Alveolar gas composition during maximal and interrupted apnoeas in ambient air and pure oxygen.

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Running title

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ABSTRACT

Introduction. We tested the hypothesis that the alveolar gas composition at the transition between the steady phase II ($\phi 2$) and the dynamic phase III ($\phi 3$) of the cardiovascular response to apnoea may lay on the *physiological breaking point* curve (Lin et al., 1974).

Methods. Twelve elite divers performed maximal and $\phi 2$ -interrupted apnoeas, in air and pure oxygen. We recorded beat-by-beat arterial blood pressure and heart rate; we measured alveolar oxygen and carbon dioxide pressures (P_{AO_2} and P_{ACO_2} , respectively) before and after apnoeas; we calculated the P_{ACO_2} difference between the end and the beginning of apnoeas (ΔP_{ACO_2}).

Results. Cardiovascular responses to apnoea were similar compared to previous studies. P_{AO_2} and P_{ACO_2} at the end of $\phi 2$ -interrupted apnoeas, corresponded to those reported at the *physiological breaking point*. For maximal apnoeas, P_{ACO_2} was less than reported by Lin et al. (1974). ΔP_{ACO_2} was higher in oxygen than in air.

Conclusions. The transition between $\phi 2$ and $\phi 3$ corresponds indeed to the *physiological breaking point*. We attribute this transition to ΔP_{ACO_2} , rather than the absolute P_{ACO_2} values, both in air and oxygen apnoeas.

KEYWORDS

Interrupted apnoea; maximal apnoea; oxygen apnoea; P_{ACO_2} ; alveolar gas; diaphragmatic contraction.

ABBREVIATION LIST

DBP	Diastolic blood pressure
HR	Heart rate
MBP	Mean blood pressure
$P_{A}CO_2$	Alveolar pressure of carbon dioxide
$P_{A}O_2$	Alveolar pressure of oxygen
$\dot{Q}$	Cardiac output
SBP	Systolic blood pressure
SpO_2	Peripheral oxygen saturation
SV	Stroke volume
TPR	Total peripheral resistance
$\Delta P_{A}CO_2$	Difference in the alveolar carbon dioxide pressure between the end and the beginning of apnoea
ϕ_1	Phase I: first dynamic phase of the cardiovascular response to apnoea
ϕ_2	Phase II: steady-state phase of the cardiovascular response to apnoea
ϕ_3	Phase III: last dynamic phase of the cardiovascular response to apnoea

INTRODUCTION

Beat-by-beat analysis of the cardiovascular responses to maximal apnoeas at rest (Costalat et al., 2013; Fagoni et al., 2015; Lemaître et al., 2008; Perini et al., 2008, 2010; Sivieri et al., 2015) allowed identification of three distinct phases: (i) a short dynamic phase, phase I (ϕ_1), lasting less than 30 s, characterised by rapid changes in blood pressure and heart rate (HR); (ii) a steady state phase, phase II (ϕ_2), lasting about two min, in which the values attained by each variable at the end of ϕ_1 stay invariant; and (iii) a further subsequent dynamic phase, phase III (ϕ_3), characterised by a continuous decrease in HR and increase in blood pressure, until the *volitional breaking point* is reached.

At the beginning of ϕ_1 , there is a sudden fall of stroke volume (SV) and of arterial blood pressure (Andersson and Schagatay, 1998; Palada et al., 2007; Perini et al., 2008), which may be generated by an immediate increase in central venous pressure related to the high lung volumes at which apnoeas are carried out (Andersson and Schagatay, 1998). The ensuing increase in HR in the initial part of ϕ_1 was seen as a baroreflex attempt at controlling blood pressure (Fagoni et al., 2015). The duration of ϕ_1 is invariant and independent of the metabolic rate (Sivieri et al., 2015) and of the size of lung oxygen stores (Fagoni et al., 2015). Conversely, the meaning of ϕ_2 and ϕ_3 is still unclear. According to Perini et al. (2008, 2010), the end of ϕ_2 might correspond to the end of the so-called *easy going phase* (Hong et al., 1971; Lin et al., 1974). If this hypothesis is correct, then the alveolar gas composition at the end of ϕ_2 should lie, on an O_2 - CO_2 diagram (Rahn and Fenn, 1955), around the *physiological breaking point* curve (Hong et al., 1971; Lin et al., 1974). Coherently with these concepts, the duration of ϕ_2 is prolonged when lung oxygen stores are increased (Fagoni et al., 2015) and reduced at exercise, when the metabolic rate is increased (Sivieri et al., 2015).

A detailed analysis of the alveolar gas composition at the end of ϕ_2 was never carried out in humans so far. The purpose of the present study was to demonstrate that the alveolar gas

composition at the end of ϕ_2 corresponds indeed to that reported at the *physiological breaking point*. In order to expand the range of investigated alveolar gas composition, experiments were carried out in air and in pure oxygen.

METHODS

Subjects

Twelve competitive divers (9 males and 3 females) volunteered for this study. They were 42 ± 7 years old, 72 ± 11 kg heavy and 176 ± 9 cm tall. All participants refrained from apnoea training and from strenuous exercise on the days of the measurements. All divers were healthy non-smokers. None had previous history of cardiovascular, pulmonary or neurological diseases, or was taking medications at the time of the study. All gave their informed consent after having received a detailed description of the methods and of the experimental procedures. The study conformed to the Declaration of Helsinki and was approved by the local ethical committee.

Experimental design

Experiments were carried out in Lignano Sabbiadoro, Italy, in an air-conditioned room at 23°C , with relative humidity between 60 and 65%, on two different days. On the first day, upon arrival in the laboratory and after instrumentation, the subject took the supine posture. Five min were allowed to achieve steady state conditions before starting the recordings. Then 10 min of resting recordings were obtained during spontaneous normal breathing; in this period, all the cardiovascular parameters were collected and were retained as control values (control). Then the subject performed two maximal apnoeas, the first of which was systematically shorter than the second: the first apnoea was thus taken as a training apnoea, the second as the experimental apnoea in air. These apnoeas were separated by a recovery

interval of two min, as described elsewhere (Fagoni et al., 2015; Sivieri et al., 2015). At the end of the second apnoea, after further two min of recovery, the subject started breathing pure oxygen. Oxygen was administered from high-pressure, high-precision cylinders, via a Douglas bag that was used as pressure buffer. After 10 min of normal breathing to clear nitrogen from the airways, and further five min of resting recordings, the subject performed the third maximal apnoea. After the end of this apnoea, the subject kept breathing pure oxygen for further two min during the recovery period.

Both in air and in oxygen, the subject underwent his/her pre-dive routine breathing before breath-holding, consisting of a couple of deep respiratory acts. A deep inspiration preceded the apnoeas, so that the lung volume at which the apnoeas started was close to the subject's total lung capacity. As a consequence, the first breathing movement at the end of the apnoeas was an expiration, which the subject performed as deep as possible, in order to allow analysis of alveolar gases from the alveolar plateau.

The cardiovascular time courses of the apnoeas carried out on the first day were immediately analysed, in order to identify ϕ_1 , ϕ_2 and ϕ_3 for each apnoea. The identification of the time, at which the transitions between ϕ_2 and ϕ_3 occurred, defined the duration of ϕ_2 -interrupted apnoeas to be performed on the following day. The procedure for the ϕ_2 -interrupted apnoeas was the same as on the first day, the unique difference being the shorter duration of the apnoeas. Each diver performed three apnoeas, two in air and one in oxygen.

Measurements

Arterial blood pressure profiles (PortaPres[®], TNO-TPD, Amsterdam, The Netherlands) were continuously recorded throughout the experiments. Peripheral blood O₂ saturation (SpO₂) was also continuously monitored by infrared spectroscopy (BioPac System Inc., Goleta, CA, USA) at an earlobe. Cardiac output ($\dot{Q}$) was continuously measured on a beat-by-beat basis

by impedance cardiography (ACKICG module, BioPac System Inc., Goleta, CA, USA). The signals were sampled at 100 Hz by using a 16-bit A/D converter (MP100 VS, BioPac System Inc., Goleta, CA, USA) and stored on a personal computer for subsequent analysis.

The time course of oxygen and carbon dioxide partial pressures throughout the respiratory cycles were calibrated against a gas mixture of known composition and continuously monitored by rapid gas analysers (O2100C and CO2100C respectively for O₂ and CO₂, BioPac System Inc., Goleta, CA, USA). The inspiratory and expiratory ventilations were measured by an ultrasonic flowmeter (Spiroson, Ecomedics, Duernten, Switzerland) calibrated with a 3 l syringe. The traces alignment was corrected for the time delay between the flowmeter and the gas analysers.

The breath-by-breath recording of respiratory data was obviously interrupted during the apnoeas. The time with flat gas flow signals provided the duration of apnoeas. The deep expiration at the end of apnoea was analysed and the alveolar partial pressures of O₂ and CO₂ determined on the alveolar plateau (P_AO₂ and P_ACO₂, respectively) were retained as representative of the mean alveolar gas composition at the end of the breath-holding.

Data treatment

Arterial pressure profiles were analysed off line, to obtain beat-by-beat values of HR, SBP, diastolic and mean blood pressure (DBP and MBP, respectively), using the Beatscope™ software (TNO-TPD, The Netherlands). SV was computed on a beat-by-beat basis by dividing each $\dot{Q}$ value by the corresponding HR value. The ratio between MBP and $\dot{Q}$ provided an estimate of total peripheral resistances (TPR).

The beat-by-beat data were analysed off-line to identify the three phases of apnoeas. An automated procedure implemented under Matlab (version 7.6.0.324, MathWorks, Natick, MA, USA) was used to this aim. The procedure was based on linear regression analysis,

allowing detection of changes in slope between successive phases; linear regression was also used to verify steady state of investigated variables in $\phi 2$, as described elsewhere (Sivieri et al., 2015).

Control values were computed from the resting recordings as the mean value over one min for each cardiovascular parameter (SBP, DBP, HR, SV, $\dot{Q}$, and TPR), for both maximal and $\phi 2$ -interrupted apnoeas. For each cardiovascular variable, in the analysis of the time course of apnoeas, the following values were highlighted: “Start”, referring to the single value just before the beginning of apnoeas; “MSP”, i.e. the single value when SBP reached its minimum during $\phi 1$; “Last 10 beats $\phi 2$ ”, i.e. the average of the last 10 beats during $\phi 2$; and “Last 10 beats $\phi 3$ ”, i.e. the average of the last 10 beats during $\phi 3$.

Statistical analysis

Data are presented as mean and standard deviation (SD). Two-way ANOVA was used to compare cardiovascular data during the time course of apnoeas, in all different conditions, and to compare control values between the two experimental sessions, carried out on different days. Tukey test was used as post-hoc test to isolate the differences when necessary. Differences within maximal and interrupted apnoeas were located by means of Student’s T test, and were considered significant when $p < 0.05$, otherwise they were considered non-significant (NS). The Stata 10.0 statistical software (StataCorp, College Station, TX, USA) was used to this aim.

RESULTS

Table 1 shows the control values for each parameter, recorded in air and oxygen apnoeas (NS), in the two different sessions (before the maximal and before the $\phi 2$ -interrupted apnoeas). The ANOVA showed no differences among the four investigated conditions.

The duration of maximal apnoeas was longer ($p<0.05$) in oxygen (527 ± 203 s) than in air (231 ± 51 s). Similarly, interrupted apnoeas were longer ($p<0.05$) in oxygen (203 ± 53 s) than in air (124 ± 39 s).

Figure 1 reports an example of the time course of HR and SBP for maximal (panels A and B) and $\phi 2$ -interrupted (panels C and D) apnoeas, in air and oxygen. The same pattern was found in all the subjects. The values attained by the cardiovascular variables at selected times during maximal and $\phi 2$ -interrupted apnoeas are reported in Table 2. Characteristics and durations of $\phi 1$ were similar in maximal as in $\phi 2$ -interrupted apnoeas, without differences between air and oxygen (NS). The initial decrease in SBP led to a minimum (minimum of systolic blood pressure, MSP) at which SBP was lower than at Start. During maximal apnoeas, MSP was attained within 5.2 ± 1.7 s in air and 6.3 ± 3.2 s in oxygen (NS). During $\phi 2$ -interrupted apnoeas, the time required to attain the MSP was 6.5 ± 1.8 s in air, and 10.0 ± 6.3 s in oxygen (NS). No significant differences with respect to day one were observed on day two for MSP and the time to attain it. During the same period, HR tended to increase, reaching a maximum at MSP, yet without being significantly different from Start, during both maximal and interrupted apnoeas, whether in air or in oxygen. At the end of $\phi 1$, SBP, DBP, and HR were the same as at Start.

In maximal apnoeas, $\phi 2$ was longer in oxygen (164 ± 47 s) than in air (101 ± 39 s); similarly, $\phi 3$ lasted 335 ± 172 s and 107 ± 33 s in oxygen and in air, respectively ($p<0.05$). The former was the case also during interrupted apnoeas: $\phi 2$ lasted 172 ± 54 s in oxygen and 100 ± 39 s in air ($p<0.05$) whereas $\phi 3$ was obviously missing in $\phi 2$ -interrupted apnoeas. Both in air and in oxygen, the HR and blood pressure values attained at the end of $\phi 1$ remained unchanged throughout the entire $\phi 2$, whether in maximal or in $\phi 2$ -interrupted apnoeas.

In maximal apnoeas, during $\phi 3$, SBP and DBP progressively increased until the end of apnoeas, both in air and in oxygen. At the end of $\phi 3$, SBP was higher than at all other

representative time periods of breath-holding. Meanwhile, HR decreased continuously during $\phi 3$ in air, to reach its minimum at the end of the maximal apnoea. This value was significantly lower than at Start and MPS. This decrease in HR was not observed in oxygen maximal apnoeas.

In air, SV and $\dot{Q}$ were lower at the MSP compared to Start. Then they slightly recovered, yet attaining values at the beginning of $\phi 2$, which however remained lower than at Start. SV and $\dot{Q}$ were stable during the entire $\phi 2$, in both maximal and $\phi 2$ -interrupted apnoeas. At the end of $\phi 3$, SV was at the same level as in Start (NS between air and oxygen apnoeas). $\dot{Q}$ followed a similar pattern, yet remaining lower ($p < 0.05$) at the end of $\phi 3$ than at Start. In oxygen apnoeas, at the end of $\phi 3$, $\dot{Q}$ returned to the same level as at Start (NS between air and oxygen apnoeas). TPR tended to increase during $\phi 1$, being higher ($p < 0.05$) during $\phi 2$ than at Start, in $\phi 2$ -interrupted apnoeas.

The alveolar gas composition at the end of maximal and interrupted apnoeas is reported in Table 3. P_{AO_2} was always higher ($p < 0.05$) in oxygen than in air, and lower ($p < 0.05$) at the end of breath-holding than at Start. In maximal apnoeas, P_{ACO_2} increased during breath-holding, resulting higher ($p < 0.05$) at the end of breath-holding than before, both in oxygen and in air. P_{ACO_2} was lower ($p < 0.05$) in oxygen than in air before the apnoeas, and it was higher ($p < 0.05$) in oxygen than in air at the end of breath-holding. Thus, the difference between the P_{ACO_2} (ΔP_{ACO_2}) resulted higher ($p < 0.05$) in oxygen than in air. SpO_2 reached a nadir of 74.4 ± 19.0 % after 24.7 ± 6.3 s the end of maximal apnoeas in air. In maximal apnoeas in oxygen SpO_2 was always 100 %. During $\phi 2$ -interrupted apnoeas, P_{ACO_2} was lower in oxygen than in air before the apnoeas, and it was similar in oxygen as in air at the end of apnoeas. Thus, the resulting ΔP_{ACO_2} was higher in oxygen than in air. SpO_2 attained a minimum, 95.8 ± 3.8 %, after 29.4 ± 9.2 s the end of $\phi 2$ -interrupted apnoeas in air. This value was higher than the corresponding value observed at the end of maximal apnoeas in air.

The P_{AO_2} and P_{ACO_2} values observed at the end of ϕ_2 -interrupted apnoeas were higher and lower, respectively, than the corresponding values observed at the end of maximal apnoeas, both in air and in oxygen.

DISCUSSION

This study was carried out to test the hypothesis, put forward by several authors (Fagoni et al., 2015; Perini et al., 2010; Sivieri et al., 2015), that the transition between ϕ_2 and ϕ_3 in maximal dry apnoeas corresponds to the end of the so-called *easy going phase* of apnoea. This condition is characterized by an alveolar gas composition that, on O_2 - CO_2 diagram (Rahn and Fenn, 1955), should lie around the *physiological breaking point* curve (Ferretti, 2001; Lin et al., 1974). According to Agostoni (1963), this alveolar gas composition should correspond to that eliciting the first involuntary diaphragmatic contractions during maximal apnoeas. To this aim, the divers performed ϕ_2 -interrupted apnoeas, the duration of which was pre-determined on the basis of the time of the ϕ_2 - ϕ_3 transition, as determined the previous day during maximal apnoeas.

The alveolar gas composition obtained at the end of ϕ_2 -interrupted apnoeas (Table 3) were plotted on an O_2 - CO_2 diagram in Figure 2. The *physiological breaking point* curve obtained by Agostoni (1963) and Lin et al. (1974) is also shown on the same Figure. Figure 2 tells that the present results lie fairly close to the *physiological breaking point* curve. Thus they appear compatible with the tested hypothesis, which therefore cannot be discarded on this basis. This being the case, the patterns followed by the cardiovascular variables during ϕ_3 might indeed be affected by the onset of diaphragmatic contractions, the intensity and frequency of which may well increase until the *volitional breaking point*. The recording of diaphragmatic contractions during ϕ_3 , which could not be carried out in the present study, would be the next inevitable step aimed at testing this corollary of the present experiments.

The $P_A\text{CO}_2$ during maximal apnoeas in oxygen was lower compared to the data reported by Lin et al. (1974). Several authors (Agostoni, 1963; Delapille et al., 2001; Lin et al., 1974) hypothesized that diaphragmatic contractions start when an absolute $P_A\text{CO}_2$ of about 47-48 mmHg is attained, starting with a $P_A\text{CO}_2$ of about 40 mmHg at the beginning of the apnoeas. Our divers probably hyperventilated before oxygen apnoeas: even if they were asked to refrain from hyperventilation manoeuvres, they started apnoeas with a $P_A\text{CO}_2$ of 28 ± 7 mmHg, so that they finished the apnoeas with lower values of $P_A\text{CO}_2$ compared to Lin et al. (1974).

As expected from previous studies (Perini et al., 2008; Sivieri et al., 2015), SpO_2 decreased in both maximal and interrupted apnoeas in air, reaching the minimum after few seconds from the end of the apnoeas. The delay between the end of apnoea and the minimum of SpO_2 is the consequence of the time required for the oxygenated blood in lung to reach the periphery, where SpO_2 was measured; this time also depends on the environmental conditions (Severinghaus and Kelleher, 1992). The reduction was greater for maximal air apnoeas than for $\phi 2$ -interrupted apnoeas as a consequence of longer duration for the former compared to the latter. In oxygen apnoeas all the divers showed a $P_A\text{O}_2$ greater than 100 mmHg, thus SpO_2 did not change, and was always 100 %.

The respiratory stimuli exerted by O_2 and CO_2 are synergic (Cunningham et al., 1986), hence the onset of diaphragmatic contractions should have different origin in oxygen from air. During apnoeas in air, the synergic action of hypercapnia and hypoxaemia would determine the attainment of the *physiological breaking point*. During apnoeas in oxygen, since SpO_2 remained at 100 %, the hypoxic stimulus was absent and the *physiological breaking point* was a consequence of increasing $P_A\text{CO}_2$ only. Since the stimuli act synergistically, one would predict that, during oxygen apnoeas, higher $P_A\text{CO}_2$ values than in air would be required to get

the *physiological* and the *volitional breaking points*. A proportionately longer duration of static apnoeas was expected in oxygen than in air, but this was not the case.

At the end of maximal apnoeas in oxygen, $P_A\text{CO}_2$ was lower than that reported by Lin et al. (1974), even if all the subjects presented a clear $\phi 3$ and attained their *volitional breaking point*, and even though the mean duration of oxygen apnoeas in the present study was twice as long as that reported by Lin et al. (1974). It is also noteworthy that the starting $P_A\text{CO}_2$ was lower in oxygen than in air, an observation that induced us to analyse also $\Delta P_A\text{CO}_2$. This variable, reported in Table 3, was higher in oxygen than in air, although the final $P_A\text{CO}_2$ did not attain the levels observed by Lin et al. (1974). Moreover, the present $\Delta P_A\text{CO}_2$ values were similar to those that can be calculated from the data of Lin et al. (1974). This analysis suggests that the attainment of the *volitional breaking point* may be the consequence of a higher $\Delta P_A\text{CO}_2$, rather than of the absolute $P_A\text{CO}_2$ values.

Similarly, during $\phi 2$ -interrupted apnoeas, i) $P_A\text{CO}_2$ at the end of the apnoeas was similar in oxygen as in air, despite the longer $\phi 2$ duration in oxygen, and ii) $\Delta P_A\text{CO}_2$ was higher in oxygen than in air. By analogy we can thus conclude that also in this case the attainment of the *physiological breaking point* might have been the consequence of a higher $\Delta P_A\text{CO}_2$, rather than of the absolute $P_A\text{CO}_2$ values. This being so, then $P_A\text{CO}_2$ would play the main role in determining the end of the *easy going phase* ($\phi 2$), the attainment of the *physiological breaking point* and the genesis of the $\phi 3$ cardiovascular responses. In this context, the hypoxia that develops during air apnoeas would just interfere by reducing the $P_A\text{CO}_2$ increase that is necessary to attain the physiological and volitional breaking points.

It is nonetheless noteworthy that the $P_A\text{CO}_2$ measured at the mouth at the end of an apnoea is likely less than predicted on the basis of the metabolic CO_2 production during the same apnoea. In fact, part of the CO_2 production is accumulated in tissues, especially blood and lung parenchyma, as a consequence of the suppression of alveolar ventilation (Farhi and

Rahn, 1960; Ferretti et al., 1991; Hong et al., 1971). Moreover, the fact that $\dot{Q}$ remained low during the entire apnoea might have contributed to the CO₂ accumulation in tissues. In sum, there might be a mismatch between the CO₂ output measured in the lungs and the CO₂ production in peripheral tissues, which makes an estimate of CO₂ concentration in the cerebrospinal fluid impossible: it cannot be excluded that this value, and thus the corresponding ventilatory stimulus, is higher than one could predict from P_ACO₂ measurements.

The evolution of all cardiovascular variables during apnoeas was in agreement with the data obtained in previous studies. ϕ_1 and ϕ_2 were similar in air as in oxygen, except for the duration of the latter, and corresponded to those reported in previous studies (Costalat et al., 2013, 2015; Fagoni et al., 2015; Perini et al., 2008, 2010; Sivieri et al., 2015; Tocco et al., 2012). If $\Delta P_A\text{CO}_2$ determines the *volitional breaking point*, the fact that $\Delta P_A\text{CO}_2$ was higher in oxygen than in air, due to the lack of the hypoxic stimulus in the former case, explains why the duration of ϕ_3 was also longer in oxygen than in air. In this context, the activation of the respiratory chemoreflexes may contribute to the continuous increase of SBP during ϕ_3 , as well as to the maintenance of high TPR, despite the bradycardia (see Table 2), with consequently low $\dot{Q}$ and SV. In fact, activation of respiratory chemoreflexes was proposed as possible mechanism implied in peripheral vasoconstriction (Leuenberger et al., 2001; Lindholm et al., 2002).

Although diaphragmatic contractions are known to increase venous return (Willeput et al., 1984), no increases in $\dot{Q}$ were found in this study. We postulate that the increased peripheral resistance and afterload during ϕ_3 , possibly due to the sympathetic activation that occur during apnoeas (Breskovic et al., 2011; Dujic et al., 2008; Zubin Maslov et al., 2014), may have counteracted the expected increase in venous return, thus maintaining low $\dot{Q}$ values. In

this context, the baroreflex mediated vasoconstriction could play a major role in eliciting the cardiovascular responses during apnoeas.

Recently, some authors (Caspers et al., 2011; Costalat et al., 2015, 2016) studied the oxygen-conserving mechanisms in elite divers as compared with non-divers during dynamic apnoeas with face immersion. On the basis of regression analysis of the time course of HR during the breath-holds, they identified an *oxygen-conserving breaking point*, defined as a sudden change in slope in the HR versus time relationship during apnoea. According to those authors, the *oxygen-conserving breaking point* triggers a phase of apnoea, present only in elite divers, in which oxygen-preserving mechanisms are activated. On the basis of the present data, we are unable to judge on the occurrence of oxygen preserving mechanisms in $\phi 3$, nevertheless, that notion of an oxygen-conserving breaking point is conceptually analogous to the present transition between $\phi 2$ and $\phi 3$.

This study is affected by some limitations. First, the sample size estimation was not computed in this study, because power analysis cannot be carried out in the present experimental condition. We are aware that the absence of power analysis prevents from ruling out the hypothesis that a higher number of subjects could have led to different conclusions. No control group was studied, since identification of $\phi 3$ requires that the subjects should be able to perform apnoeas of at least 3 min (Perini et al., 2008), and non-divers cannot hold their breath for longer than 3 min, thus $\phi 3$ is missing in them and the end of $\phi 2$ cannot be identified. We cannot exclude that the end of their static apnoea may correspond to the physiological breaking point, and this issue could be addressed in further studies. Another limitation of the study is that several factors other than alveolar gas composition, such as lung volumes (mechanical factors), sensitivity to hypercapnia or hypoxia (chemical factors), absence of respiratory movements (non-chemical factors) as well as psychology, training, exercise and altitude, may influence the onset of diaphragmatic contractions and the total time

of apnoea (Courteix et al., 1992; Delapille et al., 2001; Godfrey and Campbell, 1968; Grassi et al., 1993). The presence of these factors may explain the variability in alveolar gas composition observed at the end of apnoeas. In this context, the lung volumes (not recorded in this study) and information about their training status could have been useful for further interpretation of data.

CONCLUSION

In conclusion, the results support the hypothesis that the alveolar gas composition at the end of ϕ_2 corresponds to that reported at the *physiological breaking point*. During air apnoeas, a synergic effect of hypercapnia and hypoxia would generate ϕ_3 ; in oxygen apnoeas, the CO_2 rise would be the only active factor. In this context, the $\Delta P_{\text{A}}\text{CO}_2$ might be more important than the absolute $P_{\text{A}}\text{CO}_2$ level in determining the onset of diaphragmatic contractions.

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FIGURE LEGENDS

Figure 1

Time courses of systolic blood pressure (SBP) and heart rate (HR) of a representative subject during the four investigated conditions (air and oxygen, maximal and interrupted apnoeas).

$\phi 1$: phase I; $\phi 2$: phase II; $\phi 3$: phase III.

A: maximal apnoea in air

B: maximal apnoea in oxygen

C: interrupted apnoea in air

D: interrupted apnoea in

oxygen

Figure 2

Graphical representation of the mean values for $P_{A}CO_2$ and $P_{A}O_2$ on the O_2 - CO_2 diagram. Black line: alveolar air; grey dashed line: *physiological breaking point*; black dotted line: *volitional breaking point*; black squares refer to the $P_{A}CO_2$ and $P_{A}O_2$ values reported by Lin et al. (1974); black triangles refer to our experimental data obtained in interrupted air and oxygen apnoeas.

Table 1. Cardiovascular variables in resting condition (control), before starting the maximal (1st day) and the interrupted (2nd day) apnoeas, both in air and oxygen. Data are presented as mean and standard deviation. The averages were computed over one min during the 10 min recording. SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; SV: stroke volume; $\dot{Q}$: cardiac output; TPR: total peripheral resistances.

	1 st day maximal apnoeas		2 nd day interrupted apnoeas	
	AIR	OXYGEN	AIR	OXYGEN
SBP (mmHg)	131 ± 19	128 ± 17	122 ± 18	124 ± 16
DBP (mmHg)	70 ± 13	71 ± 12	64 ± 11	65 ± 10
HR (b·min⁻¹)	79 ± 9	79 ± 11	73 ± 9	69 ± 10
SV (ml)	95 ± 20	91 ± 22	98 ± 28	98 ± 23
$\dot{Q}$ (l·min⁻¹)	7.4 ± 1.6	7.1 ± 1.7	7.1 ± 1.9	6.6 ± 1.3
TPR (mmHg·min·l⁻¹)	13.0 ± 4.3	13.7 ± 4.6	12.8 ± 4.8	13.2 ± 3.3

Table 2. Evolution of cardiovascular variables during maximal and interrupted apnoeas, in air and oxygen. Data are presented as mean and standard deviation. Start: single value just before starting apnoeas; MSP: value at the minimum of systolic blood pressure. SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; SV: stroke volume; $\dot{Q}$: cardiac output; TPR: total peripheral resistances. * p<0.05 compared to Start; # p<0.05 compared to MSP; § p<0.05 compared to Last 10 beats ϕ 2.

		SBP (mmHg)	DBP (mmHg)	HR (b·min ⁻¹)	SV (ml)	$\dot{Q}$ (l·min ⁻¹)	TPR (mmHg·min·l ⁻¹)
MAXIMAL APNOEAS	AIR						
	Start	142 ± 18	76 ± 13	87 ± 15	94 ± 24	7.9 ± 1.5	12.6 ± 3.8
	MSP	102 ± 20*	61 ± 14	93 ± 15	58 ± 17*	5.3 ± 1.3*	14.9 ± 7.0
	Last 10 beats ϕ 2	144 ± 19 [#]	82 ± 14 [#]	79 ± 17	64 ± 14*	5.0 ± 1.4*	22.2 ± 10.1
	Last 10 beats ϕ 3	200 ± 20* ^{#§}	102 ± 16* [#]	68 ± 12* [#]	87 ± 23 [#]	5.9 ± 1.7*	25.3 ± 10.5
	OXYGEN						
	Start	125 ± 16	77 ± 11	88 ± 14	81 ± 22	6.9 ± 1.6	14.4 ± 4.4
	MSP	83 ± 18*	59 ± 13*	94 ± 16	39 ± 20*	3.5 ± 1.8*	23.8 ± 13.8
	Last 10 beats ϕ 2	124 ± 14 [#]	80 ± 10 [#]	75 ± 11	55 ± 15	4.0 ± 1.2*	25.1 ± 8.5
	Last 10 beats ϕ 3	172 ± 18* ^{#§}	94 ± 12* [#]	81 ± 18	88 ± 23 ^{#§}	6.8 ± 1.8 ^{#§}	19.2 ± 6.3
INTERRUPTED APNOEAS	AIR						
	Start	136 ± 17	70 ± 10	79 ± 9	101 ± 22	8.0 ± 2.0	12.0 ± 3.4
	MSP	95 ± 16*	54 ± 10*	83 ± 11	64 ± 11*	5.1 ± 0.9*	12.9 ± 3.3
	Last 10 beats ϕ 2	133 ± 18 [#]	74 ± 8 [#]	64 ± 8* [#]	70 ± 18*	4.7 ± 0.8*	19.2 ± 3.3* [#]
	OXYGEN						
	Start	126 ± 14	71 ± 9	72 ± 10	96 ± 28	6.9 ± 1.8	13.9 ± 4.4
	MSP	94 ± 15*	57 ± 15	77 ± 16	65 ± 32	4.9 ± 2.3	16.2 ± 6.6
	Last 10 beats ϕ 2	121 ± 13 [#]	72 ± 13 [#]	66 ± 11	71 ± 32	4.7 ± 2.5	21.8 ± 7.7*

Table 3. Alveolar gas composition measured before and after maximal and interrupted apnoeas, performed in air and in oxygen. Data are presented as mean and standard deviation. Percent change in $P_A\text{CO}_2$ at end of apnoeas is also reported. $\Delta P_A\text{CO}_2$: difference between the $P_A\text{CO}_2$ measured after and before apnoeas. Level of significance calculated by means of Student's T test for paired samples. *: $p < 0.05$ compared to maximal apnoeas in air. §: $p < 0.05$ compared to interrupted apnoeas in air.

(mmHg)	$P_A\text{O}_2$ before apnoeas	$P_A\text{O}_2$ after apnoeas	$P_A\text{CO}_2$ before apnoeas	$P_A\text{CO}_2$ after apnoeas	$\Delta P_A\text{CO}_2$	% $P_A\text{CO}_2$ change
Maximal apnoeas in AIR	133 ± 10	44 ± 12	31 ± 7	51 ± 8	20 ± 5	$69\% \pm 24\%$
Maximal apnoeas in OXYGEN	$709 \pm 14 *$	$621 \pm 35 *$	$28 \pm 7 *$	$56 \pm 7 *$	$28 \pm 10 *$	$113\% \pm 63\%$
Interrupted apnoeas in AIR	126 ± 11	84 ± 16	35 ± 6	48 ± 5	12 ± 6	$38\% \pm 24\%$
Interrupted apnoeas in OXYGEN	$692 \pm 65 §$	$683 \pm 15 §$	$30 \pm 6 §$	45 ± 7	$15 \pm 7 §$	$55\% \pm 33\%$