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The oxygen-conserving potential of the diving response: A kinetic-based analysis

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ABSTRACT

We investigated the oxygen-conserving potential of the human diving response by comparing trained breath-hold divers (BHDs) to non-divers (NDs) during simulated dynamic breath-holding (BH). Changes in haemodynamics [heart rate (HR), stroke volume (SV), cardiac output (CO)] and peripheral muscle oxygenation [oxyhaemoglobin ([HbO₂]), deoxyhaemoglobin ([HHb]), total haemoglobin ([tHb]), tissue saturation index (TSI)] and peripheral oxygen saturation (SpO₂) were continuously recorded during simulated dynamic BH. BHDs showed a breaking point in HR kinetics at mid-BH immediately preceding a more pronounced drop in HR ($-0.86 \text{ bpm}\cdot\%^{-1}$) while HR kinetics in NDs steadily decreased throughout BH ($-0.47 \text{ bpm}\cdot\%^{-1}$). By contrast, SV remained unchanged during BH in both groups (all $P > 0.05$). Near-infrared spectroscopy (NIRS) results (mean $\pm$ SD) expressed as percentage changes from the initial values showed a lower [HHb] increase for BHDs than for NDs at the cessation of BH ($+24.0 \pm 10.1$ vs. $+39.2 \pm 9.6\%$, respectively; $P < 0.05$). As a result, BHDs showed a [tHb] drop that NDs did not at the end of BH (-7.3 ± 3.2 vs. $-3.0 \pm 4.7\%$, respectively; $P < 0.05$). The most striking finding of the present study was that BHDs presented an increase in oxygen-conserving efficiency due to substantial shifts in both cardiac and peripheral haemodynamics during simulated BH. In addition, the kinetic-based approach we used provides further credence to the concept of an “oxygen-conserving breaking point” in the human diving response.

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KEYWORDS

Diving reflex; breath-holding; haemodynamics; kinetics; oxygen-conserving effect; NIRS

Introduction

Human physiological response to breath-holding (BH) is called the diving response and its main effects are bradycardia, decreased cardiac output (CO) and increased arterial blood pressure (Lindholm & Lundgren, 2009). Bradycardia is induced by increased vagal activity, whereas the peripheral vasoconstriction of selected vascular beds is linked to sympathetic discharge increase (Foster & Sheel, 2005). These protective cardiovascular mechanisms against hypoxic damages slow the depletion of lung oxygen stores and arterial oxygen desaturation through an oxygen-conserving effect during BH, thereby reducing overall O₂ uptake (Alboni, Alboni, & Gianfranchi, 2011; Lindholm & Lundgren, 2009).

In humans, most of previous and current studies dealing with the oxygen-conserving potential of the human diving response have relied on a comparison of cardiovascular adjustments between dry BH and BH with face immersion/or whole body immersion performed at varying water temperature (Andersson, Biasoletto-Tjellström, & Schagatay, 2008; Andersson & Evaggelidis, 2009; Andersson, Linér, Fredsted, & Schagatay, 2004; Andersson, Linér, Rünow, & Schagatay, 2002; Andersson & Schagatay, 1998; de Bruijn, Richardson, & Schagatay, 2009; Furedy, Morrison, Heslegrave, & Arabian, 1983; Marabotti et al., 2013; Schuitema & Holm, 1988; Sterba & Lundgren, 1988). Surprisingly, only a few have investigated the promising effects of BH training on cardiovascular

adjustments to enhance oxygen conservation. In non-divers (NDs), short-term training including repetitive BH has shown to have positive effect on BH duration without altering the magnitude of the diving reflex and the oxygen-conserving effect (Schagatay, van Kampen, & Andersson, 1999). By contrast, 2 weeks' BH training was reported to increase tolerance to hypoxia as well as to reduce the rate of arterial oxygen desaturation due to an increase in both the speed of onset and magnitude of the cardiovascular responses to static BH (Engan, Richardson, Lodin-Sundström, van Beekvelt, & Schagatay, 2013; Schagatay, van Kampen, Emanuelsson, & Holm, 2000). Long-term BH training effects upon haemodynamic adjustments were recently investigated through the comparison of highly trained breath-hold divers (BHDs) to a control group of NDs; however, no information has been reported on the oxygen-conserving effect (Tocco et al., 2012).

Besides, it has recently been demonstrated that trained BHDs and no NDs (Caspers, Cleveland, & Schipke, 2011) displayed tri-phasic diving bradycardia whether static BH were performed in air or in submersion at water surface (Costalat, Pichon, Joulia, & Lemaître, 2015). Following an initial exponential drop in HR, the diving bradycardia displayed the occurrence of an “oxygen-conserving breaking point” at mid-BH, i.e., an intriguing breaking point in heart rate (HR) kinetic at the onset of a second HR drop. The breaking point found out in HR kinetics precisely occurred at equivalent level of arterial

oxygen saturation between dry-body BH and immersed-body BH ($\approx 95\%$), despite different BH durations (Costalat et al., 2015). These additional physiological events might represent unique adaptive feature of the human diving response to increase its oxygen-conserving efficiency during hypoxemic challenge induced by prolonged BH.

The aim of this study was to quantify, through a kinetic-based approach, the oxygen-conserving potential of the diving response by comparing trained BHDs to NDs during simulated BH. We hypothesised that the magnitude of the diving response would be more pronounced in BHDs than the one in NDs due to substantial changes in both cardiac and peripheral haemodynamics, resulting in increased oxygen-conserving efficiency in BHDs.

Materials and methods

Participants

The study was performed on 16 healthy male participants split into two groups: trained BHDs ($n = 8$) and NDs, i.e., who had never been involved in any sub-aquatic activities ($n = 8$). All participants were informed about the objectives and procedures of the study, and all gave written consent prior to the start of the experiment. The study was conducted in accordance with the Declaration of Helsinki and was approved by the local research ethics committee.

Experimental design

In both groups, time courses of cardiac haemodynamics, skeletal tissue oxygenation and peripheral oxygen saturation changes were continuously recorded during a maximal dynamic BH with cold face immersion. First, every participant performed one of the most commonly used sub-maximal ergometry protocol (Astrand-Ryhming) to determine their workload during dynamic BH, i.e., 30% of each individual's peak power output (PPO). We used the nomogram of Astrand, which predicts maximal oxygen consumption ($\dot{V}O_{2\max}$) from the steady-state HR reached after 6-min of constant loading at an individually chosen work rate (Astrand, 1960). Based on predicted $\dot{V}O_{2\max}$, the workload used during dynamic BH (30% of PPO) was calculated for each participant through the existing relationship between $\dot{V}O_{2\max}$ and PPO [$PPO = (\dot{V}O_{2\max} - 0.435)/0.01141$] (Hawley & Noakes, 1992).

On a separate day, following a 10-min rest period, every participant performed their own maximal BH with cold face immersion while steadily pedaling at 30% of their PPO. During the resting phase, the participants were seated up-right on a cycloergometer (E100 P/K, Cosmed) and data such as the systolic and diastolic blood pressure, calibrations of haemodynamic parameters and baselines values were recorded. Then, both groups followed a countdown process similar to that of BH competitions with the following timing: 2 min, 1 min, 30 s and then every second of the last 10 s prior to starting. Preceding BH, the participants were asked to lean over a water-filled basin on a home-made shelf positioned slightly above the ergometer so that the participants could rest their

arms on each side of the basin. Simultaneously, the participants were asked to pedal at a constant pedal rate of 60 rpm until the end of the BH. Visual checks were systematically performed to ensure the participants had their face fully immersed, i.e., forehead, periorbital and nasal region. The pedal rate was continuously regulated through verbal notifications to the participants. BHDs were instructed not to hyperventilate before BH.

Haemodynamic measurements

CO, stroke volume (SV) and HR were estimated by bio-impedance (PhysioFlow PF-05, Manatec Biomedical, Macheren, France), a non-invasive method commonly used nowadays to determine cardiodynamic parameters at rest (Charloux et al., 2000; Tonelli, Alnuaimat, Li, Carrie, & Mubarak, 2011) and during exercise (Tordi, Mourot, Matusheski, & Hughson, 2004; Welsman, Bywater, Farr, Welford, & Armstrong, 2005). The relationship between peak CO derived from the impedance-based device and the direct Fick method has been shown to be high at rest ($r = 0.89$, $P < 0.001$), submaximal exercise ($r = 0.85$, $P < 0.001$) and maximal exercise ($r = 0.94$, $p < 0.01$). The PhysioFlow methodology has been described in detail elsewhere (Richard et al., 2001). Briefly, the bio-impedance method for determining CO uses transthoracic impedance changes (dZ) in response to an electrical current administered during cardiac ejection to calculate SV. After shaving and applying a mildly abrasive paste (Reegaponce, Bussy Saint-Georges, France) to the skin, two sets of electrodes, one transmitting and the other receiving, are applied above the supraclavicular fossa (left side) and along the xiphoid process of each participant. Another pair of electrodes is used to measure a single electrocardiogram signal (ECG). After an autocalibration over 30 heart beats, CO is then continuously calculated (beat-to-beat) by multiplying the stroke volume index (SVi) with the body surface area (BSA) and HR, which is obtained from the R-R interval determined on the ECG first derivative:

$$CO(l \cdot \min^{-1}) = HR(\text{beats} \cdot \min^{-1}) \times SVi(\text{ml} \cdot \text{m}^{-2}) \times BSA(\text{m}^2)$$

The same experimenter used this device and performed the haemodynamic recording throughout the study.

Peripheral oxygen saturation and peripheral muscle oxygenation

Peripheral oxygen saturation (SpO_2) was assessed by fingertip pulse oximetry (PalmSat 2500, Nonin Medical, Inc., USA). Changes in oxygenated haemoglobin ($[HbO_2]$), deoxygenated haemoglobin ($[HHb]$) and total haemoglobin ($[tHb]$, i.e., $[HbO_2] + [HHb]$) from the vastus lateralis were continuously estimated using dual-wavelength near-infrared spectroscopy (NIRS; Portamon, Artinis Medical Technologies, BV, The Netherlands). This commercial wireless model has been extensively used to investigate muscle oxygenation and haemodynamics (Ferrari, Muthalib, & Quaresima, 2011; Neary, 2004). Briefly, the self-contained model houses a photon detector and an emission probe, which includes three light sources

emitting each two near-infrared wavelengths (850 and 760 nm tracking [HbO₂] and [HHb], respectively). These light sources are in a spatially resolved configuration, which allows deriving an absolute measure of tissue oxygen saturation [tissue saturation index (TSI)] representing the weighted average of arterial, capillary and venous blood oxygenation in relation to the total amount of haemoglobin (Suzuki, Takasaki, Ozaki, & Kobayashi, 1999):

$$\text{TSI}(\%) = 100 \times \frac{[\text{HbO}_2]}{[\text{HbO}_2] + [\text{HHb}]}$$

[HHb] is considered as blood volume insensitive during exercise, so that it was assumed to reflect muscle O₂ extraction (Ferreira, Koga, & Barstow, 2007; Grassi et al., 2003). The contribution of both oxygenated and deoxygenated myoglobin to the NIRS signal remains under debate and current works claimed they might contribute for more than 50% of the near-infrared signal in mammalian muscles, including those of the human leg (Davis & Barstow, 2013). As a result, the terms [HHb], [HbO₂] as well as [tHb] will always refer in the present study to the concentrations of both haemoglobin and myoglobin. The device was attached over the belly of vastus lateralis using surgical straps and was wrapped into a black light-absorbing cloth to prevent probe movement during exercise and contamination from extraneous light. Data were recorded at a rate of 10 Hz and a differential path-length factor of 4 was applied to account for photons' scattering into the tissue (Matcher, Elwell, Cooper, Cope, & Delpy, 1995).

Blood lactate collection

Blood lactate samples (5 µl) were drawn from capillary finger blood by means of a hand-held portable analyser (Lactate Pro, LP, Arkay KDK, Japan) at baseline and 3 min following dynamic BH. This analyser has shown to display good accuracy (standard error of the estimate (SEE) = 1.1 mM) and reliability ($r = 0.95$) when compared to a laboratory-based device (Tanner, Fuller, & Ross, 2010).

Data analysis

Kinetics

All data sets were time-aligned in each group using cubic spline interpolation/extrapolation since BH durations within the same group were slightly different. Prior kinetic analysis, a moving average with a filter width of 0.5 s was applied on [HbO₂], [HHb], [tHb] and TSI. Then, we chose to normalise NIRS variables (except for TSI) by using the percentage change from the starting value of dynamic BH as follows:

$$\text{Relative change } (\%) = \frac{\text{Absolute value}}{\text{Absolute starting value}} \times 100$$

Changes in intra-thoracic pressure induced by BH may have modified the first mathematical derivative of the impedance signal and might have overestimated SV and CO (Bougault

et al., 2005), so that only relative haemodynamic changes for SV and CO were considered, to avoid possible bias.

According to the observed mean HR kinetic, HR kinetics of BHDs were fitted into a piecewise double-linear regression (two continuous line segments) whereas simple linear regressions were fitted to HR kinetics of NDs (Figure 1, panel a). Based on an iterative approach (Levenberg-Marquardt algorithm), these regression analyses aimed to assess the sensitivity in HR drop while in dynamic BH as well as to highlight breaking points in HR kinetics in a non-arbitrary manner. The adjusted coefficient of determination (R^2_{adj}) was used to assess goodness of fit for each regression. Regressions were performed by means of the curve fitting toolbox implemented in OriginPro software 9.0 (OriginLab, Northampton, MA, USA).

Statistical analysis

The samples were first tested for equality of variances and sphericity with Levene's test and Mauchly's test, respectively. If Mauchly's test was significant, the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity. A two-way between-within ANOVA was conducted to compare kinetics of CO, HR, SV, SpO₂, [HbO₂], [HHb], [tHb] and TSI between groups (between factors, i.e., BHDs and NDs) across time (within factors, i.e., 0%, 25%, 50%, 75% and 100% time points). Multiple pairwise comparisons adjusted with the Bonferroni correction were conducted whenever the main effect was significant. A P -value <0.05 was considered statistically significant for all analyses. Statistical analysis and graphs were performed using OriginPro software and SigmaPlot software version 12.3 (SPSS, Chicago, IL, USA), respectively. The results as shown in the tables as well as in the narrative are expressed as the mean value $\pm$ SD. For clarity purposes, graphs are expressed as percentage of total BH time in both BHDs and controls.

Results

Anthropometric data from each group along with a brief quantitative diving history of the BHDs are shown in Table 1. Ambient air temperature was similar for each group (21.9 ± 1.4 vs. $21.3 \pm 2.3^\circ\text{C}$, respectively; $P = 0.46$) as well as water temperature (15.9 ± 1.3 vs. $16.7 \pm 1.7^\circ\text{C}$, respectively; $P = 0.13$). BH durations were longer in BHDs than in NDs (77.5 ± 10.2 vs. 51.5 ± 17.2 s, respectively; $P < 0.05$). PPO were similar between

Table 1. Anthropometric data of breath-hold divers (BHDs) and non-divers (NDs).

	Participants ($n = 16$)	
	BHDs ($n = 8$)	NDs ($n = 8$)
Age (years)	38.8 ± 8.6	36.0 ± 7.8
Height (cm)	177.6 ± 6.6	178.3 ± 6.5
Body mass (kg)	79.5 ± 15.0	77.5 ± 15.5
Body fat mass (%)	13.6 ± 7.4	12.4 ± 8.1
YAP (years)	6.4 ± 4.4	
BH training ($\text{h} \cdot \text{wk}^{-1}$)	3.7 ± 1.1	
Dynamic PB (m)	114.5 ± 40.2	
Static PB (s)	303.0 ± 61.7	

Values are mean $\pm$ SD. BH, breath-holding; YAP, years of apnea training; PB, personal best performance.

BHDs and NDs (328.4 ± 76.3 vs. 283.9 ± 49.1 W, respectively; $P = 0.23$) as well as workloads (30% of PPO) during dynamic BH (97.0 ± 23.8 vs. 85.2 ± 14.7 W, respectively; $P = 0.36$).

Kinetics

The time course of each haemodynamic parameter, arterial oxygen saturation and peripheral muscle oxygenation as well as their respective time points used for time-series analysis are presented in Figures 1 and 2. Based upon double-linear regression analysis, BH is separated in two phases according

to the point in time at which the “oxygen-conserving breaking point” occurred. Both groups showed a progressive decrease in HR throughout BH ($P < 0.05$, Figure 1, panel a). However, the significant interaction from two-way ANOVA and subsequent particular comparisons revealed that decreased HR was more pronounced in BHDs than in NDs at the end of BH (51.5 ± 8.3 vs. 79.1 ± 15.4 bpm, respectively; $p < 0.01$) due to an additional drop in HR occurring at mid-BH ($P < 0.05$; Figure 1, panel a). As a result, BHDs reached at the end of BH an absolute HR lower than their initial resting value (51.5 ± 8.3 vs. 72.8 ± 5.4 bpm, respectively; $P < 0.01$) whereas

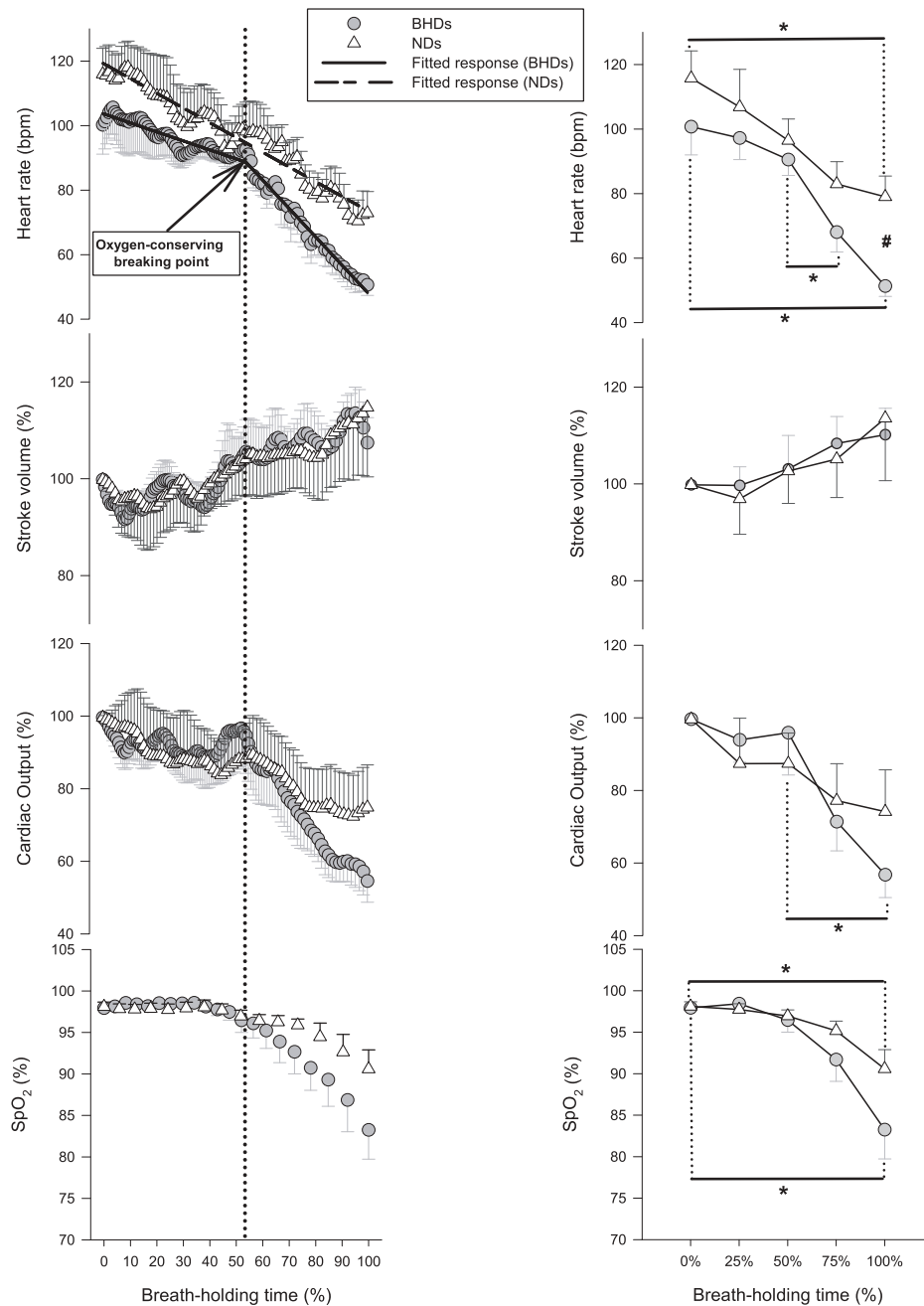


Figure 1. Mean ($\pm$ SE) time courses of heart rate (panel a), stroke volume (panel b), cardiac output (panel c) and peripheral oxygen saturation (panel d) during simulated breath-holding expressed as percentage of total breath-holding time in BHDs and NDs. Breath-holding is separated into two phases with a vertical dotted line passing through the “oxygen-conserving breaking point”. Note that the graph at the top left-hand corner describes the beat-to-beat HR reduction during dynamic BH, onto which regression-based analyses were performed to highlight the breaking point only observed in BHDs. * $P < 0.05$ vs. precedent time point; # $P < 0.05$ between groups.

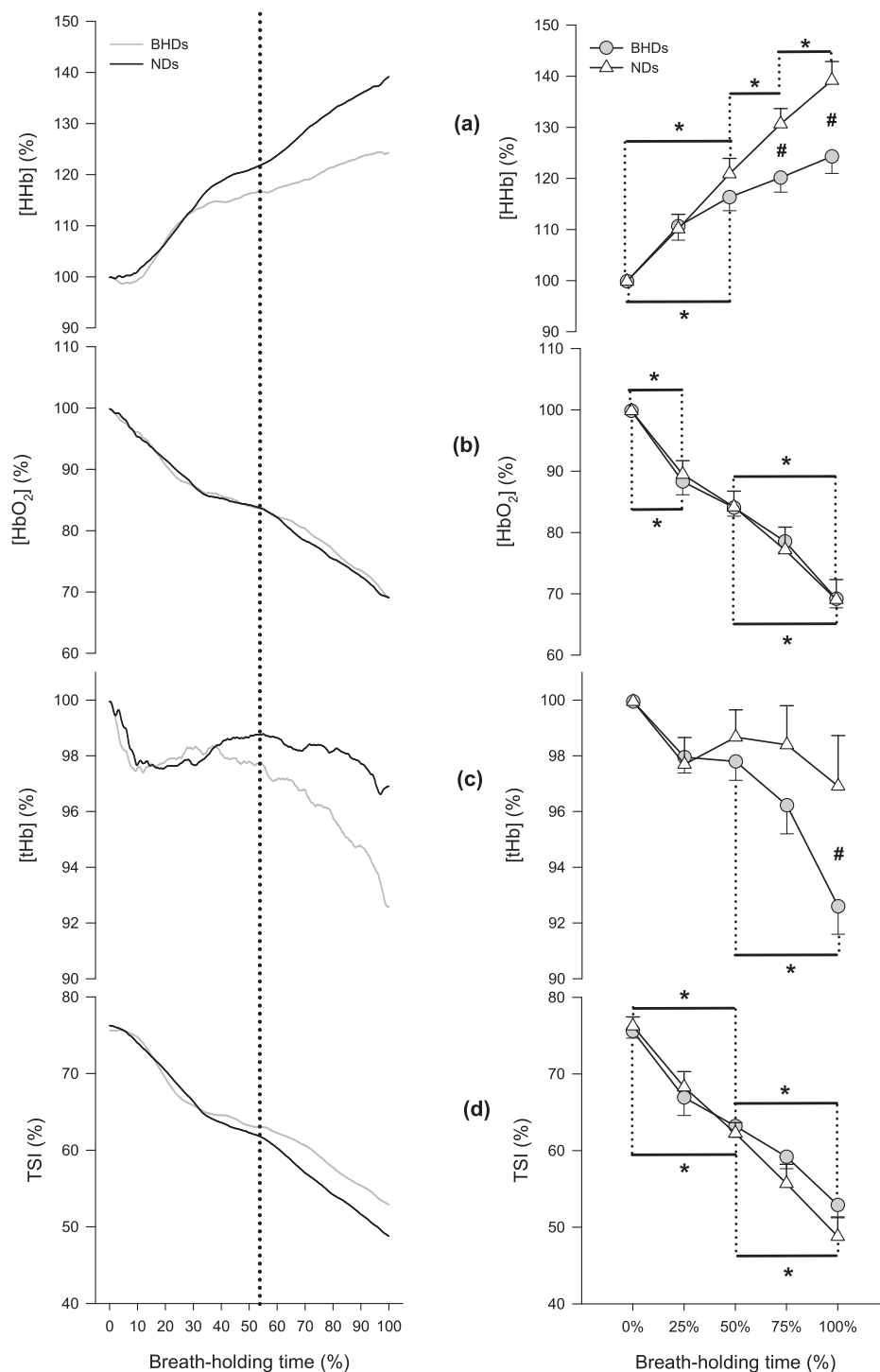


Figure 2. Mean time courses of deoxygenated haemoglobin ([HHb], panel a), oxygenated haemoglobin ([HbO₂], panel b), total haemoglobin ([tHb], panel c) and tissue saturation index (TSI, panel d) during simulated breath-holding expressed as percentage of total breath-holding time in BHDs and NDs. In BHDs, note that the decreased blood flow ([tHb]) in the left graphs is almost concurrent with the breaking point found out in HR kinetic, as shown by the vertical dotted line passing through the “oxygen-conserving breaking point”. * $P < 0.05$ vs. precedent time point; # $P < 0.05$ between groups (standard errors are not showed for clarity purpose).

NDs did not (79.1 ± 15.4 vs. 80.9 ± 16.3 bpm, respectively; $P = 0.37$). As SV remained unchanged throughout BH in both groups (all $P > 0.05$; Figure 1, panel b), only BHDs reached at the end of BH a CO percentage lower than their respective BH starting values ($-42 \pm 16.5\%$, $p < 0.01$; Figure 1, panel c).

Mean SpO₂ ended below its BH starting values in both BHDs (98.2 ± 1.2 vs. $83.5 \pm 9.2\%$, $P < 0.01$; Figure 1, panel d) and NDs

(98.3 ± 0.8 vs. $90.8 \pm 5.0\%$, $P < 0.05$; Figure 1, panel d) although no interaction was found in SpO₂ between groups regardless of the BH time point ($P = 0.14$). Similarly, the nadir SpO₂ reached following BH were similar between BHDs and NDs (80.5 ± 8.1 vs. $89.0 \pm 4.7\%$, respectively; $P = 0.13$). [HbO₂] and TSI decreased in a similar manner from their respective BH starting values for BHDs and NDs (all $P < 0.05$, Figure 2, panel b and d). Two-way

Table 2. Regression parameters and their respective confidence limits describing heart rate behaviour during dynamic breath-holding in BHDs and NDs.

Parameters	Double-linear regression ($R^2_{adj} = 0.98$)		Linear regression ($R^2_{adj} = 0.94$)	
	BHDs	LCL – UCL	NDs	LCL – UCL
HR _{S1} (bpm.% ⁻¹)	-0.23	-0.32 – -0.14	-0.47	-0.52 – -0.42
O _{2bp} (% of BH time)	53.6	48.9 – 58.3	–	–
HR _{S2} (bpm.% ⁻¹)	-0.86*	-0.96 – -0.78	–	–

O_{2bp} ("Oxygen-conserving breaking point"), time delay at which heart rate kinetic changes its behaviour; HR_{S1}, slope (sensitivity) of the decrease in heart rate before O_{2bp} for breath-hold divers and all along breath-holding for non-divers; HR_{S2}, slope (sensitivity) of the decrease in heart rate following O_{2bp}; R^2_{adj} , adjusted coefficient of variation; BHDs, breath-hold divers; NDs, non-divers; BH, breath-holding; LCL, lower confidence limit (95%); UCL, upper confidence limit (95%). * $P < 0.05$ vs. HR_{S1}.

ANOVA indicated interactions between groups and time points for [HHb] ($P < 0.01$) and [tHb] ($P < 0.05$). More specifically, Bonferroni post-hoc comparisons indicated that BHDs had a lower increase in [HHb] than NDs had from time points representing 75% of BH duration ($+20.3 \pm 8.8$ vs. $+30.7 \pm 7.9\%$, respectively; $P < 0.05$) until the end of BH ($+24.0 \pm 10.1$ vs. $+39.2 \pm 9.6\%$, respectively; $P < 0.05$). As a result, BHDs showed a drop in [tHb] from their respective BH starting values whereas NDs did not at the end of BH (-7.3 ± 3.2 vs. $-3.0 \pm 4.7\%$, respectively; $P < 0.05$).

Blood lactate concentration

Two-way ANOVA revealed interaction in blood lactate concentration between groups and time ($P < 0.05$). Particular comparisons showed that lactate were higher in BHDs than in NDs following dynamic BH (3.58 ± 0.82 vs. 2.3 ± 0.74 mM, respectively; $P = 0.01$) despite similar baseline values (1.75 ± 0.18 vs. 1.5 ± 0.33 mM, respectively; $P = 0.10$).

Linear regressions

R^2_{adj} , slope estimates and their respective confidence intervals describing HR kinetics in both groups are reported in Table 2. At the breaking point found in HR kinetics, SpO₂ percentages were similar between BHDs and NDs (96.5 ± 4.4 vs. $96.9 \pm 1.2\%$, $P = 0.59$).

Discussion

The main finding of the present study was that BHDs showed a more pronounced diving response during simulated dynamic BH than NDs. Indeed, kinetic analyses revealed that the enhanced magnitude of the diving response was due to additional physiological events unique to BHDs, i.e., simultaneous shifts in both cardiac and peripheral haemodynamics occurring approximately at mid-BH. These led to promote overall oxygen-conserving efficiency in BHDs.

For BHDs, regression analysis identified a breaking point in HR kinetic at the onset of a second more pronounced drop in HR whereas NDs displayed a steady HR decrease throughout BH. Moreover, the time course analysis for [HHb] revealed a progressive increase at mid-BH in both groups, however less significant in BHDs than in NDs (Figure 2, panel a). This phenomenon reveals a progressive decrease in oxygen extraction to the vastus lateralis in BHDs. At the breaking point in HR kinetic, only BHDs showed a drop in [tHb] as a result of a

[HbO₂] decrease similar in both groups associated with an [HHb] increase less pronounced in BHDs. Changes in [tHb] report the volume of blood in the muscle where near-infrared signal is assessed (Van Beekvelt, Colier, Wevers, & Van Engelen, 2001). Under constant haematocrit conditions, change in regional blood volume reflects change in regional blood flow, hence in oxygen delivery to the muscle studied (Perrey, 2008; Van Beekvelt et al., 2001). As the drop in TSI was similar between both groups (Figure 2, panel d), reduced oxygen extraction to the working muscle observed in BHDs may only be explained by reduced muscle blood flow. In our view, decreased vastus lateralis blood flow is likely to be due to a substantial rise in sympathetic-mediated peripheral vasoconstriction in several muscle vascular beds since an increase in muscle sympathetic nerve activity has been previously recorded during BH in BHDs (Heusser et al., 2009). Significant and in some instances massive increase in muscle nerve sympathetic activity has also been reported in healthy NDs during simulated BH with cold face exposure (Shamsuzzaman et al., 2014). Accordingly, it might be assumed that the likelihood of an increased peripheral sympathetic activity in both groups would lead to an oxygen-preserving efficiency only significant in BHDs, as indicated by the increase in oxygen extraction to the working muscle being less pronounced in BHDs than in NDs (Figure 2, panel a). Additionally, increased blood lactate concentrations in BHDs at exercise intensity normally not correlated with lactic acid accumulation are in accordance with this hypothesis. Considered as an oxygen-conserving mechanism (Ferretti, 2001), arterial blood lactate accumulation from muscles whose blood flow is restricted supports the idea of a shift to a predominance of anaerobic glycolysis pathway in BHDs (Andersson et al., 2004; Schagatay, 2010). Increased post-BH lactate concentration indirectly supports the recent results of Roecker et al. who have demonstrated that BHDs had greater shift in glycolytic pathway than NDs during a standard incremental cycle exercise test protocol (Roecker et al., 2014). During BH, according to the mean [tHb] kinetic (Figure 2, panel c), a metabolic shift might have precisely occurred when the skeletal muscle blood flow started to decrease, i.e., at the "oxygen-conserving breaking point", a phenomenon possibly facilitated by a higher proportion of fast-twitch glycolytic fibres (IIx) in BHDs (Bae et al., 2003).

Earlier investigations with NIRS have observed, amongst others, skeletal muscle desaturation preceding arterial oxygen desaturation during prolonged static BH in both BH-trained and untrained participants, therefore supporting an

oxygen-conserving effect in humans (Palada et al., 2007; Valic et al., 2006). However, either study failed to report changes in muscle blood volume [tHb] throughout BH. Since only based on static conditions, these results also suggest that exercise-induced stimuli during BH are key factors to track changes in muscle blood flow ultimately altering oxygen delivery to the working muscles. As a result, this observation supports, at least for BHDs, the idea that the oxygen-conserving effect is relatively more pronounced in dynamic BH than in static BH (Butler & Woakes, 1987). However, it should be noted that [HbO₂] showed similar kinetics in both groups throughout BH, so that this NIRS variable might not be representative of peripheral oxygenation while in dynamic BH. Indeed, as [HbO₂] is known to be blood-flow sensitive (Grassi et al., 2003), it is not possible to tell whether its drop resulted from oxidative metabolism pathway or BH-induced vasoconstriction. Consequently, [HbO₂] fails to discriminate the relative contribution of either mechanism when muscle blood flow ([tHb]) is bound to differ between groups, as in the present study.

To our knowledge, this is the first study reporting on a breaking point in HR kinetics for BHDs performing dynamic BH. By contrast, no such breaking point could be identified for NDs as single linear regression was sufficient to describe their HR kinetics (Table 2 and Figure 1, panel a). In BHDs, it is noteworthy that this particular point in HR kinetic, i.e., the “oxygen-conserving breaking point” occurred at almost similar BH time (50–55% of BH duration) and SpO₂ level (95–96%) than the breaking point recently reported during prolonged static BH in trained BHDs (Costalat et al., 2015). In the present study, the drop in muscle blood flow (i.e., drop in [tHb]) was almost concurrent with the breaking point in the HR kinetic, so that giving further credence to the emerging concept of an “oxygen-conserving breaking point” (Costalat et al., 2015). Additionally, these concurrent haemodynamics events at mid-BH demonstrated that BHDs had an increased diving response associated with reduced rate of peripheral oxygen desaturation, which are thought to reflect the oxygen-conserving effect (Engan et al., 2013; Joulia, Lemaître, Fontanari, Mille, & Barthelemy, 2009). Interestingly, the “oxygen-conserving breaking point” immediately preceding changes in both HR and peripheral haemodynamics occurred at similar level of arterial oxygen saturation in both groups (Figures 1 and 2). This breaking point in HR kinetic might be concomitant with increased sensory discharge frequency of the carotid sinus nerve where chemoreceptors are located (González, Almaraz, Obeso, & Rigual, 1992). Therefore, additional haemodynamics events might result from increased chemosensitivity to hypoxia in BHDs due to exposures to repeated bouts of hypoxemia during their training. Nevertheless, the onset of involuntary breathing movements, i.e., physiological breaking point, was not recorded in the present study. Involuntary breathing movements result from stimulation of the respiratory drive due to carbon dioxide accumulation (Lin, Lally, Moore, & Hong, 1974), thereby leading to an increase in venous return, and thus SV, which maintain cerebral perfusion (Dujic et al., 2009). Progressive BH-induced hypercapnic challenge and subsequent increased SV should also be considered as potential contributors to the reduced HR through a baroreflex mechanism in the

latter stage of prolonged BH (Sivieri et al., 2015). In the present study, SV did not increase at the end of dynamic BH in both groups, a phenomenon which is contrary to what is usually observed during resting BH (Costalat, Coquart, Castres, Tourny, & Lemaître, 2013; Cross et al., 2013).

The cardiovascular response to dynamic eupneic exercise is characterised by increases in HR and SV resulting in increased CO associated with a concomitant increased muscle blood flow to meet the metabolic demands of working skeletal muscle (Smith, Mitchell, & Garry, 2006). These adjustments are primarily mediated by alterations in sympathetic and parasympathetic neural activity through several neural mechanisms working in concert among which central command, muscle metaboreflex and arterial baroreflex play a major role (Fadel, 2013). Theoretically speaking, BH with exercise should exert co-activation of sympathetic and parasympathetic limbs, therefore leading to “autonomic conflict” (Shattock & Tipton, 2012). In practice, most of studies have demonstrated that the diving reflex is powerful enough to override the cardiovascular responses to exercise (Bergman, Campbell, & Wildenthal, 1972; Butler & Woakes, 1987; Lemaître, Lafay, Taylor, Costalat, & Gardette, 2013; Wein, Andersson, & Erdéus, 2007). Lower absolute HR reached by BHDs than their respective resting values at the end of BH are in accordance with previous studies performed during simulated dynamic BH (Wein et al., 2007), thus giving further credence to the claim that the diving response refers to the most powerful autonomic reflex known (Panneton, McCulloch, & Sun, 2000). Finally, our results suggest that the trainable part of the diving response mostly pertains to HR since, as previously mentioned, no difference was found in SV between the two groups. This is in accordance with the only work that evaluated this aspect under fairly similar experimental conditions (Tocco et al., 2012).

The breaking point and subsequent haemodynamic adjustments were only observed in BHDs and might be interpreted as unique adaptive features against hypoxemic challenge in the human diving response. Besides, enhanced ventilatory responses to hypoxia without altered hypercapnic ventilatory responses have recently been observed in BHDs (Costalat, Pichon, Coquart, Bauer, & Lemaître, 2014). Collectively, these results suggest that BH training might increase the sensitivity to hypoxia to promote overall oxygen-conserving efficiency while in BH. Finally, NIRS showed that muscle blood flow was restricted in active muscles during the hypoxic phase of dynamic BH, a phenomenon likely involved in the increased post-BH lactate concentration. Given the very low intensity used during dynamic BH, our results suggest that BH training might be of interest to increase, or at least maintain, glycolytic metabolism in athletes participating to return-to-sport program. These early results are encouraging but further investigations involving specific BH training are essential to clarify its physiological effects in NDs.

Limitations

A number of limitations have to be considered in the present study. One could question the indirect method we used to estimate both $\dot{V}O_{2\max}$ and PPO to ultimately determine each

individual's workload during dynamic BH. It has indeed been shown that the correlation between predicted $\dot{V}O_2\text{max}$ and measured $\dot{V}O_2\text{max}$ was rather low (Hawley & Noakes, 1992) along with a tendency to underestimate its measure in men (Hartung, Krock, Crandall, Bisson, & Myhre, 1993). In our study, all participants strictly followed the same protocol to estimate their own $\dot{V}O_2\text{max}$ and PPO; it has therefore been hypothesised that underestimation of both these measures likely occurred in the two groups, thus minimising the effects of this approximation on data interpretation.

During prolonged eupneic exercise, it has recently been demonstrated that NIRS variables might be affected by the interference of cutaneous circulation on muscle blood volume (Messere & Roatta, 2013; Miyazawa et al., 2013). Besides, the diving reflex is known to alter skin blood flow during static and dynamic BH either with or without face immersion (Andersson, Schagatay, Gislén, & Holm, 2000; Andersson et al., 2004). As a result, changes in [tHb] during BH might not represent the real haemodynamic changes in muscle blood flow similarly to what was previously reported in the literature during eupneic exercise. However, we found significant elevations in post-BH blood lactate measurements which indirectly suggest that changes in [tHb] resulted from changes in oxygen delivery to contracting muscle. Consequently, it is reasonable to assume that these changes cannot be solely attributable to decreased BH-induced skin blood flow.

Conclusion

In summary, the kinetic-based analysis developed in the present study demonstrated that BHDs elicit a more pronounced diving response during dynamic BH through substantial shifts in both cardiac and peripheral haemodynamics that ultimately promoted overall oxygen-conserving efficiency in BHDs. The breaking point identified in HR kinetics was concomitant with the drop in peripheral haemodynamics, thus giving further credence to the emerging concept of an "oxygen-conserving breaking point" in the human diving response.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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