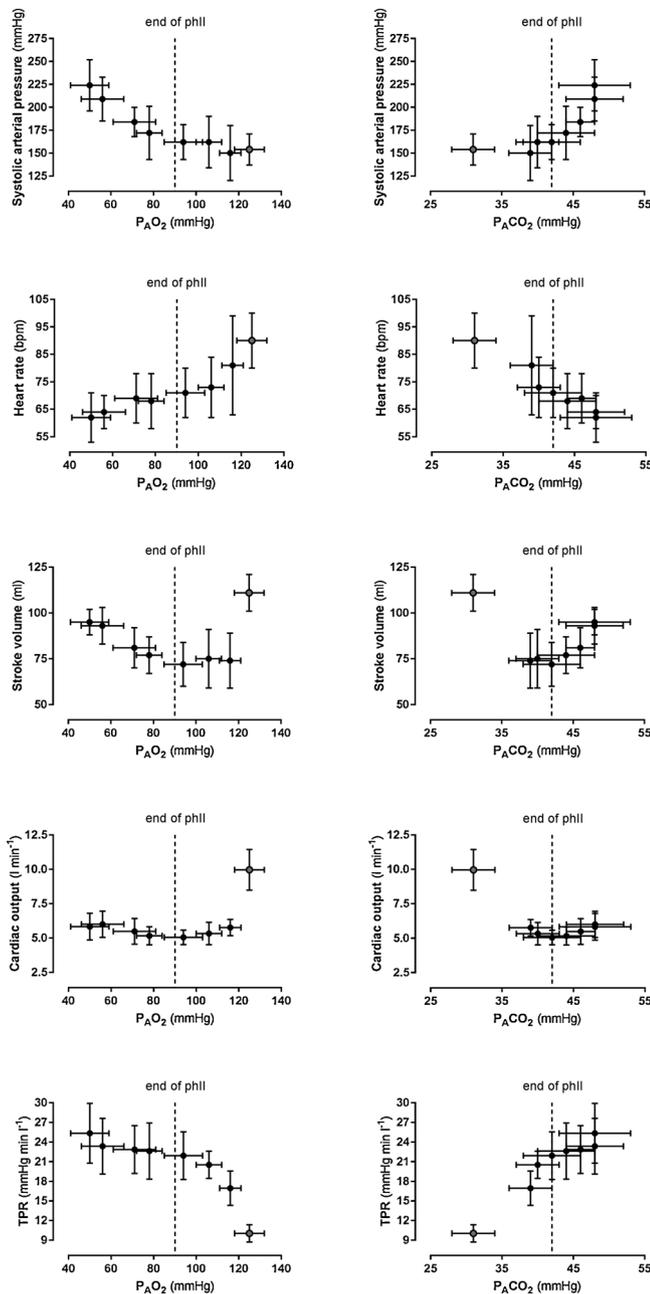


Fig. 6. Systolic arterial pressure, heart rate, stroke volume, cardiac output, and total peripheral resistances (TPR) plotted as a function of the end-apnoea alveolar partial pressure of oxygen ($P_{A}O_2$, left column of panels) and of carbon dioxide ($P_{A}CO_2$, right column of panels) in apnoeas performed in air. Grey dots represent the pre-apnoea values. The corresponding end of phase II of apnoea (end of phII) is represented as a dashed vertical line.

apnoea, possibly as a consequence of the elevated central venous pressure at high lung volume, which reduces venous return. The subsequent partial recovery of Q_s in air was coupled with the decrease in f_H , thus resulting in stable \dot{Q} values in the apnoea duration range from 30 s to the maximum. Conversely, in pure oxygen, the slower decrease of f_H caused a continuous increase of \dot{Q} from 30 s on. Assuming that the initial fall in \dot{Q} is linked to the fall in venous return due to both the high intrathoracic pressure and the stop in the pumping effect of the ventilation, some authors speculated that a constantly low \dot{Q} in air might reduce the pulmonary oxygen uptake, thus preserving the lung oxygen stores (Andersson et al., 2004; Andersson and Evagelidis, 2009; Lindholm et al., 2002; Linér and Linnarsson, 1994). Support of this hypothesis would require an analysis of ventilation-perfusion

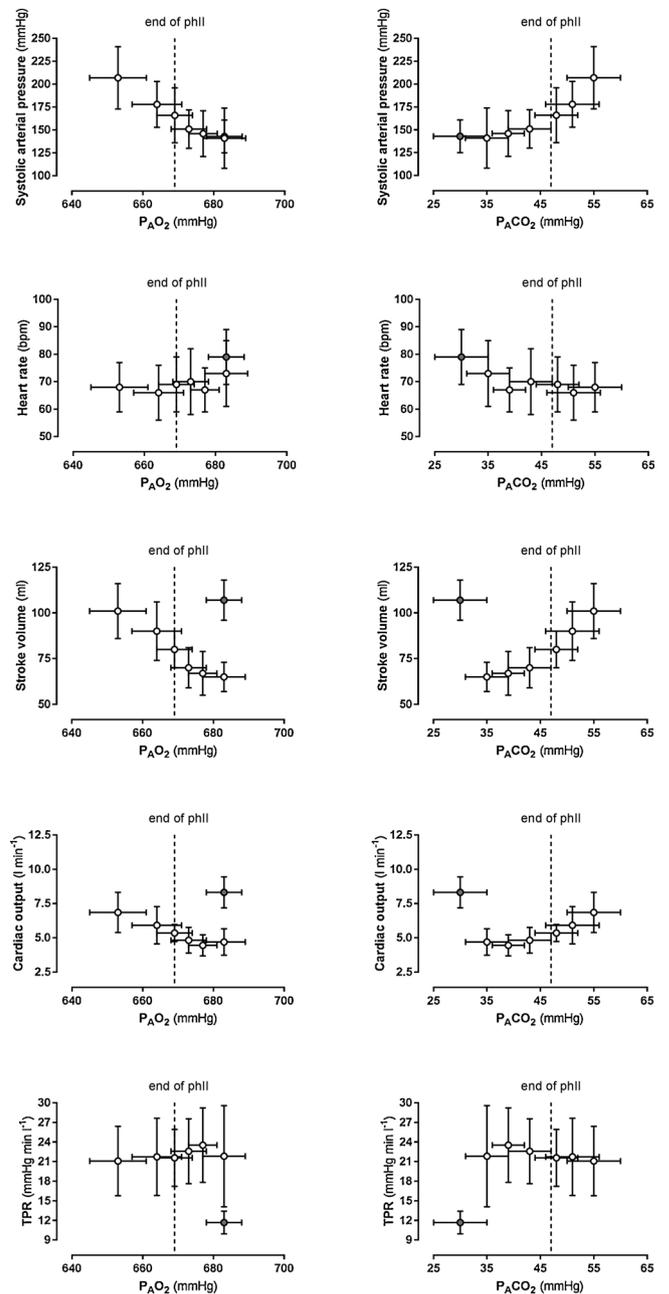


Fig. 7. Systolic arterial pressure, heart rate, stroke volume, cardiac output, and total peripheral resistances (TPR) plotted as a function of the end-apnoea alveolar partial pressure of oxygen ($P_{A}O_2$, left column of panels) and of carbon dioxide ($P_{A}CO_2$, right column of panels) in apnoeas performed in pure oxygen. Grey dots represent the pre-apnoea values. The corresponding end of phase II of apnoea (end of phII) is represented as a dashed vertical line.

inequality, which could not be carried out in the present study.

The correction of SAP after attainment of the minimum SAP in phase I is coupled to an increase of TPR, which in fact was higher at 30 s than in PRE. Thereafter, TPR continuously increased in air, while it flattened in oxygen. Desaturation of arterial blood in air may suggest that the TPR increase results from vasoconstriction related to diving response mechanisms. Whatever the case, the difference between final and initial TPR was higher in air than in oxygen ($15 \pm 5 \text{ mmHg min l}^{-1}$, and $9 \pm 4 \text{ mmHg min l}^{-1}$, respectively, $p < 0.05$).

To summarise, the patterns of the cardiovascular responses to apnoea analysed in this paragraph suggest that: i) the increase in partial

pressure of CO₂ may trigger the rise of SAP in phase III; ii) mechanisms similar to a diving response appear in air, but are less accentuated in oxygen; iii) consequently, different \dot{Q} patterns appear in oxygen with respect to air. Although the present results are only indirectly related to the practice of breath-hold diving, any understanding of the mechanisms underlying cardiopulmonary regulations during breath-holding may concur in improving safety in diving.

4.2. On the physiological and the conventional breaking point curves

The creation of the conventional and the physiological breaking point curves is the result of the work of many physiologists. However, to the best of our knowledge, it has been only partly modelled (Lin, 1987). We believe that identifying an equation describing the two curves can ease the comparison between experimental data of different studies.

Considering the effects of a slight enlargement of the alveolo-arterial O₂ gradient negligible, we may develop an analogous for P_AO₂ Hill's model of the oxygen equilibrium curve (Hill, 1910; Roughton, 1964). On the other hand, we may assume that P_ACO₂ increases linearly with blood carbon dioxide concentration. On these premises, we are authorized to treat experimental data as a set of (x, y) points on a P_AO₂ – P_ACO₂ graph by means of the following equation:

$$P_A CO_2 = a \frac{P_A O_2^h}{b^h + P_A O_2^h} \tag{1}$$

Where *a* is the maximum P_ACO₂, *b* is the P_AO₂ at the half of the maximum value of P_ACO₂, and *h* is an equivalent of Hill's slope. Values for parameters *a*, *b* and *h* were obtained by fitting Eq. (1) to data obtained in similar experimental conditions by others (Agostoni, 1963; Lin et al., 1974), covering a wide range of alveolar gas compositions on both the conventional and the physiological breaking point curves. The results are shown in Table 1. Coefficient *h* resulted somewhat lower than Hill's coefficient for the oxygen equilibrium curve. We speculate that this may be due to the impact of heterogeneity of ventilation-perfusion distribution, which carries along an extremely large alveolo-arterial O₂ difference in oxygen with respect to air (Rahn and Fenn, 1955). Since we deal with alveolar gases, we must admit possible distortion of the relationship as compared with the predictions which may be made from the oxygen equilibrium curve.

Conventional and physiological breaking point curves constructed using the parameters reported in Table 1 were then plotted in Fig. 8. On the same Figure we finally added the mean alveolar gas compositions observed in the present study at the end of phase II (dots) and at the end of maximal apnoeas (triangles). Fig. 8 shows that the alveolar gas composition at the end of maximal apnoeas in air falls on the theoretical conventional breaking point curve, whereas the corresponding point in oxygen falls under the theoretical curve. Fagoni et al (2017) hypothesized that the attainment of the conventional breaking point could be related to the increase in P_ACO₂ rather than the absolute P_ACO₂ value. This hypothesis is supported by the observation that the P_ACO₂ value at the conventional breaking point is lower when apnoeas are preceded by hyperventilation (Klocke and Rahn, 1959; Lindholm and Lundgren, 2006), and is higher when breath-holds are performed in

Table 1

Best fitting parameters of Eq. (1) for the physiological and the conventional breaking point curves. The goodness of fit is expressed as the coefficient of determination. BP: breaking point; *a*: maximum P_ACO₂; *b*: P_AO₂ at the half of the maximum value of P_ACO₂; *h*: Hill's slope; R²: coefficient of determination.

	Physiological BP	Conventional BP
<i>a</i>	48	63
<i>b</i>	21	24
<i>h</i>	2.4	2.1
R ²	0.98	0.98

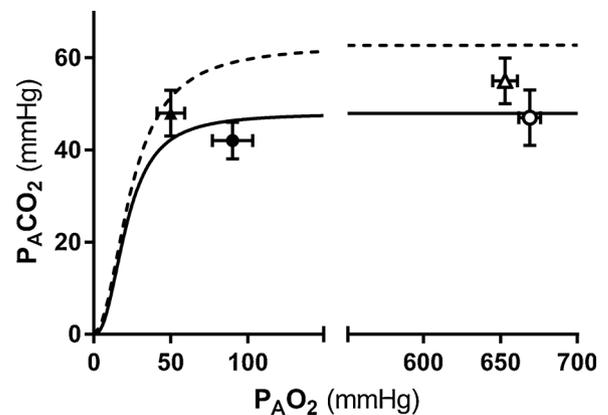


Fig. 8. Conventional and physiological breaking point curves are represented, respectively, as a dashed and as a solid line. These curves are drawn from Eq. (1) and results obtained in Table 1. Mean and standard deviation of the alveolar gas composition at the end of the phase II of the cardiovascular responses to apnoea (dots) and at the end of maximal apnoeas (triangles) are plotted. Full dots and triangles represent data in air, and open dots and triangles those in pure oxygen. P_AO₂, P_ACO₂: alveolar partial pressures of oxygen and of carbon dioxide, respectively.

hypercapnia (Kelman and Wann, 1971). Nonetheless, the conventional breaking point may be also subjected to psychological factors (Lin et al., 1974). Conversely, when we compared the modelled PBP curve and the alveolar gas composition at the end of phase II, it appears that the point in oxygen falls on the theoretical PBP curve, whereas the point in air falls under the theoretical PBP curve.

The data presented in Fig. 8 may well be discussed in the context of ongoing hypotheses on the meaning of the PBP curve. Parkes (2006) criticised the physiological meaning of the PBP, claiming that there is some tonic activation of the diaphragm during all breath-holding, but only rhythmic diaphragmatic activity can be detected. However, Fig. 8 may also suggest, as already proposed by others (Taboni et al., 2018), that the end of phase II may systematically precede the first detectable diaphragmatic contraction, yet being correlated to it.

The hypothesis that the alveolar gas composition at the end of phase II may correspond to that occurring on the PBP curve was put forward by several previous studies (Fagoni et al., 2017, 2015; Perini et al., 2008; Sivieri et al., 2015). In the context of this hypothesis, we may state that the end of phase II in oxygen may indeed represent the first detectable diaphragmatic contraction, whereas the point in air seems to anticipate it (Taboni et al., 2018). In fact, the present study represents a step forward with respect to the studies mentioned here above, because we were the first, as already pointed out, to perform serial measurements of alveolar gas composition in conjunction with the continuous determination of cardiovascular recordings during breath-holding. So, the evidence that we provide in support of this hypothesis is stronger than that obtained by previous studies. Notwithstanding, all the aforementioned hypotheses may eventually be clarified by succeeding in performing direct invasive electromyographic analysis of diaphragm activity during breath-holding.

The mathematical representation of the conventional and the physiological breaking point curves by means of Eq. (1) has, however, a limitation. In fact, Eq. (1) does not take the Bohr and the Haldane effects into account, the mathematical modelling of which has been recently investigated (Malte and Lykkeboe, 2018). Nevertheless, the simplicity of Eq. (1) may ease the comparison of results obtained with different methods and experimental procedures and the understanding of how different initial conditions, e.g. metabolic rate, alveolar gas composition, and lung volume, affect the attainment of physiological and conventional breaking points.

4.3. Methodological considerations

In breath-hold studies on human, the choice of using trained divers and non-divers is not trivial. Trained divers are able to perform longer apnoeas (Hong et al., 1970) with a more intense bradycardic response (Hong et al., 1970), probably due to the presence of a final f_H decrease in the cardiovascular responses which lacks in non-divers (Lemaître et al., 2008; Perini et al., 2008). These differences between trained divers and non-divers may be a consequence of the higher lung volumes that can be reached by the formers (Andersson and Schagatay, 1998b; Ferretti et al., 2012; Lemaître et al., 2008) or a greater psychological tolerance to the struggle phase of apnoea (Hentsch and Ulmer, 1984). Thus, it cannot be undoubtedly stated whether this is a form of adaptation or not. Nevertheless, when the phase II – phase III transition is under study, only trained divers can be used, because only trained divers can sustain diaphragmatic contractions during phase III (Perini et al., 2008).

Finally, we note that results obtained during series of resting apnoeas of progressively increasing duration can contribute to our understanding of deep breath-hold dives in the sea only indirectly. It is known that water immersion and underwater swimming imply a sum of physiological responses that are unique (Fitz-Clarke, 2018), and that deep diving entails changing in alveolar partial pressure of gases according to depth (Ferretti, 2001). However, our choice was not fortuitous: on one hand, the analysis of cardiovascular parameters and of the alveolar gas composition during apnoea in dry conditions can isolate the physiological responses to apnoea itself and, on the other hand, the possibility of recording all main cardiovascular parameters on a beat-by-beat basis non-invasively is nowadays limited under water.

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