Eur J Appl Physiol (2015) 115:119–128
DOI 10.1007/s00421-014-2992-9

ORIGINAL ARTICLE

A beat-by-beat analysis of cardiovascular responses to dry resting and exercise apnoeas in elite divers

Andrea Sivieri · Nazzareno Fagoni · Aurélien Bringard · Michela Capogrosso · Renza Perini · Guido Ferretti

Received: 15 May 2014 / Accepted: 28 August 2014 / Published online: 13 September 2014

Abstract
Purpose Cardiovascular responses during resting apnoea include three phases: (1) a dynamic phase of rapid changes, lasting at most 30 s; (2) a subsequent steady phase; and (3) a further dynamic phase, with a continuous decrease in heart rate (HR) and an increase in blood pressure. The interpretation was that the end of the steady phase corresponds to the physiological apnoea breaking point. This being so, during exercise apnoeas, the steady phase would be shorter, and the rate of cardiovascular changes in the subsequent unsteady phase would be faster than at rest.
Methods To test these hypotheses, we measured beat-by-beat systolic (SBP), diastolic, and mean blood pressures (MBP), HR, and stroke volume (SV) in six divers during dry resting (duration 239.4 ± 51.6 s) and exercise (30 W on cycle ergometer, duration 88.2 ± 20.9 s) maximal apnoeas, and we computed cardiac output ($\dot{Q}$) and total peripheral resistance (TPR).
Results Compared to control, at the beginning of resting (R1) and exercising (E1) apnoeas, SBP and MBP decreased and HR increased. SV and $\dot{Q}$ fell, so that TPR remained unchanged. At rest, HR, SV, $\dot{Q}$, and SBP were stable during the subsequent phase; this steady phase was missing in exercise apnoeas. Subsequently, at rest (R3) and at exercise (E2), HR decreased and SBP increased continuously. SV returned to control values. Since $\dot{Q}$ remained unchanged, TPR grew.
Conclusions The lack of steady phase during exercise apnoeas suggests that the conditions determining R3 were already attained at the end of E1. This being so, E2 would correspond to R3.

Keywords Heart rate · Arterial blood pressure · Cardiac output · Baroreflexes

Abbreviations

DBP	Diastolic blood pressure
E1	Rapid transition phase of apnoea at exercise
E2	Readjustment phase of apnoea at exercise
HR	Heart rate
MBP	Mean blood pressure
$\dot{Q}$	Cardiac output
R1	Rapid transition phase of apnoea at rest
R2	Steady state phase of apnoea at rest
R3	Readjustment phase of apnoea at rest
SaO_2	Arterial oxygen saturation
SBP	Systolic blood pressure
SD	Standard deviation
SV	Stroke volume
TPR	Total peripheral resistance
$\dot{V}E$	Expired ventilation
$\dot{V}O_2$	Oxygen uptake

Introduction

The cardiovascular responses to breath-holding have been investigated for long. Bradycardia is a well-known

Communicated by David C. Poole.

A. Sivieri · N. Fagoni · M. Capogrosso · R. Perini · G. Ferretti
Dipartimento di Scienze Cliniche E Sperimentali, Università di Brescia, Brescia, Italy

N. Fagoni
Dipartimento di Specialità Medico-chirurgiche, Scienze Radiologiche e Sanità Pubblica, Università di Brescia, Brescia, Italy

A. Bringard · G. Ferretti (✉)
Département des Neurosciences Fondamentales, Université de Genève, Geneva, Switzerland
e-mail: guido.ferretti@unige.ch

phenomenon, reported in almost all studies carried out so far. Its intensity is accentuated by face immersion, especially in cold water (Andersson et al. 2000), and is stronger in elite divers than in non-diver controls (Andersson and Schagatay 1998). Cardiac output is correspondingly decreased (Bjertnæs et al. 1984; Palada et al. 2008), whereas arterial blood pressure is increased (Bjertnæs et al. 1984). Most of the studies report either values from the measurements obtained before and at the end of apnoeas, or mean values at various breath-holding times. Descriptions of the beat-by-beat dynamics of cardiovascular changes during breath-holding, whether dry or on the water surface, are scanty. Observations during short apnoeas in air (Palada et al. 2007) or with face immersion (Andersson and Schagatay 1998; Andersson et al. 2000) suggested that a transient fall in blood pressure associated with tachycardia may precede the typical cardiovascular response. In apnoeas prolonged to the breaking point, hypertension and bradycardia were described as continuously developing phenomena (Andersson and Schagatay 1998; Andersson et al. 2000).

More recently, continuous beat-by-beat observations, during apnoeas prolonged to the volitional breaking point, were made on elite divers who could sustain resting apnoea for longer than 3 min (Perini et al. 2008, 2010) and have demonstrated the occurrence of three distinct phases of the cardiovascular response to apnoea: (1) a dynamic phase of rapid changes, lasting no more than 30 s (phase I); (2) a steady phase, lasting about 2 min, in which the blood pressure and heart rate (HR) values attained at the end of phase I are maintained invariant (phase II); and (3) a further dynamic phase, in which there are continuous decrease in HR and increase in blood pressure, linear with time, prolonged to the attainment of the volitional breaking point (phase III). The interpretation was that the end of the steady phase could represent the physiological breaking point (Hong et al. 1971). In fact, the amplitude of HR and blood pressure oscillations was increased in the subsequent phase, suggesting the occurrence of a new source of fluctuation, perhaps related to the onset of diaphragmatic contractions.

In this study, we test the hypothesis that the end of the steady state phase of the cardiovascular response to apnoea is imposed by the attainment of the physiological breaking point. During exercise, the increase in metabolism accelerates the rate at which the oxygen stores are emptied and the CO_2 stores are replenished in the body. Therefore, if the tested hypothesis was correct, the time necessary to attain the O_2 and CO_2 levels characterising the physiological breaking point and raising the first diaphragmatic contractions would be shorter than at rest. If this is so, although we may well expect to find the three phases of cardiovascular response to apnoea also in exercise, the duration of the steady phase should be reduced in apnoeas during light exercise as compared with resting apnoeas. Moreover, the rate at which the cardiovascular variables change in the subsequent unsteady phase would be steeper in exercise than at rest. These direct experimental consequences of the above hypothesis were not tested so far. Several studies investigated the cardiovascular responses to breath-holding during exercise (Bjertnæs et al. 1984; Breskovic et al. 2011; Lindholm et al. 2002; Nishiyasu et al. 2012; Smeland et al. 1984; Tocco et al. 2012), but to the best of our knowledge, no study ever described these responses on a beat-by-beat basis.

The aim of the present study was to investigate the cardiovascular responses to breath-holding during light dynamic exercise in humans on a beat-by-beat basis, in an attempt at testing some cardiovascular consequences of the general hypothesis formulated above.

Methods

Subjects

Six male competitive divers volunteered for this study. Their age was 37.2 ± 7.3 years, and they were 73.1 ± 9.5 kg heavy and 176 ± 4 cm tall. All divers were non-smokers and had no previous history of cardiovascular, pulmonary or neurological diseases. None of them was taking medications 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. Local ethical approval was obtained.

The ability of sustaining maximal apnoeas lasting longer than 3 min at rest was an inclusion criterion for this study, since only apnoeas of such duration allow a clear identification of the third dynamic phase. This reduced the number of subjects who could participate in the study, which may be seen as a limitation, although it did not prevent from attaining significant changes in the investigated variables at various times during breath-holding, as detailed in the Results section.

Experimental procedure

All tests were carried out in a room at 25–26 °C, in the late afternoon. Upon arrival in the laboratory, after instrumentation, the subject took the sitting posture. Five minutes was allowed to achieve steady state conditions for ventilation, HR and gas exchange, identified by visually inspecting the data on the screen; then, 10 min of measurements was obtained with the subject sitting at rest and spontaneously breathing (quiet rest). Then, the subject was asked to perform two successive prolonged apnoeas. The second

apnoea was requested to be maximal. Subjects undertook their pre-dive breathing routine before breath-holding, generally consisting of two-to-three deep respiratory acts. All apnoeas were initiated with a lung volume close to the subject's total lung capacity, so that the last breathing movement before breath-holding was a deep inspiration and the first breathing movement at the end of apnoeas was an expiration, which the subjects were asked to perform as deep as possible. Then, he moved on the cycle ergometer, where, after 3 min of quiet resting, he started pedalling at a power of 30 W, i.e. at the minimal power that was applicable to the cycle ergometer. After the attainment of the exercise steady state, which again was identified by stable ventilation, HR and gas exchange, 1 min of recordings during regular breathing was allowed. Then, two maximal apnoeas were performed, with a recovery interval of 2 min between them. Again, two-to-three deep respiratory acts preceded the apnoea start, and the last breathing movement before breath-holding was a deep inspiration. After the end of the second apnoea, the subject kept exercising for 3 min. Both at rest and at exercise, the longest apnoea was retained for further analysis.

Methods

Arterial blood pressure profiles (PortaPres, TNO-TPD, Amsterdam, The Netherlands) were continuously recorded throughout the experiments. Arterial blood O_2 saturation (SaO_2) was also continuously monitored by infrared spectroscopy (BioPac System Inc., Goleta, CA, USA) at an earlobe. 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. Oxygen uptake ($\dot{V}O_2$) and expired ventilation ($\dot{V}E$) were monitored by means of a metabolic cart (Quark b^2, Cosmed, Italy) whenever the subject was breathing.

Data treatment

Arterial pressure profile and respiratory traces were analysed offline. Beat-to-beat values of HR, systolic, diastolic and mean blood pressures (SBP, DBP and MBP, respectively) were computed. MBP was obtained as the integral mean of each blood pressure profile. The duration of each apnoea for each subject was calculated as the time over which the flow meter recorded no respiratory air flows. The pulse pressure profile was analysed by means of the Modelflow model implemented in the Beatscope™ software (TNO-TPD, The Netherlands) to obtain the stroke volume (SV) (Wesseling et al. 1993). The method was evaluated against echo-Doppler and found to be highly reproducible in a variety of conditions, with slight systematic overestimate with respect to echo-Doppler (Van Lieshout et al. 2003). Cardiac output ($\dot{Q}$) was then calculated on a beat-by-beat basis as the product of SV times the corresponding HR. The ratio between MBP and $\dot{Q}$ was calculated to estimate total peripheral resistance (TPR). Metabolic rate and ventilation were calculated at rest and exercise steady state as the mean of the breath-by-breath values over 1 min of regular breathing.

The beat-by-beat data were analysed offline to identify the different phases of apnoeas (Perini et al. 2008). The blood pressure data were fitted using a nonlinear least squares procedure (Levenberg–Marquardt algorithm, Levenberg 1944; Marquardt 1963), implemented under MATLAB (version 7.9.0, MathWorks, Natick, MA, USA). The equations of two consecutive straight lines that have an intersection point were used as the fitting model. The slopes and y-intercepts of the two lines and the coordinates of their intersection point were obtained by minimising the squared difference between the model function and the actual blood pressure data. Initial guesses of the parameters of the model were entered after visual inspection of the data.

Statistical analysis

Data are presented as mean and standard deviation (SD). One-way ANOVA for repeated measures was used to evaluate the effect of time of apnoea on various variables at rest and exercise, after having checked for normal distribution using the Kolmogorov–Smirnov test. Tukey's test was used as post hoc test to isolate the differences when necessary. Paired t test was used to compare the duration of breath-holding at rest versus exercise. Differences that were below the $p < 0.05$ were considered significant.

The negative HR versus MBP relationships during the initial part of phase I were analysed by linear regression. Sequences of at least three consecutive beats on which HR and MBP varied in opposite directions were used. The slopes of these regression lines were retained as the "dynamic" sensitivity of arterial baroreflex (BRS) (Adami et al. 2013). The Stata 10.0 statistical software (StataCorp, College Station, TX, USA) was used.

Results

Apnoeas at rest

In quiet rest, $\dot{V}O_2$ was 246 ± 47 ml min^{-1} and $\dot{V}E$ was 9.0 ± 2.3 L min^{-1}. The corresponding $\dot{V}CO_2$ and $\dot{V}E$ were 238 ± 44 ml min^{-1} and 9.0 ± 2.3 L min^{-1}, respectively, with values of gas exchange ratio suggesting slight

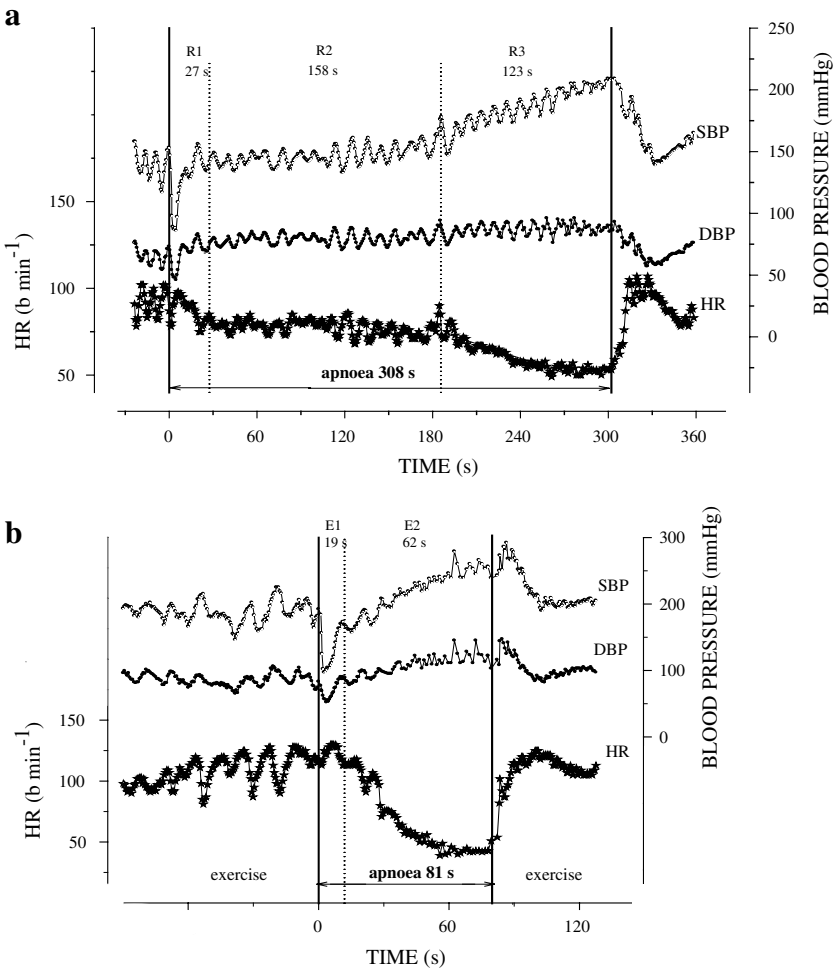

Fig. 1 Heart rate (HR) and systolic blood pressure (SBP) recordings obtained on one subject during maximal apnoea at rest (**a**) and during maximal apnoea at exercise (**b**)

Table 1 Cardiovascular variables at quiet rest and at steady state of light exercise (mean ± SD)

	SBP (mmHg)	DBP (mmHg)	MBP (mmHg)	HR (b min^{-1})	SV (ml)	$\dot{Q}$ (L min^{-1})	TPR (mmHg min L^{-1})
Resting	135 ± 20	68 ± 10	87 ± 11	84 ± 10	97 ± 21	8.1 ± 1.8	11.5 ± 3.4
Exercise	159 ± 16	74 ± 9	97 ± 11	91 ± 9	102 ± 13	9.4 ± 1.9	10.8 ± 2.1

hyperventilation in two subjects. Mean duration of maximal apnoea at rest was 239.4 ± 51.6 s. An example of HR and SBP recordings obtained on one subject during maximal resting apnoea is shown in Fig. 1 (panel a). All subjects followed the same patterns, although in some cases, the time course of TPR was less accentuated than in the reported example. Values of all variables obtained in quiet rest are reported in Table 1. During the hyperventilation that preceded breath-holding, HR grew to attain 96 ± 6 min^{-1} at the beginning of apnoea ($p < 0.05$ with respect to control). The corresponding SBP and DBP were 154 ± 19 and 77 ± 12 mmHg, respectively (NS with respect to control). SaO_2 was 0.998 ± 0.004.

The evolution of all variables during maximal breath-holds at rest is reported in Figs. 2, 3 and 4 (panel a), and Table 2. The first unsteady phase of breath-holding (rapid transition phase, R1) lasted 27.9 ± 1.6 s. During R1, SBP and DBP fell remarkably, to attain a minimum of 90 ± 26 mmHg ($p < 0.05$ with respect to both quiet rest and beginning of apnoea) and 53 ± 11 mmHg ($p < 0.05$ with respect to beginning of apnoea) after 5.7 ± 1.9 s. Correspondingly, HR increased to a maximum of 103 ± 6 min^{-1}. The corresponding SV was 53 ± 18 ml, so that $\dot{Q}$ at the nadir of SBP resulted equal to 5.4 ± 1.9 L min^{-1}. Since MBP was 63 ± 13 mmHg, TPR turned out equal to 12.6 ± 4.0 mmHg min L^{-1}. All these values except TPR were significantly different from those observed both during quiet rest and at the beginning of apnoea (Table 2).

An example of an individual relationship between HR and MBP during the initial phase of apnoea, before the

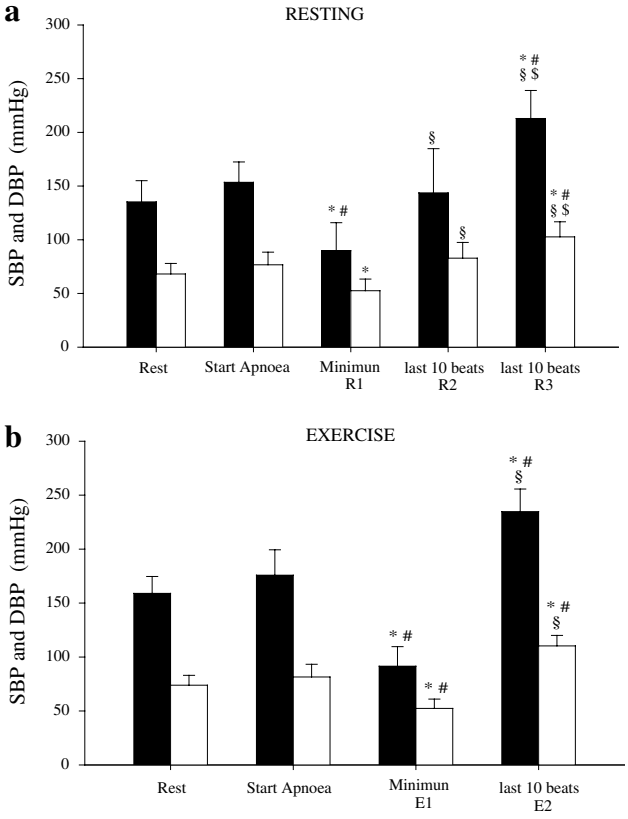

Fig. 2 Evolution of systolic blood pressure (SBP) and diastolic blood pressure (DBP) during apnoea at rest (**a**) and apnoea at exercise (**b**). $^{*}p < 0.05$ with respect to rest; $^{\#}p < 0.05$ with respect to start apnoea; $^{\S}p < 0.05$ with respect to minimum R1 (**a**) or minimum E1 (**b**); $^{\$}p < 0.05$ with respect to R2

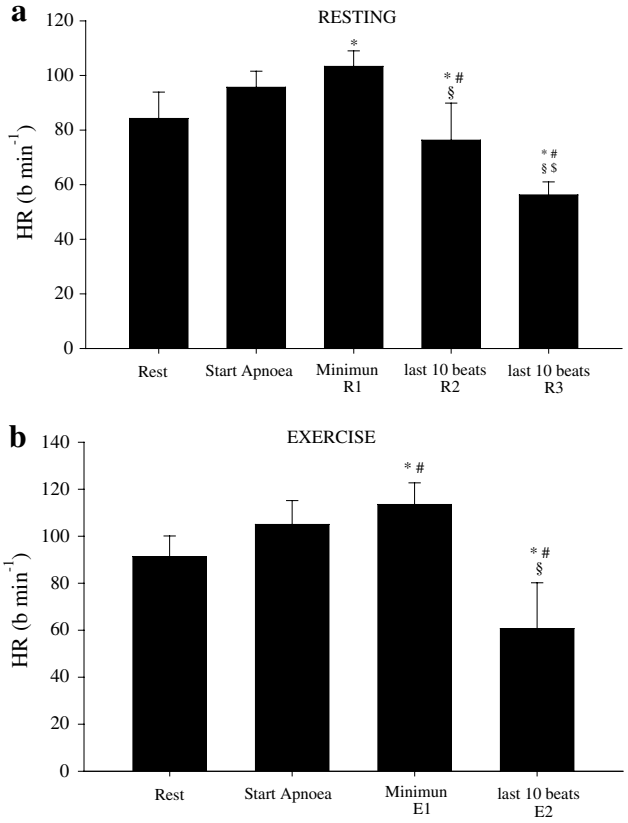

Fig. 3 Evolution of heart rate (HR) during apnoea at rest (**a**) and apnoea at exercise (**b**). $^{*}p < 0.05$ with respect to rest; $^{\#}p < 0.05$ with respect to start apnoea; $^{\S}p < 0.05$ with respect to minimum R1 (**a**) or minimum E1 (**b**); $^{\$}p < 0.05$ with respect to R2

attainment of the minimum of SBP, is shown in Fig. 5. The figure includes a series of consecutive beats in which MBP decreased and HR increased with respect to the immediately preceding beat. Application of linear regression analysis provided a slope of −0.563. This value was taken as indicative of the spontaneous baroreflex sensitivity for the subject at stake during apnoea R1. Similar relationships were obtained on all subjects. The slopes of the obtained individual regression lines are reported in Table 4.

After the attainment of the minimum of SBP, the values of the cardiovascular variables underwent partial recovery, to attain a steady state level (R2) that lasted 84.9 ± 33.5 s. The re-establishment of SBP was accompanied by a drop of HR. The mean slopes of the individual relationships between HR and MBP during the recovery part of R1 are also reported in Table 4. As long as HR decreased, SV increased, and the opposite patterns followed by HR and SV were compensatory, so that during R2, the steady $\dot{Q}$ was not different from that found at the minimum of SBP in R1. As a consequence, R2 was characterised by higher TPR values than before apnoea or in R1. HR, SV and $\dot{Q}$ were lower than in quiet rest.

R2 was followed by a new unsteady phase (readjustment phase, R3), wherein HR decreased steadily and continuously, whereas SBP and DBP increased. At the end of apnoea, HR was significantly lower than in all the previous conditions. The reverse was the case for SBP and DBP. The SV increase compensated for the fall of HR, so that $\dot{Q}$ did not vary during R3. As a consequence, TPR continuously increased. The slopes of the individual relationships between HR and MBP during R3 are also reported in Table 3.

SaO_2 started to decrease in R2. It reached a value of 0.945 ± 0.067 at the end of R2. SaO_2 kept decreasing during the entire R3, to attain a minimum of 0.710 ± 0.149 at 7.5 ± 2.3 s from the end of apnoea.

Apnoeas at exercise

Before apnoea, the steady state $\dot{V}O_2$ during exercise was 862 ± 160 ml min^{-1}, i.e. thrice as high as in quiet rest. The corresponding $\dot{V}CO_2$ and $\dot{V}E$ were 698 ± 127 and 20.5 ± 3.9 L min^{-1}, respectively. Mean duration of maximal apnoea during exercise was 88.2 ± 20.9 s. An

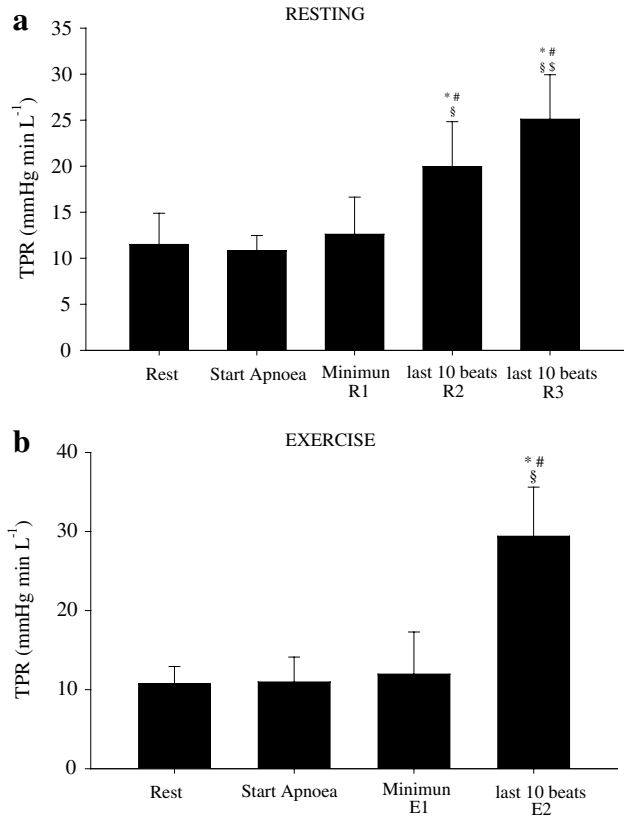

Fig. 4 Evolution of total peripheral resistance (TPR) during apnoea at rest (**a**) and apnoea at exercise (**b**). $^{*}p < 0.05$ with respect to rest; $^{\#}p < 0.05$ with respect to start apnoea; $^{\S}p < 0.05$ with respect to minimum R1 (**a**) or minimum E1 (**b**); $^{\$}p < 0.05$ with respect to R2

example of HR and SBP recordings obtained on one subject during maximal apnoea at exercise is shown in Fig. 1 (panel b). All subjects followed the same patterns. Values of all variables obtained during exercise at steady state before apnoea are reported in Table 1. In the minute that preceded the apnoea, no hyperventilation was observed.

The evolution of all variables during maximal breathholds at exercise is reported in Figs. 2, 3 and 4 (panel b), and Table 2. After the beginning of apnoea, we observed a pattern comparable to that of R1 (E1), although the minimum of SBP was attained within a shorter time (3.6 ± 1.1 s, $p < 0.05$). In E1, SBP attained a minimum of 92 ± 18 mmHg and DBP a minimum of 52 ± 9 mmHg, whereas HR attained a maximum of 114 ± 9 min^{-1}. At the minimum of SBP, SV and $\dot{Q}$ were respectively 51 ± 18 ml and 5.7 ± 1.8 L min^{-1}. Since MBP was 61 ± 10 mmHg, TPR turned out equal to 12.0 ± 5.3 mmHg min L^{-1}. E1 lasted 21.3 ± 6.9 s. The slopes of the individual relationships between HR and MBP, determined during E1 before—descending SBP—the point of minimum SBP, are reported in Table 3.

After the completion of E1, we found no steady state phase. At the beginning of the following phase (E2), which lasted 66.8 ± 22.8 s, SBP and DBP had returned at their initial values, whereas HR was higher than in control, similar to that attained at the minimum of SBP in E1. The recovery of SV was responsible for the increase in $\dot{Q}$, which attained values closer to yet still significantly lower than those observed before apnoea. During E2, a continuous fall of HR and an increase in SBP and DBP occurred, similar to what was found in R3. At the end of apnoea, HR was 61 ± 19 min^{-1}, SBP was 235 ± 21 mmHg and DBP was 110 ± 10 mmHg. These values were significantly different from those found in all other conditions. As in R3, the SV increase compensated for the fall of HR, so that $\dot{Q}$ did not vary during E2. As a consequence, TPR continuously increased. SaO_2 started to drop after ~30 s of apnoea to attain a minimum of 0.74 ± 0.14 at 8.3 ± 0.9 s from the end of apnoea.

The values observed at the beginning of R3 and of E2 are compared in Table 4. Due to exercise, HR and SBP were higher at the start of E2 than of R3, whereas MBP and DBP did not differ significantly. If any, DBP tended to be lower at the start of E2. SV was the same in both cases, so that $\dot{Q}$ was more elevated due to higher heart rate. TPR was lower at start of E2 than of R3. At the end of E2, with respect to the end of R3, SBP, MBP and DBP were significantly higher, whereas SV was significantly lower. No significant differences were found concerning HR, $\dot{Q}$ and TPR.

Table 2 Evolution of SV, $\dot{Q}$ and TPR during apnoea at quiet rest and at steady state of light exercise (mean ± SD)

Resting	Rest	Start	Minimum R1	Last 10 beats R2	Last 10 beats R3
a					
SV (ml)	97 ± 21	96 ± 13	53 ± 18$^{*\#}$	68 ± 19$^{*\#}$	100 ± 10$^{\S\$}$
$\dot{Q}$ (L min^{-1})	8.1 ± 1.8	9.1 ± 1.1	5.4 ± 1.9$^{*\#}$	5.1 ± 0.6$^{*\#}$	5.6 ± 0.4$^{*\#}$
TPR (mmHg min L^{-1})	11.5 ± 3.4	10.8 ± 1.7	12.6 ± 4.0	20.0 ± 4.9$^{*\#\S}$	25.1 ± 4.8$^{*\#\S\$}$
Exercise	30 W		Start	Minimum E1	Last 10 beats E2
b					
SV (ml)	102 ± 13		92 ± 27	51 ± 18$^{*\#}$	90 ± 14§
$\dot{Q}$ (L min^{-1})	9.4 ± 1.9		9.6 ± 2.5	5.7 ± 1.8$^{*\#}$	5.2 ± 0.9$^{*\#}$
TPR (mmHg min L^{-1})	10.8 ± 2.1		11.0 ± 3.2	12.0 ± 5.3	29.5 ± 6.2$^{*\#\S}$

* $p < 0.05$ with respect to rest

$^{\#}$ $p < 0.05$ with respect to start apnoea

§ $p < 0.05$ with respect to minimum R1 or minimum E1

$^{\$}$ $p < 0.05$ with respect to R2

Table 3 Parameters of the relationships between heart rate (HR) and mean blood pressure (MAP) during the first phase, before and after the attainment of the minimum of systolic blood pressure (minSBP) at rest and at exercise

Subject	Apnoea at rest						Apnoea at exercise		
	R1 (before minSBP)			R1 (after minSBP)			E1 (before minSBP)		
	Slope	N_{beats}	R	Slope	N_{beats}	R	Slope	N_{beats}	R
1	–	–	–	−1.098	33	0.950	−0.840	6	0.885
2	−0.792	8	0.951	−0.645	7	0.854	−0.675	8	0.978
3	−0.520	7	0.953	−0.175	7	0.663	−0.273	6	0.861
4	−0.310	5	0.957	−1.379	13	0.948	−0.300	7	0.928
5	−0.563	10	0.965	−0.726	6	0.886	–	–	–
6	−0.353	6	0.976	−1.267	8	0.902	−0.663	6	0.822
Mean	−0.508			−0.882			−0.550		
S.D.	0.192			0.452			0.251		

Data are presented as slope of all individual regression lines and relative Pearson's coefficient

Nbeats number of beats used to calculate the slope

– data not available

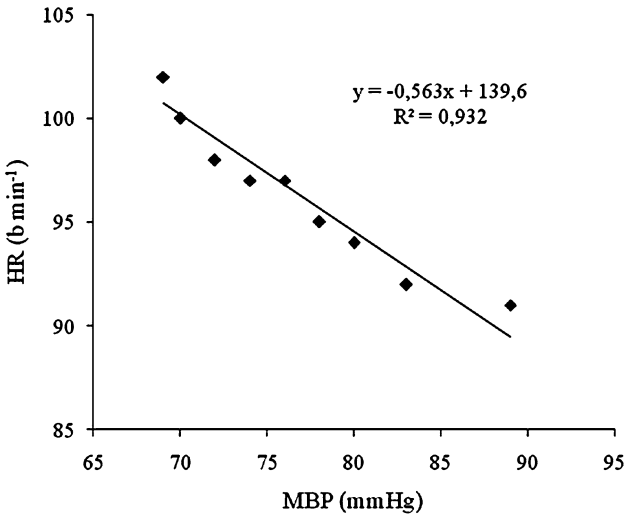

Fig. 5 Relationship between heart rate (HR) and mean blood pressure (MBP) obtained on one subject during R1, before the attainment of the minimum of systolic blood pressure (SBP)

Discussion

In this study, we tested a few direct experimental consequences of the general hypothesis that the end of the R2, i.e. the steady state phase of the cardiovascular response to apnoea at rest, corresponds to the attainment of the physiological breaking point of apnoea. This point is characterised by precise combinations of alveolar gas composition, which are attained as long as, during apnoea, the oxygen stores of the body are emptied and the CO_2 stores correspondingly filled up (Lin et al. 1974). As long as this occurs, metaboreflexes may also be activated leading to powerful respiratory stimuli (Balestra et al. 2011). The time required to attain the physiological breaking point is thus a consequence of two factors: (1) the overall amount of oxygen and CO_2 stores at the beginning of apnoea, and (2) the rate at which oxygen is consumed and CO_2 and other metabolites are accumulated in the body, which is related to the metabolic rate. Metabolic rate is higher at exercise than at rest, so that in the former case, the time required to reach the physiological breaking point would be shorter. On this basis, we expected a reduction of R2 duration during exercise apnoeas.

In fact, during exercise, we did not find something comparable to R2, i.e. a steady state period for all cardiovascular variables. As soon as the initial phase of rapid changes had been completed, we observed a continuous increase in SBP and DBP, accompanied by a continuous decrease in HR, similar to what is usually observed during the R3. The ensuing changes in SV were such as to compensate for the increase in HR, so that $\dot{Q}$ did not vary, both in E2 and in R3. At a first sight, these findings seem to contradict the tested hypothesis, in the sense that exercise apnoeas seem to be characterised by different dynamic cardiovascular responses from those of resting apnoeas. However, another explanation may be put forward: the duration of R2 was reduced to zero, because the end of E1 occurs already at or beyond the physiological apnoea breaking point.

Table 4 Cardiovascular variables (mean ± SD) during the first 10 beats of R3 (apnoea at rest) and E2 (apnoea at exercise)

	SBP (mmHg)	DBP (mmHg)	MBP (mmHg)	HR (b min^{-1})	SV (ml)	$\dot{Q}$ (b min^{-1})	TPR (mmHg $min\ L^{-1}$)
R3	148 ± 12	85 ± 7	103 ± 8	75 ± 15	69 ± 8	5.1 ± 0.8	20.5 ± 3.9
E2	161 ± 12	79 ± 7	97 ± 5	111 ± 10	70 ± 16	7.7 ± 1.7	13.0 ± 2.9
Test T	$p < 0.05$	n.s.	n.s.	$p < 0.05$	n.s.	$p < 0.05$	$p < 0.05$

Cardiovascular changes at the beginning of apnoea at rest and exercise

The characteristics of R1 and E1 were very similar, with HR and SBP showing opposite trends. This strongly suggests that these two phases are representative of the same physiological phenomena. If this is so, the size of the cardiovascular adjustments observed in R1 is independent of the rate at which oxygen stores are emptied. The rapid fall of SBP after the beginning of apnoea, which we observed both at rest and at exercise, was coherent with previous results at rest (Andersson and Schagatay 1998; Palada et al. 2007; Perini et al. 2008). According to Andersson and Schagatay (1998), this fall may be a consequence of an acute reduction of venous return related to the act of holding the breath at elevated lung volumes. The observed fall of SV is in agreement with this concept, although the observation was not accompanied by direct measures of end-diastolic ventricular volume. If this is the case, then the fall of blood pressure and of SV has a mechanical origin, and the simultaneous increase in HR is an attempt at compensating for the fall of SV and/or of blood pressure. If the former is the case, the compensation failed, as demonstrated by the remarkable reduction of $\dot{Q}$ at the minimum of SBP. The recovery of SBP, after the attainment of its minimum, was accompanied by an increase in SV and in TPR (see example in Fig. 2). This last is suggestive of strong peripheral vasoconstriction, compatible with the peripheral sympathetic stimulation. The increase in SV does not seem to have mechanical origin, since (1) the mechanical condition that may have induced the drop of blood pressure at the start of apnoea is still present, and (2) the lack of increase in $\dot{Q}$ implies a lack of increase in venous return. We therefore postulate that after the attainment of the minimum of SBP, a general stimulation of the sympathetic branch of the autonomic nervous system might have led to higher SBP, TPR and SV. In this context, HR would be exposed to two conflicting demands: on one side, sympathetic stimulation of the heart would tend to increase it; and on the other hand, baroreflex stimulation by increased blood pressure would tend to decrease it. In fact, HR recovered, but at a variable rate.

In the initial phase of R1, when blood pressure decreased, the increase in HR might be the result of baroreflex control of blood pressure. According to Perini et al. (2008), however, changes in HR do not compensate for changes in SBP, suggesting that the interaction between HR and blood pressure, normally mediated by baroreflexes, is negligible in the initial phase of apnoea. To clarify this aspect, we constructed relationships between HR and MBP like those reported in Fig. 5. These allowed computation of a dynamic baroreflex sensitivity from the slope of the linear regression lines, thus by the application of an analogous of the sequence method (Iellamo et al. 1997) in which, however, the analysis can be done over a larger number of points than usually done with the sequence method. Dynamic baroreflex sensitivity (Table 3) during the early part of R1 and E1 ranged between −0.273 and −0.840. These values correspond well to those of other studies in steady state conditions, whether with open-loop carotid cardiac baroreflex measures or with closed-loop spontaneous baroreflex evaluation with the sequence method (Akimoto et al. 2011; Fisher et al. 2009, 2010; Gallagher et al. 2006). They correspond well also to those obtained by similar procedure in another dynamic condition at the end of prolonged bed rest (Adami et al. 2013). On this basis, our postulate is that the increase in HR during the first seconds of apnoea (whether in R1 or in E1) tends to attenuate the SBP drop via arterial baroreflex stimulation. This may not be so after the attainment of the minimum of SBP, as shown by the much wider range of dynamic baroreflex sensitivity values at rest and by the difficulty of performing this computation at exercise.

The second phase of apnoea at exercise

If we admit that the present results are coherent with the tested hypothesis, as discussed above, the E2 would indeed correspond to R3. We expected it to be shorter than R3 due to higher metabolic rate at exercise than at rest, with subsequent faster depletion of oxygen stores, and this was the case. Coherently, the rate at which the cardiovascular variables changed in E2 was steeper than in R3. As R3, E2 was also characterised by a progressive reduction in HR and increase in SBP and DBP, suggesting continuous readjustment of cardiovascular haemodynamics. No differences between E2 and R3 were observed at the end of maximal breath-hold, independent of whether a subject was at exercise or at rest, except for blood pressure and SV. Notwithstanding E2 and R3 seemed characterised by the same phenomena, at the beginning of E2 HR, SBP and $\dot{Q}$ were significantly higher than those at the beginning of R3, probably due to sympathetic activation during exercise apnoeas as a consequence of either central command or exercise pressor response, which normally tend to operate in opposition to baroreflexes (Charkoudian et al. Charkoudian and Wallin 2014; Nobrega et al. 2014); moreover, vascular recruitment during exercise apnoea led to significantly lower TPR with respect to resting apnoea.

The negative relation between HR and MBP appeared also in E2, similarly to what was observed in R3. The oscillations of HR and MBP in R3 and E2, however, made the computation of HR versus MBP relations over consecutive beats impossible. Yet, this is indicative of baroreflex activity in E2 as in R3, in agreement with the results of previous studies (Perini et al. 2008). We speculate that in the final

phase of apnoea, the baroreflex activation prevailed over exercise sympathetic activity, so that HR, $\dot{Q}$ and TPR were similar in both resting and exercise apnoeas.

The SaO_2 values observed after the end of apnoeas suggest that changes in alveolar gas composition may be occurring during both E2 and R3. These changes may even take place more rapidly in the former than in the latter case, due to higher $\dot{V}O_2$ in exercise than in resting apnoeas. We therefore cannot exclude that also chemoreflex activation may contribute, at least in part, to the HR reduction in the final phases of apnoeas, as previously suggested (Foster and Sheel 2005; Perini et al. 2008, 2010). This HR fall is accompanied by a further increase in SV, which we postulate may have mechanical origin. In fact, if the tested hypothesis was correct, central venous pressure may become negative during diaphragmatic contractions in the final phase of apnoeas, thus determining an increase in venous return. The increase in SV was such as to correct the drop of HR, so that $\dot{Q}$ remained unchanged. But this carried along a further increase in TPR, suggestive of even stronger peripheral vasoconstriction.

Conclusions

In conclusion, no steady state phase occurred during exercise apnoeas, despite the lightness of the exercise carried out in this study. We postulate that in exercise apnoeas, the cardiorespiratory conditions determining R3 were already attained at the end of the first phase of rapid cardiovascular changes. If this is so, then E2 would indeed correspond to R3.

Acknowledgments The study was supported by a Grant from the University of Brescia to Guido Ferretti and by Swiss National Science Foundation Grant 32003B_144259 to Guido Ferretti. We are grateful to Dr. Nicola Sponsiello for collaboration in subjects' recruitment.

References

Adami A, Pizzinelli P, Bringard A, Capelli C, Malacarne M, Lucini D, Simunič B, Pišot R, Ferretti G (2013) Cardiovascular readjustments and baroreflex response during clinical reambulation procedure at the end of 35-day bed rest in humans. Appl Physiol Nutr Metab 38:673–680

Akimoto T, Sugawara J, Ichikawa D, Terada N, Fadel PJ, Ogoh S (2011) Enhanced open-loop but not closed-loop cardiac baroreflex sensitivity during orthostatic stress in humans. Am J Physiol Regul Integr Comp Physiol 301:R1591–R1598

Andersson J, Schagatay E (1998) Effects of lung volume and involuntary breathing movements on the human diving response. Eur J Appl Physiol 77:19–24

Andersson J, Schagatay E, Gislen A, Holm B (2000) Cardiovascular responses to cold-water immersions of the forearm and face, and their relationship to apnoea. Eur J Appl Physiol 83:566–572

Balestra C, Levenez M, Lafère P, Dachy B, Ezquer M, Germonpré P (2011) Respiratory rate can be modulated by long-loop muscular reflexes, a possible factor in involuntary cessation of apnea. Diving Hyperb Med 41:3–8

Bjertnæs L, Hauge J, Kjekshus J, Søyland J (1984) Cardiovascular responses to face immersion and apnea during steady state muscle exercise. Acta Physiol Scand 120:605–612

Breskovic T, Uglesic L, Zubin P, Kuch B, Kraljevic J, Zanchi J, Ljubkovic M, Sieber A, Dujic Z (2011) Cardiovascular changes during underwater static and dynamic breath- hold dives in trained divers. J Appl Physiol 111:673–678

Charkoudian N, Wallin BG (2014) Sympathetic neural activity to the cardiovascular system: integrator of systemic physiology and interindividual characteristics. Compr Physiol 4:825–850

Fisher JP, Ogoh S, Junor C, Khaja A, Northrup M, Fadel PJ (2009) Spontaneous baroreflex measures are unable to detect age-related impairments in cardiac baroreflex function during dynamic exercise in humans. Exp Physiol 94:447–458

Fisher JP, Kim A, Young CN, Fadel PJ (2010) Carotid baroreflex control of arterial blood pressure at rest and during dynamic exercise in aging humans. Am J Physiol Regul Integr Comp Physiol 299:R1241–R1247

Foster GE, Sheel AW (2005) The human diving response, its function, and its control. Scand J Med Sci Sports 15:3–12

Gallagher KM, Fadel PJ, Smith SA, Strømstad M, Ide K, Secher NH, Raven PB (2006) The interaction of central command and the exercise pressor reflex in mediating baroreflex resetting during exercise in men. Exp Physiol 91:79–87

Hong SK, Lin YC, Lally DA, Yim BJ, Kominami N, Hong PW, Moore TO (1971) Alveolar gas exchanges and cardiovascular functions during breath holding with air. J Appl Physiol 30:540–547

Iellamo F, Legramante JM, Raimondi G, Peruzzi G (1997) Baroreflex control of sinus node during dynamic exercise in humans: effects of central command and muscle reflexes. Am J Physiol Heart Circ Physiol 272:H1157–H1164

Levenberg K (1944) A method for the solution of certain problems in least squares. Quart Appl Math 2:164–168

Lin YC, Lally DA, Moore TO, Hong SK (1974) Physiological and conventional breath-hold breaking points. J Appl Physiol 37:291–296

Lindholm P, Nordh J, Linnarsson D (2002) Role of hypoxemia for the cardiovascular responses to apnea during exercise. Am J Physiol Regul Integr Comp Physiol 283:R1227–R1235

Marquardt D (1963) An algorithm for least-squares estimation of nonlinear parameters. SIAM J Appl Math 11:431–441

Nishiyasu T, Tsukamoto R, Kawai K, Hayashi K, Koga S, Ichinose M (2012) Relationships between the extent of apnea-induced bradycardia and the vascular response in the arm and leg during dynamic two-legged knee extension exercise. Am J Physiol Heart Circ Physiol 302:H864–H871

Nobrega AC, O'Leary D, Silva BM, Marongiu E, Piepoli MF, Crisafulli A (2014) Neural regulation of cardiovascular response to exercise: role of central command and peripheral afferents. Biomed Res Int. doi:10.1155/2014/478965 Online

Palada I, Eterovic D, Obad A, Bakovic D, Valic Z, Ivancev V, Lojour M, Shoemaker JK, Dujic Z (2007) Spleen and cardiovascular function during short apneas in divers. J Appl Physiol 103:1958–1963

Palada I, Bakovic D, Valic Z, Obad A, Ivancev V, Eterovic D, Shoemaker JK, Dujic Z (2008) Restoration of hemodynamics in apnea struggle phase in association with involuntary breathing movements. Respir Physiol Neurobiol 161:174–181

Perini R, Tironi A, Gheza A, Butti F, Moia C, Ferretti G (2008) Heart rate and blood pressure time courses during prolonged dry apnoea in breath-hold divers. Eur J Appl Physiol 104:1–7

Perini R, Gheza A, Moia C, Sponsiello N, Ferretti G (2010) Cardiovascular time course during prolonged immersed static apnoea. Eur J Appl Physiol 110:277–283

Smeland EB, Owe JO, Andersen HT (1984) Modification of the 'dividing bradycardia' by hypoxia or exercise. Respir Physiol 56:245–251

Tocco F, Crisafulli A, Melis F, Porru C, Pittau G, Milia R, Concu A (2012) Cardiovascular adjustments in breath-hold diving: comparison between divers and non- divers in simulated dynamic apnoea. Eur J Appl Physiol 112:543–554

Van Lieshout JJ, Toska K, Van Lieshout EJ, Eriksen M, Walløe L, Wesseling KH (2003) Beat-to-beat noninvasive stroke volume from arterial pressure and Doppler ultrasound. Eur J Appl Physiol 90:131–137

Wesseling KH, Jansen JRC, Settels JJ, Schreuder JJ (1993) Computation of aortic flow from pressure in humans using a nonlinear, three-element model. J Appl Physiol 74:2566–2573